Quality Assessment and Standardization of "Seetharama Watee" and "Maha Varthikava Watee"

By

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The work described in this thesis was carried out by me under the Supervision of Prof. K.K.D.S. Ranaweera Prof. M.H.A. Tissera and Prof. P.A.J. Yapa and a report on this has not been submitted in whole in part to any university or other institution for another Degree/Diploma.

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We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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AFFECTIONATELY DEDICATED

TO

My Late Mother and Father
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1S₀ authentically prepared Seetharama sample 1
2S₀ authentically prepared Seetharama sample 2
3S₀ authentically prepared Seetharama sample 3
S₁ Commercial Seetharama sample 1
S₂ Commercial Seetharama sample 2
S₃ Commercial Seetharama sample 3
S₄ Commercial Seetharama sample 4
S₅ Commercial Seetharama sample 5

1V₀ authentically prepared Maha Varthikava sample 1
2V₀ authentically prepared Maha Varthikava sample 2
3V₀ authentically prepared Maha Varthikava sample 3
V₁ Commercial Maha Varthikava sample 1
V₂ Commercial Maha Varthikava sample 2
V₃ Commercial Maha Varthikava sample 3
V₄ Commercial Maha Varthikava sample 4
V₅ Commercial Maha Varthikava sample 5

VW-Maha Varthikava Watee
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Standardization and Quality Assessment of Seetharama Watee and Maha Varthikava Watee

Tissa Hewavithana

ABSTRACT

In present context, standardization of herbal drugs is a crucial issue in herbal drug industry as there are unauthentic counterparts found in the market. Seetharama Watee (SW) and Maha Varthikava Watee (MV) are two effective herbo mineral and poly herbal drugs, recorded in Sri Lankan ancient text Watika Prakaranaya published in 1879. SW contains 28 herbs and 9 minerals and mainly effective on febrile illnesses while the MV contains 29 herbs, and very effective in curing digestive tract disorders. As commercially available products may have not been prepared according to the authentic formulas, their efficacy would not be the same as that of the authentic formulas. This work aimed at generating physico-chemical, spectrophotometric and chromatographic fingerprints for the standardization of these two drugs to confirm their authenticity. Following the authentic formulas, the pills of two drugs SW and MV have been prepared. In the preparation of SW, the powdered herbs and minerals were mixed thoroughly and ground for 7 days using 5 herbal juice extracts and 2 oils. In the preparation of MV, all herbs were purified and finely powdered and mixed thoroughly and ground using 3 juice extracts and bee’s honey. These pills were made to the size of a green gram grain and dried under shade. Three batches of the two formulations were prepared to account for seasonal changes and were compared with five commercial samples. The quality test for purity and test for the identity were considered as tools for the standardization. One way ANOVA followed by the Dunnett t-test was used in the analysis of data at 0.05 significant level. The SPSS statistical package was used for this data analysis. When considering the variation of the weight of prepared pills, the mean
weight of SW and MV 10 pills were $1.3\pm0.04$ g and $1.2\pm0.06$ g respectively. pH of the 10% aqueous solutions of two preparations were $5.4\pm0.15$ and $4.6\pm0.07$ respectively, the mean specific gravity of the mean values were $1.2\pm0.01$ and $1.2\pm0.03$, the mean values of weight loss on drying were $9.3\pm0.97$ g and $12.3\pm0.48$ g respectively. In ash content, the mean values were $10.1\pm0.59$ g and $6.8\pm0.17$ g respectively. The mean values of acid insoluble ash of the two formulations were $0.49\pm0.03$ g and $0.09\pm0.07$ g. When considering the crude fiber the mean values were $7.9\pm1.01$ g and $8.4\pm1.8$ g. Mean values of the disintegration time, friability and hardness of SW were $21.7\pm3.61$ min, $0.77\pm0.1\%$, and $1.3\pm0.08$ kg/cm$^2$ while for MV they were $30.5\pm2.8$ min, $1.1\pm0.08\%$ and $0.78\pm0.12$ kg/cm$^2$. Mean weight of the drug extract (residues) of hexane, dichloromethane, ethyl acetate and methanol of SW were $26.5\pm1.4\%$ w/w, $2.4\pm0.03\%$ w/w, $1.5\pm0.18\%$ w/w, $8.4\pm1.5\%$ w/w and $6.1\pm0.84\%$ w/w, $1.7\pm0.2\%$ w/w, $2.2\pm0.52\%$ w/w, $15.3\pm4.4\%$ w/w for MV respectively. When comparing the authentically prepared samples with the commercial counterparts using the above mentioned parameters, some samples were significantly different whereas others were not at the level of 0.05 In the IMVIC test, the microbial content of both preparations were less than the WHO recommended level. The TLC fingerprints of some raw materials were interconnected with these formulations. Aflatoxins were not present in any of the two preparations. Hg and As were present in standard levels and Pb and Cd levels were under the levels WHO recommendation limits in SW. In MV, all heavy metal contents were under the WHO recommendations. The $\lambda_{\text{max}}$ of uv/vis spectrum of SW was 287 nm; 287 nm and 345 nm for MV. Five peaks in the SW and seven peaks in the MV were identified in the HPLC fingerprints of the authentic samples. Hence, SW and MV can be standardized using the above mentioned measurements.
CHAPTER 1

1. Introduction:

1.1 Background:

Traditional herbal medicines have been used for thousands of years in countries like India and Sri Lanka. Herbs, minerals and metals are being used in disease treatments since time of immemorial (Sunita et. al, 2010; Sudha et. al, 2009; Saper et. al, 2004). The consumption of plant-based medicines and other botanicals in the West has been increased manifold in recent years. Between 1990-2000, an increase of consumption of such plant-based medicines over 380% in sales in US has been recorded (Mosiluzzaman and Choudhary, 2008). Global sales of herbal products including herbal medicines are already over USD $10^5$ millions and expected to exceed USD $10^6$ millions in the next 20 years, at the present growth rate (Hussain et. al, 2009). As per WHO report, more than 80% of the world population use medicines made of herbal and natural products. Especially in Asia, Africa, Latin America and the Middle East, people use traditional and herbal medicines, for the management of health and as primary health care to address their health care needs and concerns. In some industrialized nations, use of traditional medication is equally significant; Canada, France, Germany and Italy for instance, report that between 70% and 90% of their populations use traditional medicines under the titles “complementary”, alternative or “non conventional”(Anon, 2002,2008, Barns et. al, 2008). During last decade, importance of alternative medicine was realized and with the inclination to this, the growth rate of Ayurveda and herbal industry can be put more than 30% for the last 25 years (Sheth, 2006). The world has now started to feel the needs for longer lasing and more fundamental cures for their diseases and health related issues.
Attention now is being shifted from relief to prevention and cure, by going back to nature and using natural materials and methods of ancient times. Scientifically validated and technologically standardized botanical medicines will play an important role in the advancement of healthcare. The development of parameters for standardization and quality control of botanicals is a challenging task (Warude and Patwardhan, 2005).

The Sri Lankan indigenous medical system is deeply rooted into Sri Lankan culture and now it is very popular among foreign nationals who specially visit Sri Lanka for treatments. Although the Sri Lankan medical practice dates back to even before the Aryan civilization, it is difficult to find any documentary evidence in prehistoric period (Abeysekara, 2007; Kumarasinghe, 1962).

In Sri Lanka, Ayurveda includes Ayurveda itself, Siddha, Unani and Sri Lankan traditional medicine. Ayurveda Department is the main legal body responsible for operating Ayurveda, Siddha, Unani and traditional medical systems in the country for the benefit for the people (Anon, 1961).

In older times, the native physician treated patients on individual basis, with drugs prepared by himself according to the requirements of the patients and used the traditional technology. However, with the changes of the society due to factors like urbanization and commercialization of the industry, the scenario has changed. At present, the herbal medicines are being manufactured at large scale using various modern processing and manufacturing methods by both state and private sector pharmaceutical units. In this context, these manufacturers come across with many problems such as non availability of raw materials of required quality, authentication of raw materials, non availability of standards for the recipes, lack of proper
standardization methods for single drug and formulations and the final products, nonavailability of quality controller parameters etc. (Agrawal, 2005).

In the 21st century, Ayurveda needs changes and improvements in order to become a user-friendly healthcare system accepted by people. It has to accept the new challenges and be prepared to answer the queries related to quality, toxicity and efficacy of the herbal drugs administered to patients and how best the herbs are cultivated, collected, processed, preserved and used (Patra et al., 2009; Takate et al., 2010). Ayurveda and herbal medicine emphasize the relationship between man and plant through the development of human culture. Herbal medicines are the major remedy in traditional systems of medicine and they have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health (Sane, 2002). The development of these traditional systems of medicines with the perspectives of safety efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the healthcare systems (Mukherjee, 2006, 2003).

The need for the quality control methods for the Ayurveda and indigenous medicinal drugs prepared according to ancient systems of medicine is over looked, due to the commercialization of the Ayurveda pharmacy during the current century (Reddy, 1998; Pattanaya et al., 2010).

With the commercialization of herbal medicinal preparations, their quality has remarkably neglected as the most of the manufactures are interested mainly on their profit rather than the safety, quality and efficacy of the drugs they produced. Plant materials, when used in bulk quantity, may contain adulterants and unauthentic plant
materials as much may vary in their chemical compositions resulting in changed or reduced therapeutic effects. Different batches of raw materials collected may vary according to the factors like the materials being collected in different seasons, from locations of different agro-climatologic or different environmental surroundings or geographical locations (Ram, 2009).

The tests for strength or potency of medicines can be done through assays, which determine the quality of the drug. These tests can be categorized into chemical and physical measurements or bioassays. The standards for the quantitative strength indicate the required level of concentration. It is necessary to determine their potency by means of a biological indicator.

The tests for quality are accurate measurement of the characteristic properties of drug. Therefore, a qualitative test can be employed to demonstrate that a physical property measured for drug product is within the range of values obtained for a purified specimen of authentic drug sample. Hence, the standards for quality are given as permissible range, lower and upper limit of values for a measured properly (Tennakoon, 2002).

The tests for purity are designed to detect and quantify any contaminating substances and the adulterants in the drug preparation. The purity tests are quantitative estimates of impurities (Fernando, 1986).

The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant based products by using modern techniques and applying suitable standards (Ekka et. al, 2008).

"Assessment" or evaluation of a drug confirms its identity and determination of its quality and purity and detection of its nature of adulteration. "Standardization" is used to describe the all measures, which are taken during the manufacturing process and
quality control leading to reproducible quality. Standardization is an essential measurement for ensuring the quality control of herbal drugs (Sriwastava et. al, 2010; Kumar et al, 2009).

If the product does not meet with the requirements of the standards, then the drug is considered substandard. Thus, the standardization is a model of the characteristic property of a medicinal preparation. The increasing demand for standardized drugs on one hand and severe shortage of authentic raw materials on the other extreme have made it incumbent, therefore, there should be mechanism to guarantee the uniformity in the manufacture of herbal medicines so as to ensure quality assessment and standardization (Simha et al, 2008, Simha & Laxminarayana, 2008).

The present research intends to study on the quality assessment and standardization of well established, two effective traditional Sri Lankan formulas used in pill form, that were mentioned in ancient pill and paste book titled Watika Prakaranaya published in 1879. The first selected formula was a poly herbo-mineral preparation containing 28 herbs and 9 minerals named Seetharama Water (SW). Herbal ingredients in this preparation consist of Cuminum cyminum L. (Cumin), Nigella sativa L. (Black Cumin), Foeniculum vulgare Miller. (Fennel), Trachyspermum ammi (L.) Sprague. (Ajowan) Anethum graveolens L. (Dill), Zingiber officinale Roscoe. (Ginger), Piper nigrum L. (Pepper), Piper longum L. (Long pepper), Myristica fragrans Houtt. (Nutmeg), fruit and aril, Syzygium aromaticum (L.) Merr. & Perry (Clove), Aconitum palmatum D. Don. Saussurea costus (Falc.) Lipschitz. (Costus), Glycyrrhiza glabra L. (Liquorice). Allium sativum L. (Garlic), Coriandrum sativum L. (Coriander), Holarhena antidysenterica (Roxb.) Wall, Pieracapus santalinus Linn.f. (Red sandalwood), Aconitum heteropyllum Wall. (Atis root).
Picrorhiza kurrooa Royle ex Benth. (Picrorhiza), *Ferula assa-foetida* L. (Asafoetida) *Ocimum tenuiflorum* L. (Holy basil), *Vitex negundo* L. (Indian privet), *Toddalia asiatica* (L.) Lam. (Forest pepper), *Leucas zeylanica* (L.) R.Br., *Cleome gynandra* L., *Azadirachta indica* A.juss. (Neem) and *Acorus calamus* L. (Cinnamon sedge). When preparing this pill, all powdered herbal ingredients, and the powdered minerals, that are cinnabar, orpiment, realgar, borax, zinc ore, Alum, copper sulphate, gypsum, and rock salt are mixed together after using the purification method as in the text. Then ground with juices of the leaves of holy basil, leaves of indian privet, leaves of neem, sour orange, fresh ginger, neem oil and gee respectively for seven days and make pills in green gram fruit size and dried under a shade.

*Mesua ferrea* L. (Ceylon ironwood), *Piper betel* L. (Betel), *Vitex nigundo* L. (Indian privet). When preparing the pill the raw materials were powdered and ground using juices of betel, juice of leaves of Indian privet, fresh ginger and bee honey respectively. This preparation also makes into pill of the size of green gram fruit and dried under a shade. Both preparations were authentic popular formulas among Ayurveda and native physicians. While the *Seetharama Watee* used for various types of febrile illness, convulsions, nausea etc. *Maha Varthikava Watee* is mainly used for the alimentary tract disorders.

The both preparations have large number of herbal ingredients and specify contains herbal ingredients as well as minerals too. There is the possibility of some of the important ingredients may be omitted or inferior quality ingredients can be used by the drug manufactures, particularly large scale commercial preparations. Usage of unpurified minerals in the manufacturing process is another possibility, which may affect the safety and efficacy of the preparation. When inspecting the commercial samples, one finds that sizes of the pills are different, form one manufacturer to other, and it can be affected to the dosage of the medicine significantly.

All these indicate the importance of standardization and quality assessment with production of authentic drugs.

Test for identity can be based on chemical, physical, biological or pharmacological procedures or observations, which indicates distinctive response of the components of the drug. Therefore, an identity test can be a Thin Layer Chromatographic (TLC) fingerprint, High Performance Liquid Chromatography (HPLC) profile UV-VIS spectrophotometry or microscopical examinations. Hence, the standards for identity are qualitative criteria that ensure the presence of the proper constituents of preparation.
1.2 Objectives:

1.2.1. To study the factors affecting the quality and stability of two widely used Ayurvedic preparations namely Seetharama Watee (SW) and Maha Varthikava Watee (MV).

1.2.2. To establish standards for the above two Ayurveda preparations Seetharama Watee and Maha Varthikava Watee as a model for adapting to other Ayurveda preparations.
CHAPTER 2

2. Literature Review:

2.1. Herbal Medicine:

Herbal medicines are historically used in local community region and are well known through long usage by the local population in terms of its composition, treatment and dosage. If this category enters the market or region in the country, they have to meet the requirements of safety and efficacy laid down in the national regulations for herbal medicines. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients part of plants or other plant materials or combinations (Abeysekara, 2007; Anon, 2000).

2.1.1. Herbs:

Herbs include crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered (Anon, 2000).

2.1.2. Herbal Material:

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir baking with honey, alcoholic beverages or other materials (Mehta and Sharma, 2005; Anon, 2000).

2.1.3. Herbal Preparations:

Herbal preparations are the basis for finished herbal products and may include comminuted of powdered herbal materials or extracts, tinctures and fatty oils of herbal materials. Extraction, fractionation, purification, concentration or other physical or
biological processes produce them. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or in other materials (Anon, 2000). Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important (Patra et. al, 2010).

2.1.4. Finished Herbal Products:

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term mixture of herbal products can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituent from herbal materials, are not considered herbal.

Exceptionally in some countries, herbal medicines may also contain by tradition, natural organic or inorganic active ingredients, which are not plant origin (Anon, 2000).

2.1.5. Traditional Use of Herbal Medicines:

Herbal medicines includes herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials or combinations. Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities (Abeysekara, 2007; Anon, 2000).

The most important challenges faced by these formulations arise because of their lack of complete evaluation. Therefore, evaluation is necessary to ensure quality and purity of herbal products.
It is very important to establish a system of evaluation for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous (Sony et. al, 2010).

**2.1.6. Limitations of Herbal Medicines:**

Like in any other branch of science and technology, present scenario of herbal medicine has its own limitations arising out of its own technical constituents. Although there are a few herbal medicines, which work instantly in acute medical problems, most preparations are not varying effective to treat many acute illnesses. On the other hand, modern system of medicine has adequate paraphernalia for management of acute conditions. It has already been established by virtue of its efficacy. It may be a futile exercise to investigate and discover such methods of acute medicinal care within the framework of herbal medicine (Shrikumar, 2007).

Inadequate standardization and lack of quality specifications are another criticized aspect. One important fact is that a herbal preparation is administered for its holistic value. Each herbal ingredient in a herbal preparation has an array of chemical constituents with complex molecular formulae. This each herbal preparation is a source of poly pharmacy within itself (Shrikumar, 2007).

Literature on herbal medicines, lack of scientific data of a drug preparation, the medicinal activity claimed and their safety and efficacy assumed. Hence, there is a need to in cooperate certain parameters of the pharmacological evaluation on modern lines. WHO guide lines clearly directed that is not necessary to carry out detailed toxicological evaluation of herbs and herbal preparation originated from traditional system of medicine.
Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants are not considered as herbal medicine. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients, which are not of plant origin (Shrikumar, 2007).

2.2. Watika Prakaranaya or Treatment on pills:

This is a Sri Lankan book first published in 1879 as printed version, compiled by E.S. de Alvis a native medical practitioner. It contains number of popular and effective pills and pastes in poetry form, practiced for a long period in Sri Lanka. In the first part of the book contains 2063 poems. It also contains the purification methods of the toxic raw materials used in the formulations (Alvis, 1879). It was reprinted in 1903, 1914, 1933 and the second part published in 1894, which contains 1048 poems and reprinted in 1912.

Illeperuma Achchige Don Abraham Alvis, a relation of E.S. de Alvis, published another print giving the same name in 1948, under another publisher, with some different verses. There is a another book called Abinava Watika Prakaranaya published in 1927 by Sandanayaka Siyadoris Tissera, and it contains 3204 poems (Ponnampuruma, 2004).

The book published in 1879 was taken for this study. Two effective and popular pill form formulas in this publication named Seetharama Watee and Maha Varthikava Watee were selected.

2.2.1. Watee and gutika:

Medicines prepared in the form of tablets or pills are known as Watee and Gutika. These are made of one or more drugs of plant, animal or mineral origin.
Gutika, Vatakai, Modaka, Pindi and Watee are synonymous terms used in classics for Watee. The drugs of plant origin are dried and made into fine powders, separately. The minerals are made into Bhasma or Sindura, unless otherwise mentioned. These are put into a khalva and ground to a soft paste with the prescribed fluids. When more than one liquid is mentioned for grinding, they are used in succession. When the mass is properly ground and is in a condition to be made into pills, Gandha Dravyas, like Kasturi, Karpura, which are included in the formula, are added and ground again. The criterion to determine the final stage of the formulation before making pills is that it should not stick to the fingers when rolled. Pills may be dried in shade or in sun as specified in the texts. Pills made of plant drugs when kept in airtight containers can be used for two years. Pills containing minerals can be used for an indefinite period. Pills or Watee should not lose their original colour, smell, taste and form. When sugar, salt or Kshara is an ingredient, the pills should be kept away from moisture (Anon, 2003, 2008 a).

2.3. Seetharama Watee:

Seetharama Watee is a polyherbo-mineral preparation that contains 28 herbals and 9 minerals. It is mainly used to treat various types of fevers, body aches, convulsions, cough, asthma, head ache, vomiting and many more ailments with different Anupanas (Vehicles) (Ariyavansa & Ratnasooriya, 2006).

2.3.1. Cuminum cyminum, (fruit): Apiaceae

Annual herb with a much branched, cylindrical, striate or angular, solid smooth stems about 30 cm high branches spreading. Widely cultivated in India and China. Seeds contain volatile oil, which is composed of hydrocarbon cymol, oxygenated oil called cuminal, acymene and other terpenes.
Medicinally it is a stomachic, carminative and astringent. It is useful for dyspepsia, chronic diarrhoea and bilious nausea (Jayaweera, 2006 e).

Fruit, a cremocarp, often separated into mericarps, brown with light coloured ridges ellipsoidal, elongated, about 4-6 mm long, 2 mm wide, tapering at ends and slightly compressed laterally, mericarps with 5 longitudinal hairy primary ridges from base to apex, alternating with 4 secondary ridges which are flatter and bear conspicuous emergences, seeds orthospermous, odour umbelliferous characteristic, taste, richly spicy (Anon, 2008 a).

Loss on drying 14.9-16.2%, total ash 4.4-9.1%, acid insoluble ash 1.4-2.6%. water soluble extractive 14.7-21.5%, ethanol soluble extractive 33.3-33.6 % (Fernando, 1986).

2.3.2. Nigella sativa, (seed): Ranunculaceae

Annual herb 35-50 cm tall branching at the top stem green, round hairy 2-5 mm diameter. It is not grown in Sri Lanka and not native to India. The seed of this herb contain the alkaloids, connigelline and nigelline. The seeds are aromatic stimulant with a sharp taste having antihelmintic and carminative properties (Jayaweera, 2006 d).

Seeds, flattened, oblong, angular, rugulose tubercular, small, funnel shaped, 0.2 cm. long and 0.1 cm. wide, black, odour, slightly aromatic, taste, bitter.

Powder is black, oily to touch, under microscope show, groups of parenchyma, endosperm cells and oil globules (Anon, 2008 a).

Loss on drying at 105 °C 5.9-8.8%, total ash 3.1-5.6%, acid insoluble ash 1.2-2.7% water soluble extractive 9.1-11.9%, ethanol soluble extractive 27.3-29.3% (Fernando, 1986).
2.3.3. *Foeniculum vulgare*, (fruit): Apiaceae

A large perennial herb cultivated as an annual or biennial with a rootstock and stout, erect cylindrical bright green smooth polished much branched stem 60-120 cm high. It is extensively cultivated in India. The fruit yields a volatile oil, which contains anethol, fixed oil, pectin, and pentosan. The seeds are considered a stimulant, carminative and stomachic relieving flatulence griping of bowels and stomachache (Jayaweera, 2006 e).

Fruits, usually entire with pedicel attached, mericarps, up to about 10 mm long and 4 mm broad, five sided with a wider commissural surface, tapering lightly towards base and apex, crowned with a conical stylopod, glabrous, greenish or yellowish-brown with five paler prominent primary ridges, endosperm, orthospermous (Anon, 2006 a).

2.3.4. *Trachyspermum ammi*, (fruit): Apiaceae

A minutely pubescent herb with a fusiform root stem 30-90 cm tall erect branched leafy. It is extensively cultivated in India. The fresh plant yields a volatile oil, phellandrene and thymol, while the fruits contain p-thymol in addition. Fruits are administrated in flatulence atonic dyspepsia and diarrhoea (Jayaweera, 2006 e).

Fruit, consists of two mericarps, greyish brown, ovoid, compressed, about 2 mm long and 1 mm wide with pale coloured protuberances, 5 ridges and 6 vittae in each mericarp, usually separate, 5 primary ridges pale in colour, odour, characteristic, thymolic, taste, pungent.

Powder is oily, greyish-brown. under microscope, presence of oil globules and groups of endosperm cells, characterized (Anon, 2008 a).

TLC of the methanol extract on precoated silica gel 'G' plate 0.2 mm thick using toluene: ethyl acetate (9:3:0.7 v/v) as solvent system and developed 8 cm. On spraying with anisaldehyde sulfuric acid reagent and heating the plate at 110 °C for ten minutes;
spots appear at $R_f$ 0.45, 0.55 (blue), 0.69 (bright orange) 0.75 (blue) (Guptha et al., 2008).

Loss on drying 8.8-10.6%, total ash 5.14-8.1%, acid insoluble ash 0.6-9%, water soluble extractive 20.7-21.6%, ethanol soluble extractive 10.1-11.1% (Fernando, 1986).

2.3.5. *Anethum graviolens*, (fruit): Apiaceae

An annual or biennial herb with erect cylindrical slightly branched smooth pale green finely striated stem 30-90 cm high. It occurs wild among corn and other crops throughout Europe and in India. The principal constituent oil distilled from seed is a hydrocarbon with lemon odour and oil identical to carvol. The dried fruits are carminative and stomachic. Dill water is administrated to children for flatulence (Jayaweera, 2006).

Fruits, dark brown, often stalk attached, broadly oval and compressed dorsally: mericarps usually separate and free, 4 mm long, 2-3 mm broad and 1 mm thick, glabrous, traversed from the base to apex by 5 lighter coloured primary ridges of which 3 dorsal, slightly raised, brown, filiform and inconspicuous, 2 lateral prolonged into thin, yellowish membranous wings; odour, faintly aromatic resembling that of caraway, and a warm, slightly sharp taste, akin to caraway.

Powder is brown; shows spiral vessels, micro-rosette crystals of calcium oxalate and oil globules, aleuronic grains up to 5 μ in diameter (Anon, 2008 b; Bremness, 1989).

2.3.6. *Zingiber officinale*, (rhizome): Zingiberaceae

A perennial herb with large, solid tough, horizontal rhizome. Widely cultivated in tropical countries and it is commonly grown in Sri Lanka. The rhizome of this contains a pungent principal zingerone and shogaol, while the aroma is due to a volatile oil containing camphene, phellandrene, zingiberene, cineol and borneol.
Ginger rhizomes are used both in fresh and dry forms. They are stomachic, carminative, stimulant, diaphoretic and digestive. They are extremely valuable for dyspepsia, flatulence, colic, vomiting and other pains in the stomach and bowels (Jayaweera, 2006e).

Drug occurs as entire rhizome or in pieces, rhizome laterally compressed bearing flattish ovate, oblique branches on upper side, each having a depressed scar at its apex. pieces 5-15 cm long, 1.5-6.5 cm wide (usually 3-4 cm) and 1-1.5 cm thick, fracture, short with projecting fibres, transversely cut surface shows a wide central stele having numerous greyish cut ends of fibres and yellow secreting cells; odour, gingery; taste, pungent.

Powder is light yellow; shows thin-walled parenchymatous cells, septate fibres with oblique, elongated pits on their walls, reticulate and spiral vessels, oleoresin cells abundant, single starch grains of varying shapes with eccentric hilum, measuring 5-25 \( \mu \) in diameter. Coarse fibrous powder extracted using 95% ethyl alcohol (Rajpal, 2006a).

TLC of alcoholic extract of drug on silica gel 'G' plate using benzene: ethyl acetate (9:1 v/v) in visible light four spots are seen at \( R_f \) 0.16, 0.35, 0.63 & 0.69 (all light yellow). Under UV (366 nm) three fluorescent zones appear at \( R_f \) 0.16 (blue), 0.63 (grey) & 0.69 (grey). On exposure to iodine vapour eleven spots appear at \( R_f \) 0.03, 0.08, 0.13, 0.16, 0.35, 0.47, 0.63, 0.69, 0.76, 0.83 & 0.92 (all yellow). On spraying with vanillin sulfuric acid reagent & heating the plate for ten minutes at 110 °C eight spots appear at \( R_f \) 0.08 (violet), 0.16 (brownish violet), 0.35 (light violet), 0.47 (light violet), 0.63 (light violet), 0.69 (light violet), 0.76 (violet) & 0.92 (violet) (Anon, 2008b).

Volatile oil containing cineole zingiberol and sesquiterpene like zingiberene, bisabolene and sesqui phellandrene, gingerosol in the oleoresin (Anon, 2008b).
Loss on drying at 105 °C 8.4-11.3%, total ash 4.7-9%, acid insoluble ash 0.5-1.7% water soluble extractive 10.8-11.5%, ethanol soluble extractive 5.4-8.9% (Fernando, 1986).

2.3.7. *Piper nigrum*, (fruit): *Piperaceae*

A climbing perennial with cylindrical, dichotomously branched stems much thickened at nodes, glabrous, rooting sparingly, shining, Indigenous to South India but spread moist mid and low-country Sri Lanka. Black pepper contains an acrid resin, an oleoresin, a volatile oil, starch, gum, a fatty oil and inorganic matter besides the alkaloids, chavicine, β-methyl-pyrroline, piperidine and piperovatine. Internally, pepper is a stomachic, carminative and induces secretion of bile. It is used in dyspepsia, flatulence, gonorrhea, cough, hemorrhoids, intermittent fevers, piles and elephantiasis. (Jayaweera, 2006 d).

Fruits greyish-black to black, hard, wrinkled, 0.4-0.5 cm in diameter; odour, aromatic; taste, pungent. Powder is Blackish-grey; shows debris with a characteristic, in groups, more or less isodiametric or slightly elongated stone cells, interspersed with thin-walled, polygonal hypodermal cells; beaker-shaped stone cells from endocarp and abundant polyhedral, elongated cells from peri sperm, packed tightly with masses of minute compound and single, oval to round, starch grains measuring 5.5-11.0 μ in diameter.; having 2-3 component and a few aleuronic grains and oil globules.

TLC of the alcoholic extract on silica gel 'G' plate using toluene: ethyl acetate (7:3 v/v) shows in visible light four spots at Rf 0.05, 0.08 (both light green), 0.27 (light yellow) and 0.52 (yellow). Under UV (366 nm) ten fluorescent zones are visible at Rf 0.05, 0.08 (both light brown), 0.20 (light blue), 0.46 (blue), 0.52 (greenish yellow), 0.57 (bluish yellow), 0.66 (light blue), 0.74 (light pink), 0.82 and 0.97 (both blue).
On exposure to iodine vapour eleven spots appear at Rf 0.05, 0.08, 0.14, 0.20, 0.27, 0.34, 0.46, 0.57, 0.66, 0.74 and 0.97 (all yellow). On spraying with dragendorff reagent followed by 5% methanolic-sulfuric acid reagent nine spots appear at Rf 0.05 (light-orange), 0.14, 0.20, 0.27 (all orange), 0.46, 0.57 (both yellowish orange), 0.66, 0.74 (both orange) and 0.97 (light orange). On spraying with vanillin sulfuric acid reagent and heating the plate for ten minutes at 110 °C twelve spots appear at Rf 0.05, 0.08, 0.20, 0.27, 0.46, 0.52, 0.57, 0.66, 0.74, 0.82, 0.90 and 0.97 (all violet) (Anon, 2008 c).

Loss on drying at 105 °C 9.2-11.7%, total ash 3.4-5.4%, acid insoluble ash 0.1-2.9%, water soluble extractive 5.6-7.1%, ethanol soluble extractive 12.4-14.2% (Fernando, 1986).

2.3.8. *Piper longum*, (fruit): Piperaceae

A perennial herb with a thick, erect, jointed, branched rootstock, stem numerous 60-90 cm long, ascending or prostrate much branched, stout, cylindrical, thickened above nodes finely pubescent. Occurs in warmer parts of India, Sri Lanka etc. It is often cultivated in low country in Sri Lanka, principally in the dry regions. The fruit of this plant contains the alkaloid piperine, in addition to volatile oil and resin. The dried immature fruit-spikes and roots are used in decoction for acute and chronic bronchitis fever and cough. The root has carminative, laxative and expectorant properties (Jayaweera, 2006 d).

Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis; surface rough and composite; broken surface shows a central axis and 6 to 12 fruit lets arranged around an axis; taste, pungent producing numbness on the tongue; odour, aromatic.
Powder is deep moss green, shows fragments of parenchyma, oval to elongated stone cells, oil globules and round to oval, starch grains, measuring 3 to 8 μ in diameter (Anon, 2008 d).

TLC of alcoholic extract of the drug on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) as mobile phase. Under UV (366 nm) six fluorescent zones are visible at Rf 0.15, 0.26, 0.34, 0.39, 0.50 and 0.80. On exposure to iodine vapour seven spots appear at Rf 0.04, 0.15, 0.26, 0.34, 0.39, 0.50 and 0.93 (all yellow). On spraying with vanillin sulfuric acid reagent and heating the plate at 105 °C for ten minutes, five spots appear at Rf 0.04, 0.22, 0.35, 0.43 and 0.82. On spraying with dragendorff reagent, three spots appear at Rf 0.15, 0.26 and 0.34 (all orange) (Anon, 2008 d).

TLC of the methanolic extract on precoated silica gel 60 F254 TLC plate 0.2 mm thick using toluene: diethyl ether: dioxane (62.5:21.5:16 v/v) as solvent system and developed up to a distance of 8 cm, observed the plate under 366 nm. Spots appear at Rf 0.08, 0.10 (fluorescent blue), 0.21 (blush red), 0.28, 0.37 (blue), 0.50 (Fluorescent blue) 0.57 (blue) 0.64 (light blue) 0.71 (fluorescent blue) 0.80 (dark blue) (Guptha, et. al, 2003 a).

Loss on drying at 105 °C 10.6-13.6%, total ash 1.5-4.5% acid insoluble ash 0.005-0.3% water soluble extractive 4.4-7.9% ethanol soluble extractive 9.4-12% (Fernando, 1986).

2.3.9. *Myristica fragrans*, (fruit and aril): Myristicaceae

Moderate sized evergreen tree 8-13 m high with numerous spreading branches covered with greyish-brown rather smooth bark, young branches green. A native of Moluccas and other Indian islands. It is now cultivated in Sri Lanka. The principal constituents of nutmeg are fat, volatile oil, starch, albuminous matter etc. The expressed oil of nutmeg containing myristicin is a useful stimulant in chronic rheumatism, paralysis and sprains, while the volatile oil obtained by distilling the powdered nutmeg with water is
employed as a carminative and stimulant. The seeds of this tree are mainly used as a flavoring agent for medical and culinary purpose. They have carminative and stomachic properties and beneficial for flatulency, nausea and vomiting. The mace is used medicinally and as a condiment (Jayaweera, 2006 d).

Seed ellipsoid, 20-30 mm long and about 20 mm broad, externally greenish-brown sometimes marked with small irregular dark brown patches or minute dark points and lines slightly furrowed reticulately, a small light-coloured area at one end indicating the position of the radical a groove running along the line of raphe to the darker chalaza at the opposite end, surrounded by a thin layer of peri sperm with infoldings appearing as dark ruminations in the abundant greyish-brown endosperm, embryo, in an irregular cavity, small with two widely spreading crumpled cotyledons and a small radical odour. strong and aromatic, taste, pungent and aromatic (Anon, 2008 a).

Hexane extract on precoated silica gel 60F254 plate 0.2 mm thick TLC plate using toluene: ethyl acetate (98:2 v/v) as solvent system and developed to a distance of 8 cm, observe the plate after spraying with anisaldehyde sulfuric acid reagent and heating the plate at 110 °C for 5 minutes. Nine spots appear at R f 0.11 (dark brown), 0.17 (pink), 0.23 (greyish brown), 0.30, 0.42 (brown), 0.426 (greenish yellow) 0.61 (pinkish red) 0.73, 0.81 (dark brown) (Guptha et. al, 2003 a).

Nut- Loss on drying on 105 °C 15-18%, total ash 4.1-5%, acid insoluble ash 3.9-4.9%, water soluble extractive 7.4-9.7%, ethanol soluble extractive 12.4-14.1% (Fernando, 1986).

Aril- Loss on drying at 105 °C 12.5-13.7%, total ash 3.3-4.4%, acid insoluble ash not in detectable amount, water soluble extractive 3.9-5.5%, ethanol soluble extractive 8.7-11.8% (Fernando, 1986).
2.3.10. *Syzygium aromaticum*, (flower bud): Myristicaceae

A small tree about 10-13 m in height with numerous, horizontally spreading slender branches forming a dense pyramidal crown bark pale yellowish grey, smooth with glabrous buds. Flowers occur from January to March. The dried unexpanded flower buds, which are picked, green and spread out in the sun for few days to dry, when they become dark brown. In Sri Lanka, it is grown in the mid country up to an elevation of 2000 feet. The principle constituent of cloves is the volatile oil caryophyllin, a natural tasteless substance and eugéinin, a considerable proportion of gum, tannic acid and salicylic acid. The clove is regarded as a carminative, stomachic and stimulant (Jayaweera, 2006, d).

Flower bud measuring 10-17.5 mm in length, dark brown or dusty red, consisting of a sub-cylindrical, slightly flattened, four sided hypanthium, readily exuding oil when pressed hypanthium containing in its upper portion a two celled inferior ovary with numerous ovules attached to a axile placenta, surmounted by four thick, divergent sepals and covered by unopened corolla consisting of four membranous imbricate petals, frequently detached, enclosing numerous incurved stamens and one erect-style, odour, strongly aromatic, taste, pungent, aromatic followed by slight tingling of the tongue.

Powder is dark brown, fragments of parenchyma showing large oval, schizolysigenous oil cavities, spiral tracheids and a few rather thick-walled, spindle shaped fibres, calcium oxalate crystals in rosette aggregates, 10-15 μ in diameter, fragments of anther walls with characteristic reticulated cells pollen grains numerous, tetrahedral, 15-20 μ. in diameter (Anon, 2008 a).

Loss on drying at 105 °C 11-11.9%, total ash 4.1-4.8%, acid insoluble ash 1.2-3.2%
Water soluble extractive not in detectable amounts, Ethanol soluble extractive 5.4-8.4% (Fernando, 1986).

2.3.11. *Aconitum palmatum*, (rhizome): Ranunculaceae

Stem erect sometimes slightly flexuous in the upper part simple or nearly so, inclusive of the inflorescence 2-4 feet high, stout, hollow glabrous, shining. Leaves scattered rather distant, up to 10, rarely more, the lowest usually withered at the time of flowering, quite glabrous or the uppermost finely pubescent on the nerves below (Nadkarni, 2005).

Tubers biennial, paired, tuberous; daughter-tuber shortly conic to long-cylindrical, often irregularly shaped, 4 to more than 10 cm long, 0.75-3 cm thick simple or branched, sometimes flexuous or twisted, bearing root-fibers, some of which are thread like from the base and break off easily, while other are much thickened at base or thick cylindrical, light brown, smooth, fracture more or less horny and brownish in thickest part of full grown samples almost farinaceous and white towards the tips and in the root-branches (Kiritikar & Basu, 1981 a).

2.3.12. *Saussurea costus*, (root): Compositae

A tall robust perennial herb stems 1.2-2 m high pubescent above with radical and cauline leaves. Radical leaves are very large; it does not grow in Sri Lanka. This is extensively used in Sri Lanka and is imported from India. The chief constituents of the root are an essential oil an alkaloid saussurine, resin traces of bitter substances and small quantities of tannin, inulin, potassium nitrate, sugars etc. (Jayaweera, 2006 b).

Drug greyish to dull brown, thick, stout, fusiform to cylindrical, 7-15 cm long, 1.0-5.5 cm broad, thicker roots with collapsed centre, occasionally ridged, wrinkles longitudinal
and anatomized, rootlets rarely present, cut surface shows two regions, outer periderm ring thin, inner porous woody portion lighter in colour showing fine radial striations and often the central portion collapsed, fracture, short, horny, odour, strong, characteristically aromatic, taste, slightly bitter.

Powder is deep brown or rusty, under microscope irregular bits of yellow, brown or orange-red fragments of resins and oils associated with thin-walled parenchymatous cells, broken bits of xylem vessels with scalar form, reticulate thickening and horizontal end walls (Anon, 2008 a).

TLC of the petroleum ether extract on precoated silica gel 60 F254 plate 0.2 mm thick using toluene: ethyl acetate (9.5:0.5 v/v) as solvent system and developed to a distance of 8 cm. On spraying with anisaldehyde sulfuric acid reagent and heating the plate at 110 °C for 5 minutes. Seven spots appear at Rf 0.07 (pink), 0.16 (violet), 0.24 (pink), 0.39 (greenish blue), 0.47 (violet), 0.83, 0.90 (pink) (Guptha et. al, 2006 d).

2.3.13. Glycyrrhiza glabra, (stem & root): Fabaceae

A perennial herb with thick root stock passing below into long, straight, cylindrical, slightly tapering, smooth, flexible slightly branched roots about 1.2 cm diameter. It is not grow in Sri Lanka but roots are imported from foreign countries. The root contains glycyrrhizin, a sugar, asparagin, starch, tannic acid, and small quantity of resinous oil. In addition, it contains phytosterol and oestrogen (Jayaweera, 2006 c).

Stolon consists of yellowish brown or dark brown outer layer, externally longitudinally wrinkled, with occasional small buds and encircling scale leaves, smoothed transversely. cut surface shows a cambium ring about one-third of radius from outer surface and a small central pith, root similar without a pith, fracture, coarsely fibrous in
bark and splintery in wood, odour, faint and characteristic, taste, sweetish (Anon, 2008 a).

Loss on drying at 105 °C 27.6-29.7%, total ash 5.7-11.8%, acid insoluble ash 2.2-3.5%, water soluble extractive 8.7-9%, ethanol soluble extractive 10.7-11.3% (Fernando 1986).

pH of 1% solution 5-7, total ash maximum 10% loss on drying maximum 5%, heavy metals maximum 20 ppm.

2.3.14. *Allium sativum*, (bulb): Lilliaceae

A bulbous herb with a short flat axis giving off slender very thin papery scales which are enlarged and dilated below and bear at their axis large, oblong-ovoid, sessile bulbs pressed together with the outer ones curved to form collectively a lobed white tapering bulb. In Sri Lanka, it is cultivated in the up country districts. Medicinally it is a stimulant carminative anthelmintic, diaphoretic, diuretic and expectorant (Jayaweera, 2006 a).

Drug occurs as entire bulb or isolated cloves (bulblets); bulb sub-globular, 4-6 cm in diameter, consisting of 8-20 cloves, surrounded by 3-5 whitish papery membranous scales attached to a short, disc-like woody stem having numerous, wiry rootlets on the underside; each clove is irregularly ovoid, tapering at upper end with dorsal convex surface, 2-3 cm long, 0.5-0.8 cm wide, each surrounded by two very thin papery whitish and brittle scales having 2-3 yellowish green folded leaves contained within two white fleshy, modified leaf bases or scales; odour, peculiarly pungent and disagreeable; taste, acrid gives warmth to the tongue.

TLC of the alcoholic extract on silica gel 'G' plate using n butanol: isopropanol: acetic acid: water (3:1:1:1 v/v) shows under UV (366 nm) two fluorescent zones at Rf 0.58 and
0.72 (both light blue). On exposure to iodine vapour nine spots appear at \( R_f \) 0.18, 0.26, 0.34, 0.38, 0.46, 0.58, 0.72, 0.77 and 0.93 (all yellow); On spraying with ninhydrin reagent and heating the plate for ten minutes at 110 °C seven spots were appeared at \( R_f \) 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all pink). On spraying with vanillin-sulfuric acid reagent and heating the plate for ten minutes at 110 °C seven spots were appear at \( R_f \) 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all grey).

Volatile oil contained allyl disulphide and di allyl disulphide. It also contains allin, allicin, mucilage and albumin (Anon. 2008 c).

2.3.15. *Coriandrum sativum*, (fruit): Apiaceae

A glabrous herb 15-45 cm high is emitting a very disagreeable odour when rubbed. Leaves are pinnately divided; Cultivated throughout India and Sri Lanka. The fresh plant contains volatile oil, which consist of corriandrol-illinalool, licario, d-d-pinene, p-cymol, trepinene, dipentene, geraniol, l-borniol, \( \beta \)-phellandrene, terpinolene, n-decylaldehyde, acetic acid and decyl acid. The fruit contains volatile oil, pentosan, furfurol, pectin, vitamin C, fat, and protein starch and potassium malate. It is a refrigerant, diuretic, tonic, and aphrodisiac. It has special influence in controlling the bronchial and pulmonary secretions (Jayaweera, 2006 e).

Fruits are globular, mericarps usually united by their margins forming a cremocarp about 2-4 mm in diameter, uniformly brownish-yellow or brown, glabrous, sometimes crowned by the remains of sepals and styles, primary ridges 10, wavy and slightly inconspicuous secondary ridges 8 straight and more prominent, endosperm coelospermous, odour, aromatic, taste, spicy and characteristic.

Powder is fawn to brown, epidermal cells of pericarp when present, slightly thick-walled and many containing small prism of calcium oxalate, parenchymatous cells of
mesocarp without reticulate thickening, masses of sclerenchymatous cells of mesocarp in sinuous rows, often crossing at right angles, large tubular hexagonal rather thin-walled sclerenchymatous cells of endocarp, cells of inner epidermis with slightly sinuous anticlinal walls, thick-walled polygonal parenchymatous cells of endosperm, containing fixed oil and numerous small aleuronic grains, micro-rosettes of calcium oxalate (Anon, 2008 a).

2.3.16. *Holarrhena antidysenterica*, (stem bark): Apocynaceae

A tall slender tree with a whitish, rather smooth bark and slender drooping branchlets, which bear a smooth purplish bark and glabrous young parts. Flowers in April. Rare endemic species growing chiefly in the dry regions of low country up to 1500 feet altitude above sea level. Bark used for fever and dysentery (Jayaweera 2006 a).

Small recurved pieces of varying sizes and thickness, outer surface buff to brownish longitudinally wrinkled and bearing horizontal lenticels, inner surface brownish, rough and scaly fracture short and granular, taste, acrid and bitter (Anon, 2008 a).

TLC of the methanol extract on precoated silica gel 60 F254 plate 0.2 mm thick using cyclohexane: chloroform: diethyl amine (7:2:1 v/v) as solvent system and developed distance of 8 cm. On spraying the plate with modified dragendorff’s reagent, six spots were appeared at Rf 0.33, 0.51 (dark orange red), 0.55, 0.61, 0.69 (orange red), 0.82 (dark orange red) (Guptha et. al. 2003 a).

2.3.17. *Pierocapus santalinus*, (heart wood) Fabaceae

A small tree about 7-8 meters high, leaves alternate, estipulate, on downy petioles, always trifoliate leaflets articulated occurs in India up to 1500 feet elevation. It can be cultivate in Sri Lanka. The wood of this tree contains a colouring principle called santalic acid or santalin small quantities of santal and some kind of tannin (Jayaweera,
2006 c). Drug occurs, as irregular pieces, deep blood-red to dark purplish-red or almost black, hard, but can be easily split, odourless; taste, slightly astringent.

Powder red or purplish-red; shows a number of fibres, vessels and xylem parenchyma cells and prismatic crystals of calcium oxalate.

TLC of the alcoholic extract on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) shows in visible light, a spot at Rf 0.37 (light pink). Under UV (366 nm) five fluorescent zones were visible at Rf 0.07 (blue), 0.13 (grey), 0.3 (blue), 0.37 (grey), and 0.57 (blue). On exposure to iodine vapour eight spots appear at Rf 0.07, 0.13, 0.16, 0.26, 0.37, 0.43, 0.74 and 0.80 (all yellow). On spraying with vanillin sulfuric acid reagent and heating the plate for ten minutes at 110 °C seven spots appear at Rf 0.04 (violet), 0.07, 0.13 (both light violet), 0.37, 0.43 (both violet), 0.74 and 0.80 (both light violet) (Anon, 2008 c).

If toluene: chloroform: methanol (8:1:1 v/v) was used as solvent system for the TLC and anisaldehyde sulfuric acid as spray reagent, 6 spots appeared at the Rf 0.20, (blue) 0.26 (orange) 0.34 (pale orange) 0.38 (bluish pink) 0.50,0.54, (dark grey) (Guptha et. al, 2008 f).

Loss on drying at 105 °C 9-9.6%, total ash 1.5-3%, acid insoluble ash 0.6-0.9%, water soluble extractive 11.8-12.7%, ethanol soluble extractive 23.7-27.3% (Fernando, 1986).

2.3.18. *Aconitum heterophyllum*, (root): *Ranunculaceae*

A perennial herb with an oblong-ovoid or fusiform perpendicular rootstock, pale grey externally, white within and with numerous root herbs; stem erect 30-90 cm high, simple or slightly branched, cylindrical and pubescent above somewhat angled below. Occurs in India in the west temperate a region of Himalayas. It does not grow in Sri Lanka. The root of this plant contain the alkaloids atidine, atisine, heteratisine and
heticine. The root is used as a cure for diarrhoea, dysentery bilious complaints and vomiting in convalescence (Jayaweera, 1996 d).

Roots, ovoid-conical, tapering downwards to a print, 2.0-7.5 cm long, 0.4-1.6 cm or more thick at its upper extremity, gradually decreasing in thickness towards tapering end, externally light ash-grey, white or grey-brown, while internally starch white, external surface wrinkled marked with scars of fallen rootlet and with a rosette of scaly rudimentary leaves on top: fracture, short, starchy, showing uniform white surface, marked towards centre by 4-7 concentrically arranged yellowish-brown dots, corresponding to end of fibro vascular bundles traversing root longitudinally taste, bitter with no tingling sensation (Anon, 2008 a).

Powder is ash coloured to light brown, under microscope shows abundant simple and compound starch grains and parenchymatous cells (Anon, 2008 a).

TLC of the methanol extract on precoated silica gel 60 F 254 plate 0.2 mm thick TLC plate using toluene: ethyl acetate: diethylamine (7:2:1 v/v) as solvent system and developed to a distance of 8 cm; On spraying with modified dragenoff's regent eight spots appear at Rf 0.19, 0.2, 0.29, 0.36, 0.41, 0.59, 0.65, and 0.81(orange) (Guptha et. al. 2008 e).

2.3.19. Pierorhiza kurrooa, (rhizome): Scrophulariaceae

A low, more or less hairy perennial herb with a bitterroot stock with thickness of the fifth finger 15-25 cm long clothed with withered leaf bases. Occurs in Himalayan regions but not in Sri Lanka this is a bitter stomachic and useful in dyspepsia, fever and for many purgative preparations (Jayaweera, 2006 e).

Rhizome is 2.5-8 cm long and 4-8 mm thick, sub cylindrical, straight or slightly curved, externally greyish-brown, surface rough due to longitudinal wrinkles, circular scars of
roots and bud scales and sometimes roots attached, tip ends in a growing bud surrounded by tufted crown of leaves, at places cork exfoliates exposing dark cortex; fracture, short; odour, pleasant; taste, bitter (Anon, 2008 b).

Root is thin, cylindrical, 5-10 cm long, 0.05-0.1 cm in diameter, straight or slightly curved with a few longitudinal wrinkles and dotted scars, mostly attached with rhizomes, dusty grey, fracture, short, inner surface black with whitish xylem; odour, pleasant; taste, bitter.

Powder is dusty grey; shows groups of fragments of cork cells, thick-walled, parenchyma, pitted vessels and acetate fibres, simple round to oval, starch grains, measuring 25-104 μ in diameter (Anon, 2008 b).

For TLC ethyl acetate: methanol (47:3 v/v) was used as solvent system and vanillin sulfuric acid as spray reagent (Rajpal, 2006 a).

Loss on drying at 105 °C 9.4-11.7%, total ash 0.9-2.8%, acid insoluble ash not in detectable amounts water soluble extractive 12.9-15.5%, ethanol soluble extractive 24.1-25% (Fernando, 1986).

2.3.20. Ferula assa-foetida (Oleo-gum resin): Apiaceae

A very large herb persisting for a number of years, but perishing after it has flowered. Roots tuberous 45 cm long 10-15 cm diameter. Occurs in India, but not in Sri Lanka.

When plant is about 4-58 years old and leaves are drying off the upper part of the root is bared and the crown cut off with special knife. The exudation from this cut surface accumulates as a white milk which later turns brown on hardening. Asafoetida is composed of a volatile oil, resin and gum together with traces of malice acid etc. The volatile oil is mixture of sculpture compounds of the hydrocarbon C₆H₁₂ the resin containing ferulaic acid and umbelliferone. It is effective in flatulent colic and used
externally on ringworm. It is a powerful antispasmodic, expectorant, anthelmintic and a nerve stimulant (Jayaweera, 2006 c).

Rounded, flattened or masses of agglutinated tears, greyish-white to dull yellow, mostly 12-25 mm in diameter, freshly exposed surface, yellowish and translucent or milky white, opaque, slowly becoming pink, red, finally reddish brown, odour, strong, characteristic and persistent, taste, bitter and acrid.

TLC of alcoholic extract of the drug on silica gel 'G' plate using toluene: ethyl acetate (7:3 v/v), shows eleven spots under UV light (366 nm) at R_f 0.12, 0.22, 0.34, 0.42, 0.51, 0.55, 0.60, 0.67, 0.77, 0.85 and 0.91 (all blue). On spraying with anisaldehyde sulfuric acid reagent and heating the plate, for five minutes at 105 °C ten spots appear at R_f 0.05 (violet), 0.12 (brown), 0.22 (violet), 0.32 (brown), 0.42 (violet), 0.51 (pink), 0.60 (grey), 0.77 (pink), 0.85 (pink) and 0.94 (orange) (Anon, 2008 a).

TLC of ethanol extract of asafoetida on pre coated silica gel 60 F_254 in the solvent system using toluene: ethyl acetate: formic acid (14:08:0.15 v/v) to a distance of 8 cm. On spraying the plate with vanillin-sulfuric acid reagent followed by heating at 105 °C eleven sports appear at R_f 0.15 (bluish grey) 0.31, 0.35 (yellow) 0.45 (bluish grey) 0.48 (yellow) 0.56 (grey) 0.63 (blue) 0.66 (grey) 0.79 (orange) 0.85 (grey) 0.91 (grey) (Guptha et. al., 2008 c).

Loss on drying at 105 °C 6.5-8.7%, total ash 5-21.6%, acid insoluble ash 4.5-13.6% water soluble extractive 7.7-10%, ethanol soluble extractive 22.9-32.4%, umbelliferone content 4.9-7% (Fernando, 1986).

2.3.21. Ocimum tenuiflorum, (leaf): Lamiaceae

An annual herb 30-60 cm tall, often slightly woody below, branches numerous, spreading, sub quadrangular with spreading hair, usually purplish. Flowers from June to
August. Occurs throughout India and Sri Lanka. The plant contains an alkaloid. The leaves yield a volatile oil which consist of cineol, linalool and methyl homoanisic acid. Internally it acts as an aromatic stomachic and carminative stimulating the appetite and improving the digestion. It is useful in anorexia, chronic dyspepsia flatulence, colic acute and chronic bronchitis (Jayaweera, 2006 c).

Root - thin, wiry, branched, hairy, soft, blackish-brown externally and pale violet internally.

Stem - erect, herbaceous, woody, branched; hairy, sub quadrangular, externally purplish-brown to black, internally cream, coloured; fracture, fibrous in bark and short in xylem; odour faintly aromatic (Anon, 2008 b).

Leaf - 2.5-5 cm long 1.6-3.2 cm wide, elliptic oblong, obtuse or acute, entire or serrate, pubescent on both sides; petiole thin, about 1.5-3 cm long hairy; odour, aromatic; taste, characteristic (Anon, 2008 b).

Flower - purplish or crimson coloured, small in close whorls; bracts about 3 mm long and broad, pedicels longer than calyx. slender. pubescent; calyx ovoid or campanulate 3-4 mm bilipped, upper lip broadly obovate or suborbicular, shortly apiculate, lower lip longer than upper having four mucronate teeth, lateral two short and central two largest; corolla about 4 mm long, pubescent; odour, aromatic; taste, pungent.

Fruit - A group of 4 nut lets, each with one seed, enclosed in an enlarged, membranous, veined calyx. nut lets sub-globosely or broadly elliptic, slightly compressed, nearly smooth; pale brown or reddish with small black marking at the place of attachment to the thalamus; odour, aromatic; taste, pungent (Anon, 2008 b).
Seed - Rounded to oval; brown, mucilaginous when soaked in water, 0.1 cm long, slightly notched at the base; no odour, taste, pungent, slightly mucilaginous (Anon, 2008 b).

Powder is greenish: shows thin-walled, parenchymatous cells, a few containing reddish brown contents, unicellular and multi cellular-trichomes either entire or in pieces; thin walled fibres, xylem vessels with pitted thickenings, fragments of epidermal cells in surface view having irregular shape, oil globules, rounded to oval, simple as well as compound starch grains having 2-5 components, measuring 3-17 μ in diameter (Anon, 2008 b).

TLC of alcoholic extract of the drug on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) shows in visible light nine spots at Rf 0.03 (dark green), 0.04, 0.08 (both green), 0.12 (light green), 0.21, 0.33 (both green), 0.45 (yellowish green), 0.85 & 0.93 (both light green). Under UV (366 nm) eight fluorescent zones were appeared at Rf 0.04, 0.30, 0.33, 0.45, 0.83 (all pink) 0.85 (blue). 0.93 (pink) & 0.98 (blue). On exposure to iodine vapour eleven spots appear at Rf 0.04, 0.08, 0.12, 0.21, 0.33, 0.45, 0.54, 0.75, 0.83, 0.88 and 0.93 (all yellow). On spraying with vanillin sulfuric acid reagent and heating the plate at 110 °C for ten minutes ten spots appear at Rf 0.08 (violet), 0.12 (light violet), 0.21 (brown), 0.33 (violet), 0.45 (violet), 0.54 (blue), 0.75 (violet), 0.83 (blue), 0.93 (violet) and 0.98 (blue) (Anon, 2008 b).

Total ash not more than 16%, acid insoluble ash not less than 6%, alcohol soluble extractive value not less than 2% (Das, 2010).

TLC methanol extraction of the material applied on precoated silica gel 60 F254 TLC plate, in the solvent system toluene: ethyl acetate: formic acid (7:3:0.3 v/v) to a distance of 8 cm. On spraying the plate with anisaldehyde sulfuric acid reagent and heat the
plate at 110 °C 13 spots appear at Rf 0.18, 0.25, 0.29, 0.35 (blue) 0.45 (light purple) 0.50 (purple) 0.54 (light purple) 0.60 (light blue) 0.65 (blue) 0.74 (brown) 0.80 (pink) 0.85 (blue) 0.94 (purple) (Guptha et al. 2008 e).

2.3.22. *Vitex nigundo*, (leaf): Verbenaceae

A small slender tree or shrub branch lets quadrangular, finely pubescent. Flowers throughout the year. It is common by edge of streams especially in the dry regions of the low country in Ceylon. The leaves contain an alkaloid, nishidine, an essential oil and a resin, while the fruit contain an acid resin an astringent organic acid and mallic acid. The leaves, bark and roots are used for cough, asthma fever etc. (Jayaweera, 2006 e)

Leaves palmately compound, petiole 2.5-3.8 cm long; mostly trifoliate, occasionally pentafoliate; in trifoliate leaf, leaflet lanceolate or narrowly lanceolate, middle leaflet 5-10 cm long and 1.6-3.2 cm broad, with 1-1.3 cm long petiolule, remaining two sub-sessile; in pentafoliate leaf inner three leaflets have petiolule and remaining two sub-sessile; surface glabrous above and tomentose beneath; texture, leathery. Powder shows number of pieces or whole, uni-bi and multicellular covering trichomes, glandular trichomes, palisade tissues with hypodermis, and upper and lower epidermis. Xylem vessels with pitted walls.

TLC of the alcoholic extract on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) shows under UV (366 nm) two fluorescent zones at Rf 0.18 (blue) and 0.47 (red). On exposure to iodine vapour four spots appear at Rf 0.16, 0.47, 0.67 and 0.91 (all yellow). On spraying with vanillin sulfuric acid reagent and on heating the plate for ten minutes at 105 °C four spots appear at Rf 0.07, 0.47, 0.58 and 0.67 (all blue) (Anon, 2008 c).
If the ethyl acetate: water: acetic acid (8:1:1) us as solvent system for TLC six spots were appeared at \( R_f \) 0.16, 0.23, 0.28, 0.35, (all brown) 0.46, (dark brown) 0.96 (brown) (Anon, 2008 c).

2.3.23. *Toddalia asiatica*, (leaf): *Rutaceae*

A small erect or scrambling or climbing shrub with cylindrical, glabrous or slightly to mentose stem set with re curved, compressed, sharp prickles. Flowers from January to April. Occurs in India and Sri Lanka. It is very common in Sri Lanka from sea level to about 6000 feet elevation. The leaves of this plant yield a volatile oil with citronella and linalool (Jayaweera, 2006 e).

2.3.24. *Leucas zeylanica*, (leaf): *Lamiaceae*

An annual herb with erect stem 30-60 cm tall and long spreading branches from base overtopping the main stem, which is quadrangular and hispid-hairy. Flowers from September to February. It is very common in Sri Lanka on waste ground. This plant contains an essential oil and a volatile alkaloid combined with an acid. An aromatic stomachic and carminative, useful in anorexia acute and chronic dyspepsia flatulence and colic (Jayaweera, 2006 c).

2.3.25. *Cleome gynandra*, (leaf): *Capparidaceae*

An erect branched annual herb 60-120 cm high stem shaggy with long white spreading hair, leaves palmately compound 5 foliate leaflets. It is very common in waste and cultivated ground in the low country. It is common in all tropical countries. The leaves contain an alkaloid, volatile oil having the properties of garlic or mustard oil (Jayaweera, 2006 b). Seeds, small, 1-2 mm in diameter, kidney shaped, surface rough, dark brown or black (Anon, 2008 a).
2.3. 26. *Azadirachta indica*, (leaf): Meliaceae

A tall tree with spreading branches, a straight brown barked trunk, and glabrous young parts. Flowers are between March and May. A common tree contains the alkaloid paraisine, the bark margosine. The oil extract from the seed contains margosic acid and a bitter principle (Jayaweera, 2006 d).

Leaves are compound, alternate, rachis 15-25 cm long, 0.1 cm thick; leaflets with oblique base, opposite, estipulate, lanceolate, acute, serrate, 7-8.5 cm long and 1.0-1.7 cm wide, slightly yellowish-green; odour, indistinct; taste, bitter.

Powder is green; shows vessels, fibres, rosette crystals of calcium oxalate fragments of spongy and palisade parenchyma (Rajpal, 2005 b).

Bark varies much in thickness according to age and parts of tree from where it is taken; external surface rough, fissured, and rusty-grey; laminated inner surface yellowish and foliaceous, fracture, fibrous; odour, characteristic; taste, bitter.

Powder is reddish-brown; shows numerous prismatic crystals of calcium oxalate, phloem fibres with narrow lumen and pointed ends; cork cells, stone cells mostly in groups, lignified rectangular to polygonal, having wide lumen and distinct striations, simple starch grains, measuring 2.75-5 μ in diameter. Leaves contain quercitin, kaompferol and their glycosides, meliantriol, nibadinol (Rajpal, 2005 b).

TLC of alcoholic extract of the drug on silica gel 'G' plate using chloroform: ethyl acetate: formic acid (5:4:1 v/v) shows under UV (366nm) three fluorescent zones at $R_f$ 0.72 (blue), 0.86 (blue), and 0.90 (green). On spraying with 5% methanolic phosphomolybdic acid reagent and heating the plate for about ten minutes at 105 °C four spots appear at $R_f$ 0.20, 0.45, 0.63 and 0.90 (all blue) (Anon, 2008 b).
2.3.27. *Acorus calamus*, (rhizome): Araceae

An aromatic marshy herb with stout creeping and branching root stock. The rhizome contains an alkaloid mainly choline bitter glucosides acorin and calamine A, an essential oil, calamol, resin, gum, starch and tannin. The essential oil is said to contain asarone, palmitic and heptoic acids, ester of palmitic acid with some pinene, camphene, asaraldehyde, eugenol, calamine, calamerol and calameon. Medicinally the rhizome is a stomachic and carminative, in small doses. In larger doses, it is an emetic. It is used for dyspepsia, flatulence, choleric diarrhoea, cough, fever (Jayaweera, 2006 a; Anon. 1985 a).

Drug occurs in simple or rarely with thumb-like branches at nodes; sub cylindrical to slightly flattened, somewhat tortuous or rarely straight, cut pieces of 1-5 cm long, and 0.5-1.5 cm thick; upper side marked with alternately arranged, large, broadly, triangular, transverse leaf scars which almost encircle the rhizome; at nodes leaf sheath mostly having an appearance present; lower side shows elevated tubercular spots of root scars; light-brown with reddish-tinge to pinkish externally, buff coloured internally; fracture, short; odour, aromatic; taste, pungent and bitter (Rajpal, 2006 a).

Powder is buff coloured; shows fibres, reticulate, annular vessels, and simple spherical starch grains, measuring 3-6 μ in diameter.

The extraction solvent is methanol or ethanol and the temperature is taken to (65-70) °C. The material with solvent is agitated for two hours. The extraction is monitored by either TLC or HPLC (Rajpal, 2006 a).

TLC of alcoholic extract of the drug on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) shows two spots at RF 0.14 (violet) and 0.73 (violet) on spraying with vanillin.
sulfuric acid reagent and heating the plate for ten minutes at 105 °C (Anon, 2008 b; Hossain, 2008).

2.3.28. Orpiment- Harithala, (Yellow arsenic): \( \text{As}_2S_3 \)

Found in mines of China, Persia and Iran. Orpiment is a compound of sculpture and arsenic and has two varieties namely Patra harithala and Pinda harithala. Patra harithala alias Ranhiriyal used for this formulation (Anon, 1994).

Orpiment has been used as a Chinese Materia medica for nearly long as realgar. \( \text{As}_2S_3 \), a major ingredient of orpiment could also be either externally or orally (Lu et. al, 2002).

Loss on drying (at 105 °C) 0.10-0.14%, arsenic content in non purified form 35.65%-45.72%, arsenic content in purified form 33.24-46.13% (Fernando, 1986).

Purification method-

Patra harithala type of orpiment should be made into small pieces, tied in a Pottili (into a bolus form) with the help of a piece of cloth and cooked in Dola Yantra for eight hours using buffalo milk. Thereafter, it should be again cooked using new sample of buffalo milk and put into sesame oil for ten hours and taken for the medicinal preparations (Alvis, 1879).

Patra harithala type of orpiment should be made into small pieces, tied into a bolus form, and cooked in dola yantra for three hours by adding limewater. Thereafter, it
should be again cooked in same dola yantra for adding the juice of Benincasa hispida. Sesame oil and decoction of triphala one after other. Thereafter, pieces of yellow arsenic should be well washed with warm water, dried in the sun and made to powder (Dash, 1996).

2.3.29. Zinc ore – Rasaka, (Calamine): ZnCO₃

It is used as a component in some indigenous medicinal preparations. It is of two types, viz dardura and karavelaka (Dash, 1996; Choudhary, 2001).

Purification method –

Small pieces of calamine in the size of green gram mixed with cows ghee and tied into bolus form with the help of a piece of cloth and cooked in Dola Yantra for 7 times using cows urine. Sometimes put into cows urine and boiled as a purification method (Alvis, 1879).

Pieces of calamine should be heated over flame and immersed in lemon juice for seven times. Thereafter it should be washed with hot water and dried under the sun (Dash, 1996).

2.3.30. Blue Vitriol – Thutta: (CuSO₄ 5H₂O)

It is a blue coloured crystalline copper mineral, which is available occasionally in native form also. Nowadays it is prepared artificially by combining copper with sulfuric acid (Joshi & Rao, 2003).

Loss on drying 16.5-20.5%, Cu content of non-purified form 29.78-30.79%, Cu content of purified from 36.2-37.99% (Fernando, 1986).

Purification method –

Boil with human urine and dry (Alvis, 1879).
Copper sulphate should be impregnated with decoction of *triphala* and triturated. This process should be repeated thrice (Dash, 1996).

### 2.3.31. Rock salt-Saindhava Lavana: (NaCl)

Another name for Rock salt is Halite. Usually the rock salt crystals are cubes. It is often colourless, but other substances in the Rock salt can cause other colours like red, caused by radioactive radiation. Rock salt can also be blue and violet. Not only common salt is made of Rock salt, but also sodium and chlorine for the chemical industry. Rock salt can be found in many countries. Some important places with Rock salt are in the USA, in Peru, in Germany, in Austria and in Spain (Anon. 1994).

**Purification method**—Purification is not necessary.

### 2.3.32. Cinnabar – *Hingula*: (HgS)

Hingula is a compound of mercury and sulphur. It is pinkish red in colour, and heavy, on its broken surface white lines of mercury are found. It is naturally available in mines. However, mostly artificially prepared hingula is available in market and used as medicine. Cinnabar has been used for 2000 years in Traditional Chinese medicines and Ayurvedic medicines. The mercury in these traditional medicines can still be thousand folds higher than what considered safe in Western countries including the U.S.A. The question becomes, is cinnabar toxicologically similar to common mercurials (Lie *et al.*, 2008).

Loss on drying 0.17-0.24%, mercury content of non purified form 71.24-74.53%, mercury content of purified form 45.69-48.98% (Fernando, 1986).

**Purification method**—

Crushed to small pieces impregnated and triturated by adding lime juice and kept under sunrays to heat, and then filtered the lime juice. This process repeat for seven days. At the
end of every day, the residue containing in the mortar was filled with limejuice to cover and kept for overnight, before starting the process of tituration next day. Everyday tituration should be done continuously for at least eight hours. After the seventh day of tituration, the processed hingula should be left in the mortar to dry up and it should be scrapped with the help of sharp blade (Alvis, 1879).

Hingula should be impregnated and triturated by adding lemon juice for seven days. At end of every day, the mortar containing hingula should be filled up with lemon juice and kept overnight before starting the process of triturating next day. Everyday triturating should be done continuously for least eight hours. After the seventh day of triturating the processed hingula should be left in mortar to dry up (Dash, 1996).

2.3.33. Alum sulphate – Sphatika: $K_2SO_4Al_2(SO_4)\cdot24H_2O$

Alum is the common name for the hydrated salts of potassium. In market two types of spatika are available that are white and reddish white in colour. For external use crude alum is used and for internal use it should be purified (Joshi & Rao, 2003; Choudhary, 2001). This mineral is imported from various parts of India.

Purification method – Alum has to be cleaned of its physical impurities. Then it is heated in an iron container, until a whitish powder is formed, when the water of crystallization evaporates (Dash, 1996).

2.3.34. Realgar – Manashila, (Arsenic disulphide): $As_4S_4$ / $As_2S_2$

Not present in Sri Lanka. It is imported from India it is not in pure form mostly it is a mixture of minerals specially associated with orpiment. Colour is red. There are three verities. Realgar the mined ore, composed mainly contains about 90% tetra-arsenic tetra sulphide ($As_4S_4$), has been used as a traditional medicine in china for more than 1500 years (Lu et. al., 2002; Choudhary, 2001).
Loss on drying at 105 °C 0.58-0.65%, arsenic content in non purified form 36.06%-43.31%, arsenic content in purified form 20.35-30.42% (Fernando, 1986).

Purification method –
Crushed to small parts, tied in a pottali (into a bolus form) with the help of a piece of cloth, and cooked in Dola Yantra using cows’ urine. Thereafter, it should be washed four times using the juice of musk-lime and then taken for the medicinal preparations (Alvis, 1879).

Should be impregnated and triturated with juice of either Sesbenia grandiflora, ginger, lemon or Eclipta alba for seven days. Thereafter, it should be dried, made into powder (Dash, 1996).

2.3.35. Borax- Pushkara, (Tankana): Na$_2$B$_4$O$_7$.10H$_2$O
Tankana is known to Indians long back and referred in Charaka and Susrutha Samhithas as well as in later Rasahasra texts. It is one of the many important Kharas described in those texts. It is white or sometimes with blue or grey tinge, soluble in water and import from India (Satputa, 2003; Choudhary, 2001; Dash, 1996).

Purification method-
Put powdered borax put into limejuice, kept for a day, and washed using hot water (Alvis, 1879).

Clean and kept in iron pan and heated. When the water of crystallization is evaporated, it swells and comes out from fragile masses (Dash, 1996).

2.3.36. Gypsum –Godanthi: CaSO$_4$.2H$_2$O
It is a white shining and stony material having lamellae and crystalline structure with massive, flat, elongated and generally prismatic crystals (Anon, 1994; Choudhary, 2001).
Purification method-
Washing with hot water (Joshi & Rao, 2003; Dash, 1996).

2.3.37. *Citrus aurantium*, (fruit): Rutaceae
A glabrous tree of medium size 7-10 m high with long but not very sharp spines. Probably a native of Cochin China and cultivated in India and Sri Lanka. The juice of the fruit contains mucilage, sugar, citric acid and inorganic salts. The fruit is used as a diaphoretic and purgative (Jayaweera, 2006 e).

2.3.38. Fresh ginger, (described under 2.3.6)

2.3.39. Neem oil:
Neem oil derived from crushing the seeds is antidermatonic, a powerful antihelmintic and is bitter in taste. It has a wide spectrum of action and is medicinal in nature. It is mild antiseptic when applied externally. Extraction of oil is done, using the deconticated kernels. After grinding in a mill or pounded in a mortar the powder moistened with little amount of water until it sticks together. Knead this past for several minutes and press firmly, oil come out. In this way half the oil in the kernels, which could be 100-150 mL per kg can be gained.
Moisture content and insoluble impurities 0.1, specific gravity at 30 °C 0.908-0.934 (Das, 2010).

2.3.40. Cow’s ghee:
It is a semisolid substance colour in light yellow and manufactured by using cow’s milk. Five types are mentioned and cow’s ghee is the great among other kinds of ghee (Anon, 1994 b). It is mainly use as carrier that is called yogavahi for herbs and bhasmas, for its supreme penetrating qualities and thus ability to carry these substances deep into the *dhatu* or tissues.
2.4 Maha Varthikava Watee:

Maha Varthikava Watee contains 29 herbal ingredients and mainly used for vomiting, Stanni roga, stomachache, flatulence, diarrhoea, head ache, sore throat etc.

2.4.1. Cuminum cyminum, (fruit) (described under 2.3.1.)

2.4.2. Nigella sativa, (seed) (described under 2.3.2.)

2.4.3. Allium sativum, (bulb) (described under 2.3.14.)

2.4.4. Trachyspermum ammi, (fruit) (described under 2.3.4.)

2.4.5. Trigonella foenum-graecum, (fruit): Leguminosae

An annual herb 30-60 cm high with an erect, slightly branched, cylindrical, hollow, smooth or slightly pubescent stem. Occurs in India extending through Persia. It can be cultivated in Sri Lanka. The seed contains alkaloid trigonelline with mucilage, tannic acid yellow colouring matter, fixed and volatile oil and a bitter extractive, diosgenin, gitogenin, a trace of trigogenin and vitamin A. Fenugreek seeds are considered carminative and aphrodisiac. They are used for dyspepsia, diarrhoea, dysentery and rheumatism (Jayaweera, 2006 c).

Seed oblong, rhomboidal with deep furrow running obliquely from one side, dividing seed into a larger and smaller part, 0.2-0.5 cm long, 0.15-0.35 cm broad, smooth, very hard; dull yellow; seed becomes mucilaginous when soaked in water; odour, pleasant; taste, bitter.

Powder is yellow; shows groups of palisade parenchymatous cells, aleuronic grains, oil globules, endosperm and epidermal cells of testa (Anon, 2008 b).

TLC of the methanol extract on precoated silica gel 60 F254 plate 0.2 mm thick using n-hexane : ethyl acetate (8:2 v/v) as solvent system and developed up to the distance of 8 cm. Spray the plate with anisaldehyde sulfuric acid reagent and heat the plate at
110 °C for five minutes four spots appear at Rf 0.36 (greenish brown), 0.41, 0.58 (bluish), 0.91 (dark blue) (Guptha et al., 2006 d).

Loss on drying at 105 °C 9.7-12.9%, total ash 3.2-4.9%, acid insoluble ash 0.02-2.8% water soluble extractive 15.5-17.8%, ethanol soluble extractive 11.7-13.2% (Fernando, 1986).

2.4.6. Zingiber officinale, (rhizome) (described under 2.3.6.)

2.4.7. Ferula assa-foetida, (exudate) (described under 2.3.20.)

2.4.8. Piper longum, (fruit) (described under 2.3.8.)

2.4.9. Piper nigrum, (fruit) (described under 2.3.7.)

2.4.10. Solanum virginianum, (root): Solanaceae

An annual prostrate herb, stem somewhat zig-zag, nearly glabrous with numerous yellow long prickles. Flower throughout the year; Occurs throughout India and dry and sandy places in Sri Lanka. The plant contains alkaloid, solasodine, an unidentified alkaloid is found in leaves, stem and roots. The root is valued as an expectorant and used in cough, asthma, colic fever, loss of appetite and pain in chest (Jayaweera, 2006 e). Root-10-45 cm long, few mm to two cm in diameter, almost cylindrical and tapering, bearing a number of fine longitudinal and few transverse wrinkles with occasional scars or a few lenticels and small rootlets, transversely smoothened surface shows a thin bark and wide compact cylinder of wood. fracture, short, taste, bitter (Anon, 2008 a).

Stem-herbaceous, prickly with prominent nodes and internodes, green when fresh, young branches, covered with numerous hairs, mature ones glabrous, furrows more prominent in young stem appearing almost circular towards basal region, stem pieces 8-10 mm thick of variable length, external surface light green, when dry, surface yellowish green and smooth, transversely smoothened surface shows a very thin bark and prominent
wood, centre shows a large and distinct, pith, mature and dry stem often with hollow pith, fracture short to slightly fibrous.

Leaves- Petiolate, estipulate, ovate-oblong or elliptic, sinuate or sub-pinnatifid, sub-acute hairy. 4-12.5 cm long and 2-7.5 cm wide, green, veins and midrib full with sharp prickles, odour and taste not distinct (Anon, 2008 a).

Flower- Ebracteate, pedicellate, bisexual, pentamemrous, regular, complete, bright blue or bluish purple, calyx-persistent, gamosepalous, tube short, globose, linear-lanceolate, acute, hairy, 0.5-1.3 cm long and densely prickly, corollagamopetalous, lobes deltoid, acute, hairy. 1-2 cm long and purple in colour, stamens 5, epipetalous, basifixed, filament short 1-1.5 mm long, anther, oblong lanceolate, 0.7-0.8 cm long, ovary superior, ovoid, glabrous, bilocular with axile placentation having numerous ovules (Anon, 2008 a).

Fruit- Berry globular, measuring 0.8-1 cm in diameter, surrounded by persistent calyx at base unripe fruits variegated with green and white strips, ripe fruit shows different yellow and white shades.

Seeds- Circular, flat, numerous, embedded in a fleshy mesocarp about 0.2 cm in diameter, glabrous taste, bitter and acrid.

Powder is greenish, under microscope shows single or groups of stone cells, groups of aseptate fibre with tapering ends, pitted vessels, groups of spongy parenchyma, fragments of palisade tissue, anisocytic stomata, stellate hairs and simple, rounded to oval starch grains measuring 2.75-11 μ in diameter (Anon, 2008 a).
2.4.11. *Terminalia chebula*, (fruit pericarp): Combretaceae

A moderate-sized tree with a very thick, grey-brown bark, crooked trunk and many spreading branches drooping at the extremities and pubescent young parts. Grow in India, Burma and Sri Lanka. In Sri Lanka it is rather rare in the dry districts of low-country up to 2500 feet altitude. It is gregarious and abundant in certain areas. The fruits contain a mixture of gallic acid and tannic acid, apparently derived from an organic acid, chebulinic acid they also contain a greenish oleoresin, which is termed myrobalanin. The pericarp of dry fruit is use as a purgative (Jayaweera, 2006 a).

Intact fruit yellowish-brown, ovoid, 20-35 mm long, 13-25 mm wide, wrinkled and ribbed longitudinally, pericarp fibrous, 3-4 mm thick, non-adherent to the seed, taste, astringent.

Powder is brownish in colour, under microscope shows a few fibres, vessels with simple pits and groups of sclereids (Anon, 2008 a).

TLC of the methanolic extract on precoated silica gel 60 F 254 plate 0.2 mm thick using chloroform: ethyl acetate: formic acid (2.5:2:0.8 v/v) as solvent system and developed 8 cm, then scan the plate under UV 254 nm and 366 nm. On spraying with 5% ferric chloride four spots appear at Rf 0.11, 0.15 (pale blue), 0.34 (greyish blue), 0.50 (dark blue) (Guptha et. al, 2003 a).

2.4.12. *Terminalia bellarica*, (fruit pericarp): Combretaceae

A large deciduous tree with a straight buttressed trunk and long horizontal branches bark brown and young parts glabrous. It is not very common in Sri Lanka confined to the moist and intermediate regions of low country. The pericarp of the dry fruit is an ingredient in many drugs for variety of diseases (Jayaweera, 2006 b).
Fruit nearly spherical to ovoid, 2.5-4.0 cm in diameter, fresh ripe fruits slightly silvery or with whitish shiny pubescent surface, mature fruits grey or greyish brown with slightly wrinkled appearance, rind of fruit shows variation in thickness from 3-5 mm, taste, astringent (Anon, 2008 a).

TLC of the metanolic extract on precoated silica gel 60 F$_{254}$ plate 0.2 mm thick using chloroform: ethyl acetate: formic acid (2.5:2:0.5 v/v) as solvent system and developed 8 cm, and plate observed under UV 254 nm and 366 nm. Spray the plate with 5% ferric chloride solution in methanol four spots appear at $R_f$ 0.11 (pale blue), 0.15 (pale grey), 0.34 (pale greyish blue) (Guptha et. al. 2003 a).

2.4.13. *Phyllanthus emblica,* (fruit): Euphorbiaceae

A small or middle size tree about 10 m high with a crooked trunk and spreading branches; bark thin, grey with numerous bosses whence arise the leaf-bearing branchlets, young parts pubescent. Grows in tropical and subtropical parts of India and Sri Lanka. In Sri Lanka, it is very common in exposed places on patana lands in the moist regions up to 4000 feet altitude (Jayaweera, 2006 b).

Drug consists of curled pieces of pericarp of dried fruit occurring either as separated single segment; 1-2 cm long or united as 3 or 4 segments; bulk colour grey to black, pieces showing, a broad, highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface, external surface shows a few whitish specks, occasionally some pieces show a portion of stony testa (which should be removed before processing); texture rough, cartilaginous, tough; taste, sour and astringent.

Fruit, globose, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines; greenish when tender, changing to light yellowish or pinkish colour when mature, with a few
dark specks: taste, sour and astringent followed by delicately sweet taste (Anon, 2008).
toluene: ethyl acetate (93:7 v/v) used as solvent system and vanillin sulfuric acid used as spray reagent (Rajpal, 2005 b).

2.4.14. *Syzigium aromaticum*, (flower bud) (described under 2.3.10.)

2.4.15. *Myristica fragrans*, (fruit & aril) (described under 2.3.9.)

2.4.16. *Fumaria parviflora*, (whole plant): Fumaraceae

Root - Buff or cream coloured, branched, about 3 mm thick, cylindrical; taste, bitter.

Stem - Light green, smooth, diffused, hollow, about 2 to 4 mm thick; taste, bitter and slightly acrid.

Leaf - Compound, pinnatifid, 5 to 7 cm long, divided into narrow segments; segments 5 mm long and about 1 mm broad, linear or oblong, more or less glaucous, acute or sub acute; petiole, very thin, 2.5 to 4.0 cm long; taste, bitter.

Flower - Racemes with 10 to 15 flowers, peduncle up to 3 mm, pedicels about 2 mm, flowers about 7 mm long, bract much longer than the pedicels; sepals 2, white, minute, about 0.5 mm long, triangular ovate, acuminate; corolla in 2 whorls with very small 4 petals, each about 4 mm long; inner petals with a purple or green tip; outer petals with narrow spur, without purple spots stamens 3+3, staminal sheath subulate above, about 4 mm long, stigma 2 lipped (Anon, 2008 d).

Fruit - Capsule, 2 mm long and slightly broader, sub round, obovate, obtuse or subtruncated, obscurely apiculate, rugose when dry; nutlets globose, up to 2 mm long, single seeded.

Powder is light greenish-brown; shows fragments of parenchyma; tracheids, fibres, and vessels having simple pits and spiral thickenings; anomocytic stomata and wavy walled epidermal cells in surface view (Anon, 2008 d).
TLC of the alcoholic extract on silica gel 'G' plate using chloroform: methanol (8:2 v/v) shows under visible light, one spot at $R_f$ 0.93 (green) under UV (366 nm) eight fluorescent zones were visible at $R_f$ 0.07 (blue), 0.13 (blue), 0.29 (light blue), 0.50 (light pink), 0.60 (light yellow), 0.67 (yellow), 0.79 (blue) and 0.93 (pink). On exposure to iodine vapour twelve spots were appeared at $R_f$ 0.07, 0.10, 0.13, 0.19, 0.29, 0.50, 0.60, 0.67, 0.74, 0.79, 0.86 and 0.93 (all yellow). On spraying with dragendorff reagent followed by 5% methanolic sulfuric acid reagent one spot appeared at $R_f$ 0.07 (orange) (Anon, 2008 d).

2.4.17. *Caesalpinia bonduc*, (seed): Caesalpiniaceae

A stout climbing shrub with finely grey pubescent stems and straight small prickles flowers during February. Occurs in the tropical regions of India, Sri Lanka etc. It is rather common in the low country in Sri Lanka specially near the seacoast. The seed of this shrub yields a fixed oil known as bonduc nut oil. It contains a bitter principle called bonducin, which is a resinous acid, two phytosterolins, sucrose, saponins, some alkaloid and a mixture of aliphatic acids. This shrub has anti-suppurative properties. The seeds are anti helminintic against round worms. The seeds and the whole plant are emetic and are used for fever hydrocele and dropsy (Jayaweera, 2006 e).

Seeds globose or rounded, smooth, shiny, 1.2 to 2.5 cm in diameter; slightly flattened on one side due to close pressing of adjacent seeds; hilum and micropyle close together; hilum surrounded by a dark area around 4 mm in diameter, usually with a whitish or yellowish remnant of funiculus; micropyle near the periphery of the dark area; seed coat greenish-grey to bluish-grey, lineate, shiny; 100 seeds weigh from 225 to 250 g (Anon, 2008 e).
Powder is color light yellow through mustard to brown, coarse and free-flowing; bitter in taste and possessing tamarind-like odour. Parts of vessels showing scalariform thickenings and groups of narrow, palisade cells with light line are present; groups of cells of height from 150 to 250 μ the sub-epidermal layers of seed coat having 10 to 12 μ, squarish bearer cells and up to 150 μ long osteosecreids; cotyledon cells (up to 35 μ) showing fixed oil when mounted in Sudan III (Anon, 2008 e).

TLC of the alcoholic extract on precoated silica gel 'G' plate (0.2 mm thick) using toluene: ethyl acetate: acetic acid (5:4.5:0.5 v/v), shows under UV (366 nm) spots at Rf 0.13 (light blue), 0.28 (dark blue), 0.63 (pink), 0.92 (pink); on spraying with anisaldehyde sulfuric acid reagent and heating the plate for ten minutes at 110 °C spots appear at Rf 0.30 (brown), 0.64 (bluish purple), 0.72 (purple), 0.80 (purple), 0.89 (grey) (Anon, 2008 e).

TLC of the hexane extract on precoated silica gel 60 F 254 TLC plate 0.2 mm thick using n-hexane: ethyl acetate (6:4) as solvent system and developed to the distance of 8 cm. On spraying with anisaldehyde sulfuric acid reagent and heating the plate for ten minutes at 110 °C spots were appeared at Rf 0.16 (purplish red), 0.20 (bluish violet), 0.24 (bluish brown), 0.55 (greenish brown), 0.70 (brown), 0.95 (bluish black) (Gupta et al, 2005 b).

2.4.18. Carum cavi, (fruit): Apiaceae

A biennial or annual herb with a brown tapering root, often branched below and an erect, slender, cylindrical, hollow, faintly striate, smooth, much branched stem, branches ascending. Flowers in June. Cultivated in northern parts in India at very high elevations. Caraway fruits contain a volatile oil, which is a mixture of two liquid oils, carvol and carvone. Carvone is a hydrocarbon, while carvol is oxidized oil. The fruit is
carminative and stimulant, usually given in children's ailments for flatulence and stomach ailments (Jayaweera, 2006 e).

Seeds are hard, surface smooth, ellipsoid, flattened, greyish to reddish brown, 4-6 mm long and 4 mm wide, micropyle prominent, taste, somewhat astringent.

Powder is whitish in colour, under microscope shows broken pieces of testa, parenchymatous cells and starch (Anon, 2008 a; Bremness, 1989).

TLC of the methanol extract on precoated silica gel 60 F254 TLC plate 0.2 mm thick using toluene: ethyl acetate: methanol: acetic acid (9:0.5:0.5:0.3 v/v) as solvent system and developed 8 cm, spray plate with vanillin sulfuric acid reagent and heating the plate for ten minutes at 110°C, spots appear at Rf 0.22 (pink), 0.26, 0.41 (brown), 0.49 (blue), 0.53, 0.73 (pink) (Guptha et. al, 2008, e).

2.4.19. **Cinnamomum verum**, (bark): Lauraceae

A moderate sized or large tree with a rather thick, reddish bark, glabrous young parts and finely silky buds. Flowers in February. Indigenous to Sri Lanka, commonly cultivated in the moist low country. It is also cultivated in India, Myanmar and Malay Peninsula. The chief constituent of cinnamon is the essential oil, which consist of cinnamic aldehyde with variable proportions of hydrocarbons. The bark contains besides the oil, sugar, mannite, starch, mucilage and tannic acid. The bark of this tree is used for dyspepsia, flatulence, diarrhoea, dysentery, vomiting, bronchitis, gangrene of the lungs and phthisis (Jayaweera, 2006, c).

Bark pieces about 0.5 mm thick, brittle, occurs as single or double, closely packed compound quills, up to a meter or more in length and up to about 1 cm in diameter. outer surface, dull yellowish-brown, marked with pale wavy longitudinal lines with occasional small scars or holes, inner surface darker in colour, striated with
longitudinally elongated reticulation, fracture, splintered, free from all but traces of cork, odour, fragrant, taste, sweet, aromatic with sensation of warmth (Anon, 2008 a).

2.4.20. *Acorus calamus*, (rhizome) (described under 2.3.27.)

2.4.21. *Seasamus indicum*, (seed): Padaliaceae

An annual herb with erect stem 30-60cm high, puberulous with long ascending branches from base, stems and branches obtusely quadrangular in the upper part, furrowed. Flowers from May to August and some times throughout the year. Cultivated in all warmer parts of the world, including India and Sri Lanka. It is widely grown and naturalized in the dry zone, especially along roadsides, abandoned fields and waste areas in Sri Lanka. The seed of this herb contain a fixed oil, saccharose, pentosan, lecithin, choline, phytine, conglutine, globuline, legumine etc. The seeds are nourishing, diuretic and lactagogue (Jayaweera, 2006 d).

Seed white, brown, grey or black, flattened ovate in shape, smooth or reticulate, 2.5 to 3 mm long and 1.5 mm broad, one side slightly concave with faint marginal lines and an equally faint central line; taste, pleasant and oily. Powder is blackish coloured; shows palisade-like cells in surface view, parenchyma cells, aleurone grains and oil globules (Anon. 2006 d).

TLC of alcoholic extract on silica gel ‘G’ plate using toluene: ethyl acetate (9:1 v/v) shows under UV (366 nm) three fluorescent zones at \( R_f 0.57, 0.64 \) (both light blue) and 0.72 (blue). On exposure to iodine vapour five spots appear at \( R_f 0.08, 0.57, 0.64, 0.72 \) and 0.94 (all yellow). On spraying with vanillin sulfuric acid reagent and heating the plate for ten minutes at 110 °C seven spots appear at \( R_f 0.08, 0.57, 0.64, 0.72 \) (all violet). 0.76, 0.84 (both light violet) and 0.94 (violet) (Anon. 2006 d).
TLC of n-hexane extract on precoated silica gel 60 F254 TLC plate and developed in the solvent system toluene: ethyl acetate (9:1 v/v) until the solvent rise up to a distance of 8 cm. When spraying with anisaldehyde sulfuric acid reagent followed by heating at 105 °C seven spots appear at Rf 0.19, 0.23 (light brown) 0.28 (blue) 0.33 (red) 0.42 (light green) 0.71 (dark blue) 0.83 (light violet) (Anon, 2008 f).

2.4.22. *Brassica juncea*, (seed): Cruciferae

Erect annual herb with stem 0.4-1 m high, glabrous or with few bristles at the base, much branched often purplish up to the pedicels. In Sri Lanka it occurs as weed in vegetable plantations. The pale yellow oil extracted from the seeds contains sinigrin. The fixed oil is mustard oil and the volatile oil is an allyl mustered oil (Jayaweera. 2006, b).

Seeds small, oblong, pale or reddish-brown, bright, smooth, 1.2-1.5 mm in diameter; under magnifying glass it is seen to be minutely reticulated; taste, bitter and sharp.

Powder is yellow in colour with brown particles and oily, slightly bitter and sharp in taste; shows frequently thick-walled, fragments of reddish-brown cells of hypodermis, yellowish hyaline masses (Anon, 2008, c).

2.4.23. *Emblica ribes*, (seed): Myrisinaceae

A large scandent shrub, old stem with thick brown bark set with conical mamilliform processes, climbing by means of reflexed lateral twigs, which are deciduous except the basal part, which remains as a woody reflexed spine, branches very long and slender and the bark of young branches nearly white, very smooth and shining with large lenticels. Flowers occur in between June and November. Occurs throughout India and is rather common in the lower mountain moist regions up to 4,000 feet altitude in Sri Lanka. The plant contains embeline. The fruit is a carminative, stomachic, anthelmintic
and beneficial against intestinal warms, dyspepsia and skin diseases. The powdered fruit with bees’ honey is given to prevent worm trouble (Jayaweera, 2006, d).

Fruit brownish-black, globular 2-4 mm in diameter, warty surface with a beak like projection at apex, often short, thin pedicel and persistent calyx with usually 3 or 5 sepals present, pericarp brittle enclosing a single seed covered by a thin membrane, entire seed, reddish and covered with yellowish spots (chitra tandula), odour slightly aromatic, taste, astringent. Powder is reddish, under microscope shows reddish parenchyma and stone cells (Anon, 2008, a).

TLC of the chloroform extract on precoated silica gel 60 F 254 plate 0.2 mm thick using toluene: ethyl acetate: acetic acid (5:4:1 v/v) as solvent system and developed up to distance of 8 cm. Visualize the plate under UV at 366 nm, three spots appear at Rf 0.71 (dark brown), 0.80 (fluorescent white), 0.91 (light pink), (Guptha et. al, 2008 e).

2.4.24. *Santalum album*, (heart wood) Santalaceae

A small tree 6-10 m high with numerous, opposite, slender, drooping branches. Bark is smooth greyish brown young twigs glabrous. Flowers from March to May Indigenous to Mysore in India and cultivated in Sri Lanka. The heartwood of this tree contains a volatile oil the chief constituent of which is alcohol santalol. It acts as a diaphoretic and checks haemoptysis (Jayaweera, 2008, c).

Yellowish-brown to pale-reddish orange, heavy, dense, hard but split easily; transversely smooth surface shows alternating light and dark concentric zones with numerous pores, traversed by very fine medullary rays; odour, persistently aromatic; taste, slightly bitter.
Powder is light brown and aromatic; shows pitted vessels with tails, isolated or associated with fibres, fragments of fibres, square to rectangular-shaped parenchyma, prismatic crystals of calcium oxalate, and numerous oil globules (Anon, 2008, c).

TLC of the alcoholic extract on silica gel 'G' plate using toluene: ethyl acetate (93:7 v/v) shows on exposure to iodine vapour six spots at Rf 0.05, 0.10, 0.27 (all yellowish brown), 0.60 (dark brown), 0.82 and 0.91 (both yellowish brown). On spraying with anisaldehyde sulfuric acid reagent and heating the plate for about ten minutes at 110 °C six spots appear at Rf 0.05, 0.10, 0.27 (all bluish violet), 0.60 (violet) 0.82 and 0.91 (both bluish violet) (Anon, 2008 c).

TLC of n-hexane extract on silica gel 60 F254 plate using toluene: ethyl acetate (93:7v/v) shows on spray the plate with vanillin sulfuric acid reagent and heat the plate at 105 °C nine spots were appeared at Rf 0.15 (blue) 0.18 (red) 0.30 (violet) 0.43 (greyish violet) 0.62 (orange) 0.80 0.84, 0.92, 0.97 (violet) (Gupta, 2008 f).

2.4.25. **Acasia chundra**, (dried water extract): **Leguminosae**

Moderate size tree 9-12 m high with short, somewhat crooked trunk and numerous irregular straggling branches, dark brown or dark grey, rough, red and fibrous within young branches smooth or pubescent with a pair of sharp, hooked brown prickles just below the position of the stipules of each leaf. Flowers occur in May and June. It is not found in Sri Lanka.

An extract of bark is an astringent remedy for chronic diarrhoea and desentery. It is used in passive uterine haemorrhages, and mucous discharges, in addition to hoarseness of the throat.

Heartwood, light red, turning brownish-red to nearly black with age, attached with whitish sapwood, fracture hard, taste, astringent.
Powder is brown coloured, under microscope shows a number of xylem fibers, vessels, crystal fibers, prismatic crystals of calcium oxalate (Anon, 2008 a).


A fair-sized pyramidal tree, which is much, branched and with pale and smooth bark, young branches virgate, thickened at nodes. Flowers regular, bisexual, axillary, white, scented, solitary, nearly sessile, large 7.5-10 cm diameter. Flowers from April to June. Occurs in India and Sri Lanka and common in the moist low-country forests. The flowers are used for cough with expectoration, bleeding piles and uterine haemorrhages (Jayaweera, 2008 c).

Stamen consists of anther, connective and filament; coppery or golden brown; filament united at base forming a fleshy ring; each stamen 0.9-1.9 cm long; anther about 0.5 cm long, linear, basifixede, containing pollen grains; filament 0.8-1.0 cm long; slender, filiform, more or less twisted, soft to touch, quite brittle; connective not visible with naked eye; odour, fragrant; taste, astringent (Anon, 2008 b).

TLC of n-hexane extract on silica gel 60 F254 plate using toluene: ethyl acetate: formic acid (5:4.5:0.5 v/v) solvent system to a distance to a distance of 8 cm. Observe the developed plates under UV 254 nm and after spraying the plate with vanillin sulfuric acid reagent and heat the plate at 105 °C five spots appear at Rf 0.19 0.22 (light blue) 0.35 0.78 (dark blue) (Gupta *et al*, 2005 c).

2.4.27. *Piper betel*, (leaf): Piperaceae

A perennial vine with semi-woody stems, climbing by many short adventitious rootlets, stem very stout, much thickened at nodes, young parts glabrous. It is cultivated in garden in low country in Sri Lanka. The chief constituent of the leaves of this plant is a volatile oil known as betel oil. It contains two phenols, betel phenol (chavibetol)
chavicol and cardinene. The leaves also contain an alkaloid arkakene, and terpene and sesquiterpene. They are stimulant, antiseptic sialogogue, carminative astringent, and aphrodisiac. The juice of the leaves is a stomachic and febrifuge (Jayaweera, 2006 d). Chemical and anti bacterial activities of six main cultivars of piper betel namely Galdalu, Mahamenaru, Kudamenaru, Ratadalu, Nagavalli, and Malabulath were studied (Arambewela et.al, 2005).

Leaf varies greatly in size, 7.5-20.0 cm, ovate cordate, entire, glabrous, apex acuminate to acute, lamina membranous, upper surface deep green and lower surface lighter in colour, primary or sub-primary nerves usually 7, sometimes 5-9; odour, aromatic; taste, slightly pungent.

Powder is greyish-green; shows polygonal epidermal cells in surface view, simple pitted vessels and a few uniseriate hairs, anisocytic type of stomata, palisade and spongy parenchyma cells and simple pitted vessel (Anon, 2008; Senarathna, 2001).

TLC of the alcoholic extract on silica gel 'G' plate using toluene: ethyl acetate (9:1 v/v) shows in visible light five spots at Rf 0.11 (green), 0.18 (light green), 0.23 (yellow), 0.34 (grey) and 0.61 (greyish green). Under UV (366 nm) seven fluorescent zones are visible at Rf 0.11, 0.16 (both pink), 0.23 (brown), 0.34 (pink), 0.43 (pink), 0.61 (pink) and 0.76 (grey). On exposure to iodine vapour, seven spots appear at 0.08, 0.11, 0.18, 0.34, 0.61, 0.76, and 0.88 (all yellow). On spraying with vanillin sulfuric acid reagent and heating the plate for ten minutes at 110 °C seven spots appear at Rf 0.08, 0.11, 0.18 (all the three greenish grey), 0.34 (grey), 0.43 (violet), 0.61 and 0.76 (both light green) (Anon, 2008 c; Guptha et. al, 2008 g).

2.4.28. Vitex nigundo, (leaf) (described under 3.2.22.)
2.4.29. Bee’s honey:

Bee’s honey is a saccharine substance produced by the hive bee, *Apis mellifera* and other species of *Apis* in the cell of the honeycomb. It is reddish brown, fruity odour, sweet syrupy liquid.

Loss on drying 34.9-36.2%, total ash 0.009-0.11%, water soluble extractive 80-80.7%, ethanol soluble extractive 38-42.3% (Fernando, 1986).

2.5. Standards:

Standard is a numerical value, which quantifies the parameter and thus denotes the quality and purity of a material. The criteria or the parameters which is considered for making the standard is intimately related to the factor which is responsible for the expected quality and purity of the material. In case of a synthetic drug the quality and purity depend on the most of biologically active chemical ingredient present in it, which is capable of producing the therapeutic effect in the expected level. But this procedure is not always true with natural drugs. The natural drugs are derived from plant, animal and mineral sources. Therefore, these are more complex than synthetic drugs in their chemical structure and composition. In Ayurveda, the therapeutic effect produced by a crude drug is not always attributed to a single chemical substance. The therapeutic effect produced by an Ayurvedic dosage form is always multidimensional. That is the main therapeutic effect is always accompanied by some supportive effects also. The efficacy of a drug combination depends on the purity, chemical nature, potency, rate of absorption, metabolic transformation and elimination. Different pre and therapeutic processing procedures of the various drugs are indicated for maintaining the purity, to increase the potency, easy metabolic transformation and elimination. Therefore, the
standardization of a medicine begins from the selection of crude drugs itself (Reddy, 1998).

2.5.1. Standardization Aspects:

There are three specific stages where standardization steps should be attempted separately. They are the areas related to the raw material aspects, processing aspects and finally product quality aspects (Gour and Sharma, 1997; Kumar, 2011). When the first two areas are properly monitored and controlled, then the third and important area of product quality will automatically be taken care of. That is not to say that there is no need for product quality checks, it is only intended to highlight the importance of raw material and process control measures. Raw material quality assurance measures can actually be perceived as part of the process control measures. But the practice is to have a separate set up for the purpose. An Ayurvedic preparation of medicine involves multi-step procedures and many plant and mineral drugs. The complete composition increases the difficulties of standardization and subsequent quality control of the finished product. It is therefore essential to document and standardize the botanical and chemical characters of each ingredient. It is thus obvious that the standardization and optimization in the preparation stage and quality control in a later stage is a complicated task, if the genuine formulations and concepts of Ayurveda are to be kept untouched (Reddy, 1998). Standardization expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality (Bhowmik et. al, 2009).

2.6. Quality Assessment:

It can be conducted at three levels

(a) Ensuring that the materials used for the preparations are authentic and of the
required standard (Standardization of crude drugs).
(b) Ensuring that the preparation is made according to accepted specification (Standardization of the Pharmaceutical process).
(c) Ensuring that the final product confirms to set standards (Standardization of finished products) (Gour and Sharma, 1997).

2.6.1. Standardization of Crude Drugs:

Standardization of crude drugs is a laborious effort because of many factors, which directly influence the quality and purity of crude drugs. The present status of Standardization techniques practiced in different Ayurvedic drugs manufacturing firms are not competent enough to establish a unique Standardization system for crude drugs. The therapeutic activity of a crude drug exclusively depends on the presence of active ingredients and their concentration. The development of active ingredients in a crude drug depends on the interaction of environmental and ecological factors and our ancestors were well aware about these things. As Acarya Charaka gives details about the Bhesaja Pariksa That is the tests for the drugs as the nature of drug, the properties, the prabhava, in which place it is grown, in which period it is grown, regarding collection, storage, the process, dosage etc.(Sharma, 1998).

However, standardization of crude drugs is not an easy procedure, because almost all crude drugs are in natural origin. In case of a natural drug, the therapeutic efficacy is a total effect of its chemical constituents. Therefore, the quantity and purity refers to the total profile of the drug rather than any of its character. Therefore, a multi dimensional approach is essential for standardizing a crude drug. various parameters regarding different characters to be considered. The selection of parameters depends on various aspects of the crude drug (Simha et. al, 2008; Reddy, 1998).
E.g. For herbs –

(1) Name: (a) Botanical (b) Regional

(2) Synonyms:

(3) History and Introduction:

(4) Classical reference: (a) Rasa (b) Guna, (c) Virya (d) Vipaka (e) Prabhava (f) Karma.

(5) Sources: (a) Botanical (b) Geographical

(6) Organoleptic:

(7) Anatomical:

(8) Physical:

(9) Chemical:

(10) Biological:

(11) Adulteration trends:

(12) Varieties: (a) Natural (b) Commercial

In ancient time, the method of botanical description was not prevalent as at present. Thus for this, they have been stressed on the paryayas (Synonyms) which indicates specific characters, morphological as well as pharmacological characters of the plant. Initially the Nighantus contained only synonyms, which depicted plan with Akruti, Nama, Rupa etc. Later on, gradually properties, actions and therapeutic uses were also added.

Standardization of crude herbal drugs may include-

(1) Identification - For this Macroscopic and microscopic examinations show be done, for this new technological advances like IR, UV, GLC, TLC, HPTLC, finger prints, chemical markers, biomarkers should be used. Nowadays this is called passport data of raw drugs.
(2) Time and mode of collection - The proper time of harvesting or collection particularly important, since the change in the nature and quantity of biologic active constituents vary greatly due to various factors like heredity, autogenic environment and methods of cultivation, collection etc.

(a) A general idea suggested in *Sharanghdhara Samhitha*

i. Bark - should be collected in spring before the vegetative process begins.

ii. Leaves and Flowers buds - Should be collected usually about the time on flowering and before the maturation of the fruits and seeds.

iii. Rhizomes and root - should be collected in the fall after the vegetative processes have ceased.

iv. Flowers - should be collected prior to or just about the time of pollination.

v. Fruits - may be collected either before or after ripening period.

vi. Seeds - should be collected when fully matured.

(b) Mode of collection - May be collected by hand labour or mechanical devices (Kumarasingha, 1962).

(3) Drying - Before putting in to pharmaceutical processing, drugs should be dried. For proper and successful drying involves two main principles

1. Temperature control

2. Regulation of airflow

For this purpose two methods can be adopted

(A) Natural - Sun, shade, Air drying

(B) Artificial - (i) Heating at temperature between (40-60) °C (ii) Freezing - At temperature about (-55 °C) herbs are dried.

(4) Garbling - It is the final step in the preparation of crude drug. Garbling consists of
the removal of extraneous matter.

(5) Packing - Packing of drug is dependent upon their final disposition.

(6) Storage and presentation - Proper storage and presentation are important factors in maintaining a degree of quality of the drug.

One of the main obstacles in standardization of Ayurvedic drugs is the non-availability of an adequate and regular supply of authentic crude drugs. The cultivation of medicinal plants with improved methods like tissue culture etc. at both in home garden level and the commercial sale should be encouraged by developing regional drug farms with collaboration of state forest research institutes.

This is all about the herbal drugs. Rasausadhis (minerals) are used in Ayurvedic system of medicine for cure of various ailments since time immemorial. An exhaustive study of the materials of the parthiva (earth) includes various mineral substances that are employed as Rasadravyas. The Standardization of formulations used in Rasacakitsa, is one of the most important aspect to ascertain their quality control. Therefore, the Standardization of Rasasvaushda is essential from the beginning, starting from the collection of raw materials. Hence, it becomes obvious that the nomenclature of these minerals of Rasadravyas known to Indian Medical system should be understood in terms of modern Mineralogy in order to update our Ayurvedic concepts. As there is lot of description available in Rasa texts regarding the identification of minerals which can be considered as the fundamentals for standardization. However, there is a great need for further research to examine the properties of all these minerals in order to make precise identification and Standardization. There is considerable confusion in the raw materials originally recognized by Ayurvedic physicians and the one now being supplied by various drug agencies. Hence, it is very essential to get into modern
disciplines of mineralogy for the clear identification of these raw materials. The raw materials should be identified according to the characteristics given in the ancient texts and co-related with modern mineralogy (Das, 2010; Reddy, 1998).

2.6.2. Standardization of Pharmaceutical Process:

With regard to the processing aspects, the situation is equally complicated. In Ayurveda, there are so many methods for drug formulations. Even though *Pancavidha Kasaya kalpana* (five types of decoction) are the basic for drug formulation, other *Kalpanas* like *Curna, Avaleha, Asava, Arista, Vati, Snehas, Sarkara* etc. and in the field of *Rasashastra, Bhasmas, Pottali, Parpati, Kupipakva rasayana* etc. are some of the important formulations. In every drug formulation, Acarya has given particular tests for standard product preparation. For *Avaleha* this specifies the thread form stature of the cooked item; also, it dips down in the water. For *Sneha Kalpana* the tests like *Phenodgamana* (Formation of foams) that is the starting of foam in case of *Taila* (oil) and *Phenasamāṇi* that is disappearance of foam in case of *Sarpi* (Ghee) another test is for the residue material, for *Kalka* (paste) when Kalka is rubbed into thumb and index finger it forms *Varti* (Roll) etc. For *Asavariṣṭa* (fermented preparations) match test, limewater test, smell, sound etc., in case of *Bhasma* the colour of the *Bhasma* changes according to *puta*, indication of specific *puta* for specific drugs, are some of the parameters (Sannd et al, 1980).

These ancient parameters can be considered during the process of standardization. As far as the manufacturers view is concerned, the manufacturer may usually produce varieties of medicines and mostly the batch sizes will be quite small. The largeness of the product variety and smallness of the batch size make it quite difficult to effective implement technological innovations. Another problem for process standardization is
subjectivity of parameters. There are many subjective parameters, which play a critical role in the processing steps. The concept of "Tantumatwam" which decides the finality of Avaleha processing. This specifies the thready structure of the substance, to be ascertained by touch and visual examination. It may be considered whether equipmental intervention is possible here to monitor the viscosity or fluidity.

There are similar other subjective parameters which may also have to be considered. Application of objective methods to replace subjective is a vital element of standardization. However, while designing technological aids to control the processing, care must be taken to see that such aids should not neglect the fundamental tenets of the Ayurvedic system. The basic step of processing is the preparation of an aqueous extract, so application of organic solvents can be employed in spite of their proven effectiveness. Thus, a balanced view has to be taken while implementing modern methods as a part of process modernization and standardization. Computer aided measures may become very handy in such efforts. By the use of computers, intermittent checking of materials and processes, monitoring, and controlling batch activities can be possible.

Even then, manual intervention will be very important in the processing of Ayurveda medicines. So while processing of an Ayurvedic formulations following points should be kept in mind.

- Batch processing records, measurement records.
- Prevention of product mix up.
- Simultaneous measurements by two personnel.
- Use of clean/dry equipment tags.
- In-process records of all process parameters.
Records of physical examination of product in processes.
Reports of in process analysis.
Necessary measures for safer operations.
Adequate thermal/Pressure/Electrical monitoring and controlling.
Extraction recovery systems-to minimize loss of extractable.

So manufacturing process, standardization can be rationalized to consume time, cost, and labour, to improve yield and conform to Good Manufacturing Practice (GMP) without comprising on the basic rationale of official methods and ensuring compliance with the set standard (Muliani, 1982).

2.6.3. Standardization of Finished Products:

The quality of finished product refers to the intrinsic value of drug and purity means absence of adulteration. For every drug coming in the market, it is nearly impossible to have a watch on every step of drug preparation, just from identification up to presentation.

Most of the pharmacies are manufacturing medicines according to their own methods and standards consequently there is a wide variation in the quality of products manufactured by different firms. It is, therefore necessary to lay down standards for testing the prepared medicines. The screening of finished products under standard parameters by adopting suitable analytical methods may be sufficient to rule out any adulteration or discrimination done at any phase of drug preparation. Though it is also very difficult for Ayurvedic drugs because, until now, very few drugs for which there are established standard parameters are available to screen and hence it is of prime importance to develop and establish standard parameters for the same. The fundamentals for this concept of finished product standards are available in texts. For
the test, it is mentioned that when Sneha is put on fire does not produce sound called sama paka sneha. When fingers are pressed over Avaleha it, give impression of fingers and it is having desired colour, smell etc. For Asava and Arista desired smell, colour etc. In case of Bhasmas, detailed examinations are given such as colour, rekhamapurnata, varitarata, unama, fineness, soft powdered, apunarbhava (Irreversible), niruttha, taste, lusterless etc. There is no doubt that these reflect the pancaabhautika constitution and quality of those particular drugs that must be present in the final product obtained. Again, the same problem that is discussed in process standardization is the subjectivity of the parameters, which are not much accepted nowadays and hence objectivity to these parameters should be given to widen the acceptance as well as to provide scientific basis to the Ayurvedic drugs. A number of methods depending upon the nature of test drug can be adopted for the purpose of standardization (Das, 2010; Muliani, 1982).

2.7. Standardization Using the Quality Assessment Methods:

The word ‘standardization’ implies the process, procedures and methods by which optimum conditions are ensured for obtaining a predetermined result consistently. It may refer to a manufacturing process, or to the calibration of equipment or to any prepared medicament (Gour, 2000).

Standardization is an essential factor for poly herbal formulations in order to assess the quality of drugs based on the concentration of their active principle.

It is very important to establish a system of standardization for every plant based medicines in the market, since the scope of variation in different batches of medicine is enormous. Plant materials when used in bulk quantity may vary in its intrinsic chemical constituents, which occur in the plant raw materials due to various factors, such as stage
of the plant, different seasons and the time of harvesting, site and conditions of different
environment, and geographical locations under which the plant has been grown (Ram
et al., 2009; Sriwastava, 2010).

The general standardization protocols to determine the percentage of active
medicaments could not be followed for Ayurvedic herbal preparations. The procedures
have to be modified in order to make the preparation safe. This is because of few
reasons like this that are; most of the preparations are polyherbal or herbo-mineral
preparations. Even a single herb is used in the preparation, the single herb will contain
multiple constituents, bioactive chemical constituents are not known and the principle of
holistic approach does not permit assaying a single marker (Jarlald, 2007).

Therefore, it is difficult task to standardize these indigenous medicines because majority
of them have the complex formulations of natural origin. It is difficult to locate and
isolate a single active ingredient and probably the formulations, as a whole is
responsible for the activity. Furthermore, the chemical natures of these preparations are
not well defined. Scientifically validated and technologically standardized botanical
medicines will play an important role in future advancement in health care. The
development of parameters for standardization and quality control of botanicals is a
challenging task. Various regulatory authorities, research organizations and botanical
drug manufacturers have contributed in developing guiding principles addressing issued
related to purity safety and efficacy.

The increasing demand of population and chronic shortage of authentic raw materials
have made it incumbent, so there should be some uniformity in the manufacture of
Ayurvedic medicines, so as to ensure quality control and leads to quality assurance
(Warude and Puatwardhan, 2005; Bhutani, 2000).
Due to complex nature and inherent variability of constituents of plants based drugs, it is difficult to establish quality control parameters and modern analytical techniques are expected to help in circumventing this problem. Quality controls of synthetic drug offer no problems with very well defined parameters of analysis. In contrast, herbal products represent a number of unique problems when quality aspects are considered. These are because of the nature of the herbal ingredients present there in, which are complex mixtures of different secondary metabolites that can vary considerably depending on environmental and generic factors. These complex positions of quality aspects of herbal drugs are further complicated by the use of combination of herbal ingredients as are being used in traditional practice. Thus, batch-to-batch variations start from the collection of raw material itself in the absence of any reference standard for identification. These variations multiply during storage and further processing (Nasreen et.al. 2010; Simha and Laxminarayana. 2008).

Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect, internationally recognized guidelines for their quality assessment and quality control are necessary. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable parameters and standards (Anon, 2000.1).

Although the standardizing includes the quality control of crude herbals, biological evaluation of particular disease area, chemical profiling of the materials and lying down specifications of finished product, here in this study only the specifications of finished product are considered.

Various parameters regarding different characters and various test methods have to be used to standardize these two medicinal formulations. Following scientific parameters
including organoleptic characters, Physico-chemical analysis, chromatographic pattern and microbiological screening were carried out as per the WHO guide lines (Pawar *et al.*, 2011; Meena *et al.*, 2010 a; Meena *et al.*, 2010 b; Meena *et al.*, 2010 c; Simha *et al.*, 2008; Simha *et al.*, 2007).

The tablet like preparations were subjected to the evaluation tests for tablets, such as the weight variation, friability, hardness (Vyas *et al.*, 2008).

Test for standardization can be chemical, physical, biological, or pharmacological procedures or observations, which indicates distinctive response of the components of the preparation. Therefore a standardization test can be thin layer chromatography (TLC) fingerprint, High performance liquid chromatography (HPLC) profile UV-VIS spectrum or microscopical examination, that establish the presence of the characteristic substance or substances in the drug preparation which need not necessarily be the active ingredients. Hence, standards for identity standardization are qualitative criteria that ensure the presence of proper constituents in the drug preparation.

The test for quality assessment is accurate measurement of the characteristic property measured for a drug. Therefore, a qualitative test can be employed to demonstrate that physical property measured for a drug product is within the range of values obtained for a purified specimen of the authentic sample of drug preparation.

Hence, the standards for quality are given as the permissible range, lower limit of values for a measured property. Tests for purity are designed to detect and quantify the contaminating substance and the adulterants in drug preparation. The standard of purity designates impurities in the drug preparation or the absence of an impurity (Fernando, 1986).
The development of these traditional systems of medicines with the perspective of safety, efficacy and quality will help to not only preserve the traditional heritage but also rationalize the use of natural products in health care (Patra, 2009).

2.7.1. Evaluation of the Quality Assessment:

It is obvious that the content of the herbal drugs are most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not.

Quality can be defined as the status of drug that is determined by identity, purity, content and other chemical, physical and biological properties or manufacturing processes.

To provide the identity and purity, criteria such as type of preparation, physical contents, adulteration, contaminants, moisture, ash content, and solvent residues have to be checked. The correct identity of the crude herbal material or the botanical quality is of prime importance in establishing the quality control of herbal drugs.

Quality assessment is a term that refers to processes involved in maintaining the quality and validity of a manufactured product.

In general, quality assessment is based on three important pharmacopoeial definitions:

a) Identity: The real herb that one it should be.

b) Purity: find out whether there are any contaminants.

c) Content or assay: The content of active constituents is in the define limits.

Some of the important parameters are stability testing, safety assessment, specific therapeutic activity analysis and estimation of the active constituents in plant raw material and finished products. The objective of WHO guidelines is to define basic
criteria for evaluation of quality, safety and efficacy of herbal medicines and therefore
to assist national regulatory authorities, scientific organizations and manufactures to
undertake an assessment of the documentation or submission in respect of such
products.

A method of identification, and where possible quantification of the plant material in
finished product should be defined. If the identification of an active principle is not
possible, it should be sufficient to identify a characteristic substance or mixture of
substances like chromatographic fingerprint to ensure consistent quality of the product.

According to WHO herbal medicines should be regarded as, finished labeled medicinal
products that contain as active ingredients aerial or underground parts of plants, or other
plant materials, or combination thereof, where in the crude state or as plant preparations
(Shrikumar. 2007. Prasad et.al., 2010).

Although the crude drugs, manufacturing process and the final product have to be
standardized, in this research the finished products of a complete herbal formula and a
herbo mineral formula were studied. The general standard methods were categorized as
physico-chemical studies, phytochemical screening, microbiological screening and
residual analysis (Gite et.al. 2010; Meena et. al. 2010; Jahan et. al, 2008; Sharma,
2006).

2.8. Physico-Chemical Parameters of the Finished Products:

Variation in weight, pH value, specific gravity, weight loss on drying at 105° C, ash
value, acid insoluble ash value, fiber content, disintegration time, hardness and
friability (Mosihuzzaman and Choudhrary, 2008; Vyas et. al, 2008; Kalsaria et. al,2010;
Meena et. al, 2010; Shah et. al. 2010 ) were taken as physico-chemical parameters of
the finish products.
2.8.1. Variation in Weight:
The sizes of most pills mentioned in Watika Prakaranayn are described as the sizes of various grains like green gram, chickpea, gingerly seed etc. The sizes of most commercial samples are different from each other. Hence, the weights of the pills are different from each other. In Ayurveda pharmacopeia, it is the weight that is mentioned, not the size (Rodrigo and Samarathunga, 2008).

2.8.2. pH (10% w/v aqueous solution):
pH is the measurement of the acidity or alkalinity of a fluid. The pH of any fluid is the measure of its hydrogen ion (H⁺) concentration relative to that of a given standard solution. The pH may range from 0 to 14, where 0 is most acid, 14 most basic, and 7 is neutral.

Mathematically, the pH is the logarithm (to the base 10) of the reciprocal of the hydrogen ion concentration, [H⁺]. The pH is approximately equal to the negative logarithm of the H⁺ ion concentration expressed in molarity. The pH value indicates the relative concentration of hydrogen atoms in the solution compared with that of standard solution. (Angadi, 2009).

Many chemical reactions require optimum pH conditions if the reaction is to proceed to completion (Jenkins et. al. 2008).

2.8.3 Specific Gravity:
The ratio of the weight or mass of a given volume of a substance to that of an equal volume of another substance (water for liquids and solids, air or hydrogen for gases) used as a standard. The values of both redetermined at the same temperature and pressure. (Angadi, 2009). The specific gravity of a substance is taken as a ratio of the
weight of substance to the weight of an equal volume of distilled water at the same
temperature. (Jenkins et.al. 2008).

2.8.4. Weight loss on Drying:
The loss on drying procedure determines the amount of evaporative material in the drug
sample (Angadi, 2009).
Procedure set forth here determines the amount of volatile matter and water. Excessive
moisture can be considered an adulterant because of its added weight as well as the fact,
that excessive moisture is conducive to the promotion of mould and bacterial growth,
subsequently, deterioration and spoilage of drug. Loss on drying of the product s usually
determined at 105 °C, and results were given as a percentage. The loss on drying at
105 °C is mainly due to water content (Fernando, 1986).

2.8.5. Total Ash Content:
When vegetable drugs are incinerated, they leave inorganic ashes, which vary with in
wide limits. It is a criterion to judge the identity and purity of crude drug.
Ashing is the process of mineralization for pre concentration of trace substances prior
to chemical analysis. Ash is the name given to all non-aqueous residues that remains
after a sample is burned, and consists mostly of metal oxides (Venkateshwarlu, et.al.
2010).
Ash is one of the components in the proximate analysis of biological materials,
consisting mainly of salty, inorganic constituents. It includes metal salts which are
important for processes requiring ions such as Na⁺ (Sodium), K⁺ (Potassium), Ca²⁺
(Calcium). The ash figure can be regarded as a general measure of quality or grade and
it is a useful criterion in identifying the authenticity of the material. When high ash
figure suggests the presence of an inorganic adulterant (Kirk et.al, 1991). There is a
considerable difference in ash content of different in drugs, but the difference is varies within narrow limits in the case of the same individual; hence an ash, determination furnishes a basis of judging the identity and the cleanliness of a drug and gives information relative to its adulteration with inorganic matter (Jenkins, 2008).

2.8.6. Acid Insoluble Ash:

The acid insoluble ash limit test is designed to measure the amount of ash insoluble to diluted hydrochloric acid. It is often advisable also to determine the acid insoluble ash. The acid insoluble ash is measure of sandy matter and maxima are prescribed for herbs and spices in US regulations. (Kirk et al. 1991) The diluted hydrochloric acid dissolves the calcium carbonate, alkali chlorides etc., leaving an acid insoluble residue that consist almost entirely of silica derived from soil, adhering to the drug (Jenkins et al. 2008).

2.8.7. Crude Fiber Content:

Crude fiber is defined as the tissue, which remains after material has been subjected to the action of hydrolyzing agent under controlled conditions, is likely to vary, both in amount and chemical nature in different drugs (Trease and Evans, 1983). The technique involves defatting the powder and boiling in turn with standard acid and alkali with suitable washing of the insoluble residue obtained at different stages. It helps to determine the excessive woody material criteria for judging purity (Metha et al. 2011). The crude fiber content of drug is the residue, consisting chiefly of cellulose that remains undissolved after successive treatment with boiling acid and alkali (Jenkins, et al. 2008).
2.8.8. Disintegration Test:

The disintegration test determines whether tablets or capsules disintegrate within the prescribed time when placed in liquid medium in experimental conditions (Anon, 2003). It is a crucial step in release of drugs from immediate release dosage forms. The rate of disintegration is influenced by the rate of influx of water into the tablets, which is also dependent on the porosity of the tablet. When porosity is high, disintegration is hardly influence by tablet formulation; otherwise, disintegration will be affected by the exipients. The main mechanisms of disintegration proposed are swelling of disintegrant resulting in development of swelling force, capillary action and annihilation of intermolecular forces resulting in development of a repulsive force between particles (Ngwuluka et. al, 2010). Factors affecting the disintegration of tablet dosage tablets are physicochemical properties of drug (solubility, particular size, solid phase characteristic, polymorphism) formulation factors, the test apparatus and the tablet manufacturing process (Savarikar et.al, 2011).

2.8.9. Hardness Test:

In general pills/ tablets should be sufficiently hard to resist breaking during normal handling, packaging and shipping and yet soft enough to disintegrate properly. Hardness of tablet is controlled by the degree of pressure applied during the compression stage and it varies with composition, thickness, shape and diameter of tablets. Tablet hardness is not an absolute indicator of strength since some formulations, when compressed into very hard tablets, tend to cap on attrition, losing their crown potions. The hardness of a tablet may be influenced by moisture and long term deterioration in hardness may occur when the tablets containing the most effective disintegrants are subjected to high humidity (Ahamad and Shaikh, 1994).
2.8.10. Friability Test:

Friability is another measure of tablets, strength or wearing quality is often determined in the standardization procedures. Adequate tablet hardness and resistance to friability are necessary requisites for consumer acceptance (Ahamed and Shaikh, 1994).

This test is intended to determine, under defined conditions, the friability of uncoated tablets, the phenomenon whereby tablets surfaces are damaged and/or show evidence of lamination or breakage when subjected to mechanical shock or attrition (Anon, 2003).

2.9. Phytochemical Screening:

Under this extractive values TLC fingerprints UV spectrophotometric measurement and HPLC patterns are discussed. Chromatography is essentially a group of techniques used for separation of the constituents of a mixture by continuous distribution or adsorption of analyte between two phases.

It is well reported that variation of fingerprints indicates that the quality and quantity of medicinal plants, therefore, they have to be developed (Andola et al., 2010).

The chemical fingerprints obtained by chromatographic and electrophoretic techniques, especially by hyphenated chromatographies, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of herbal medicine and therefore be used for authentication and identification of the herbal products. The fingerprinting approach has been recently recognized and accepted, a chemical profile, such as chromatographic fingerprint for a herbal product should be constructed and compared with the profile of a clinically proven reference product (Giri et al., 2010).
2.9.1. Extractable values:

Powdered material is extracted by Soxhlet apparatus; extraction is usually carried out using solvents in the ascending order of polarity. However, it is necessary to dry the plant residues in between changes of solvent prevent to carry over trace of the previous solvent into the next one. All extracts were individually filtered evaporated to dryness (40 °C) (Takate, 2010; Mukherjee, 2003).

Neutral or non-polar solvents extract mainly fat and waxes. While moderately polar solvents extract terpenoids and phenolics, polar solvents extract mostly alkaloids (Gite et. al, 2010; Rai et. al, 2010). The raw materials are extracted with pet ether, chloroform, ethyl acetate and methanol (Vaidu et. al, 2010). The powdered plant was extracted with petroleum ether, toluene, chloroform, acetone, methanol and distilled water using Soxhlet apparatus (Jain and Shukla, 2011). For the ethanol extractions 95% v/v has been used. It should not exceed 80 °C in any case, but it should preferably be between (60-70) °C. Occasionally, lower temperature up to 50 °C may also be required depending upon the herb and the purpose of extraction (Chaudri, 2004).

2.9.2. TLC fingerprints:

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. In 1938 first time this technique was introduced by Izmailor and Schrabier at the Ukrainian institute for experimental pharmacy. But its acceptance was not achieved until late 1950, when stall publicized the method, developed a kit of basic equipments and made them available. Since then TLC has become an important tool for both qualitative and quantitative analysis. (Hiremath, et.al,1993) The British Herbal Pharmacopeia 1996 has had an emphasis on using TLC profiles to identity characteristic and active principles of herbal materials (Yadav, 2008; Liang et.al, 2004).
Chinese drug monographs and analysis still use TLC to provide first characteristics finger print of herbs. Rather, TLC is used as an easier method of initial screening with a semi quantitative evaluation together with other chromatographic techniques (Pattanaya et.al, 2010). Control and commercial samples of this study were standardized based on their fingerprint profiles.

The alcoholic extracts are used to carry out for TLC spotting in order to keep the size of starting zones down to minimum diameter 2 to 4 mm, the application volumes are normally limited to a maximum to 5 µl for TLC when samples are applied as spots (Jork et.al, 1990).

According to Iqbal TLC of a drug is developed on silica Gel 60 GF 254 precoated plates, using ethyl acetate: methanol: water (100:15:5 v/v) as mobile phase, detected under UV light 254 nm, 366 nm and plate was sprayed with anisaldehyde sulfuric acid reagent followed by heating at 105 °C for 10 minutes (Iqbal et. al, 2010).

Karunnayaka mentioned that the Seetharama pill was prepared by the pharmaceutics according to the prescription given by Alvis (1948) and prepared samples were also obtained from number of private manufacturing organizations. Seetharama pills were extracted with a mixture of methanol: chloroform(10:1 v/v) 10 mL and the sample extract 10 µL were then applied to the chromatography and developed two dimensional thin layer chromatographic techniques using xylene: chloroform (1:1 v/v). In this test, five spots were observed under ultra violet light in the prepared sample and lesser spots in commercial samples (Karunanayake et.al, 1971).

Rf values of some raw ingredients used in preparation of Seetharama Watee and Maha Varthikava Watee were given in many texts like Indian Pharmacopeia, Standardization
of Botanicals (Rajpal, 2006) and Quality Standards of Indian Medicinal Plants (Gupta et. al. 2003a, 2008g).

2.9.3. UV/Vis Spectrophotometric measurements:
Method based on absorption of light and its changes. The wavelength of light that a compound absorbs, is characteristic of its chemical structure. Absorption of ultra violet and visible radiation is associated with excitation of electrons, in both atoms and molecules, to higher energy states. The amount of light “l” transmitted through a solution of an absorbing species in a transparent solvent can be related to its concentration by Beer’s Law. Thus, absorption spectroscopy can be used to quantify the amount of chemical present in an unknown solution.

Ultra violet (185-380 nm), the visible (380-780 nm) the near infrared (780-3000 nm) and the infrared (3-40 μm) regions are used in analytical procedures. (Elamuthuruthy et.al, 2005).

2.9.4. High Performance Liquid Chromatography (HPLC) Profiles:
HPLC is a method of chromatographic separation in which the mobile phase is pumped into column containing stationary phase by a high-pressure pump system. The test solution injected is carried into the column by the mobile phase. All the components are separated in column and pass through the detector sequentially. The recorder integrator or data acquisition system thus record the chromatographic signal (Lohar and Singh, 2008).

HPLC is a popular method for analysis of herbal medicines, because it has been used to distinguish different types and source of drugs; there is no limitation by the volatility or stability of the sample compound. In general, HPLC can be used in the analytical
separation of herbal medicines. Reversed phase columns may be the most popular columns used in. It is necessary to notice that the optimal separation for the HPLC involves many factors, such as different compositions of the mobile phase their pH adjustment, pump pressure etc. (Giri et.al, 2010).

In the fingerprint analysis of herbal medicines, similarity is usually used as a criterion for quality assessment. Similar analysis revealed that either fingerprint was useful while their combination would be more confirmative in identification and assessment (Li et.al, 2006).

2.10. Microbiological Screening:

Microbial quality is another important issue. Materials of vegetable origin tend to show much higher level of microbial quantity than synthetic materials. The European pharmacopeia specifies that *E. coli* and *Salmonella* spp. should be absent (Smet, 1999). Various scientists have actively participated to validate Ayurvedic formulations. It is mandatory to carry out the microbiological limit test to ensure whether the product is free from risk (Kalaiselvan et al, 2011).

2.10.1. Indole Methyl Red Voges Proskauer and Citrate Utilization test: (IMViC)

Except for the lowercase i, which is added for ease of pronunciation, each of the letters in the abbreviation IMViC stands for one of these tests. I is for indole; M is for methyl red; V is for Voges-Proskauer, and iC is for citrate.

This test is used to differentiate the Gram-negative enteric bacteria of the family Enterobacteriaceae. The basis of differentiation is the biochemical characterization of bacteria. However, the IMViC test includes four different types of tests such as (Sherman, 1998).
(i) Indole production test-
Some enteric bacteria produce indole during the hydrolysis of tryptophan. Not all bacteria hydrolyse tryptophan through the production of tryptophanase. However, few bacteria do not produce indole. Indole production can be detected using Kovac’s reagent (Appendix II) that gives cherry red layer of reagent (Dubery and Maheshwari, 2002).

\[
\text{Tryptophan} \xrightarrow{\text{Tryptophanase}} \text{Indole} + \text{Pyruvic acid} + \text{H}_2\text{O}
\]

(ii) Methyl red and Vogues-Proskauer tests (MP-VP)-
The mixed fermenters produce large amount of acids and the butanediol fermentors produce the neutral end products called acetoin. Since both the tests are performed simultaneously and on same medium, (MR-VP broth) is called MR-VP test. If the bacterium produces substantial quantity of organic acids as end products on addition of methyl red (a pH indicator) the medium will remain red indicating the acidic pH of the medium. Then it shows the positive test. Upon addition of methyl red, if the medium turns yellow it shows the negative test because the pH of the medium is increased to 6 or above due to production of ethanol and acetoin (Dubery and Maheshwari, 2002).

In the Voges Proskauer tests positive reaction will show, a maroon colour band at the top of the broth in the tubes, this will diffuse into the rest of the media. The negative reaction shows no colour change in the tubes after addition of Barritt’s reagent A and B (Sherman & Cappuccino, 1998).

(iv) Citrate utilization test-
This test is used to differentiate E. coli from Enterobacter aerogenes. It is a widely accepted test for test the presence of E. coli, a faecal organism in water, which cannot utilize citrate as carbon source while E. aerogenes can use it. Secondly, citric acid is an intermediate of metabolic product of Kreb’s cycle, which oxidizes pyruvate to CO₂. The
test based on the ability of organisms to utilize citrate, as it's only source of carbon and ammonia as nitrogen (Reddy et al., 2009; Dubery and Maheshwari, 2002).

2.10.2. Mycological contaminants:

Pharmacopeia has published information on microbiological limits or absence of specified microorganisms in herbal medicines (Anon., 2000).

The herbal medicinal products, to which boiling water is added before use, can not contain more than $10^7$/g aerobic bacteria and $10^5$/g of fungi; the herbal products which boiling water is not added before use, can not contain more than $10^5$/g aerobic bacteria and $10^4$/g fungi, absence of *E. coli* and *Salmonella* (Anon., 2003).

2.10.3. Pour plate method:

In this method, the inoculums are diluted in successive tubes containing liquefied agar medium so as to permit a thorough distribution of cells/spores with the medium. The inoculated medium is then poured into sterilized Petri dishes and incubate at desirable conditions to obtain well-isolated colonies of desired organisms (Karva, et al., 2008).

Czapeck dox agar used for the cultivation and maintenance of fungal cultures. Sucrose serves as a rich source of carbohydrate. Acidic pH and high sugar concentrations are selective ingredients for fungal growth.

Sabouraud dextrose agar used for the isolation and enumeration of yeast and mould from food and clinical samples. Acidic pH and higher sugar concentration serve as selective ingredients for fungal growth (Mudili, 2007).

*Aspergillus niger* is a cosmopolitan fungus commonly known as black mould. This fungus is among the most common fungi causing food spoilage and bio deterioration of other materials. It is reported to contaminate herbal drugs during harvesting, pre and post processing practices. This fungus is one of the major fungal contaminants with high
relative density in raw materials of some herbal drugs. 54.9% of the medicinal plants analyzed did not comply with the maximum acceptable limit for fungal contamination (Gautham et. al, 2010). The microfloristic study of mould is well in progress around the world. The most common strains reported belong to *Penicillium* Link: Fr and *Aspergillus* Fr: Fr genera (Eltem et.al, 2004).

2.11. Residual Analysis:

2.11.1. Heavy metal determination:

Toxic metals or metalloids such as lead, mercury and arsenic are frequently found in traditional medicines. Three major arsenic containing minerals are used in traditional medicines. These arsenicals include orpiment, which is also called yellow arsenic and contains As₂S₃, another is realgar, which is also called red Arsenic due to deep red colour and contains > 90% arsenic disulphide As₂S₇ or As₄S₄. Arsenolite, which is the third common mineral arsenic called white arsenic contains largely As₂O₃. Orally administered orpiment is poorly absorbed and over 82% is found in feaces within 3 days, representing an unabsorbed portion of dose compared only 12% of an oral dose of sodium arsenate. Realgar also has a low solubility in water, and only 4% is bio available in physiological gastric juice or intestinal fluid (Lui et.al, 2008). The average total arsenic concentration in a Chinese traditional medicine *Niu Huang Jiedu Pian* is approximately 7±1% (i.e. 70,000 ppm), corresponding 28 mg of arsenic per pill; of this only 1 mg of arsenic finds its way into blood stream, and 40% of this absorbed arsenic (0.4 mg) is excreted in urine. The combination of arsenicals with natural compounds can modify unpleasant side effects, such as cardio toxicity or genotoxicity in normal cells (Balaz & Sedlak, 2010).
Mercury is a major toxic metal ranked top in the toxic substance list. Cinnabar, which contains mercury sulfide, has been used in Chinese traditional medicines for thousands of years, in various remedies, and 40 ppm cinnabar containing traditional medicines are still used today. Mercurials are commonly grouped as elementary, inorganic and organic. Mercury ores are often found as cinnabar, which contains 96% mercuric sulfide (HgS). Methyl mercury (CH$_3$Hg), is the toxicologically most important organic form but dimethyl mercury (CH$_3$)$_2$Hg is the most toxic mercurial. No methyl or dimethyl mercury is used in any traditional medicines. Absorption of cinnabar (0.2%) from the gastrointestinal tract is much less than mercuric chloride (7-15%) and methyl mercury (> 95%) Both crude cinnabar and synthetic mercury sulfide have very low oral bioavailability and are poorly absorbed from gastrointestinal tract as compared to mercuric chloride and methyl mercury, but are better than liquid elementary mercury (< 0.01%). Cinnabars containing traditional medicines are generally relatively non toxic at therapeutic doses. The correct preparation methods, appropriate doses, disease status, age and drug combinations are important factors impacting cinnabar toxicity. There is as yet no available report on cinnabar induced neurotoxicity in humans. This fortifies the notion that cinnabar is poorly absorbed and kidney is the major organ of mercury accumulation, despite the fact that the doses used in this study were 1000 times higher than human daily dose (2.5-5 g/kg. p.o. for 2-4 weeks). The adverse effects of cinnabar containing traditional medicines seem to be tolerable and reversible. In safety, evaluation of cinnabar containing traditional medicines, total mercury content alone is insufficient, and chemical forms of mercurial compound should be taken into consideration (Lui et. al, 2008; Chuu et. al, 2007).
The toxic kinetics data clearly demonstrate that As and Hg accumulation in liver and kidney are highly dependent on chemical form of metals, with arsenite $\gg$ realgar; and HgCl$_2$ $\gg$ Cinnabar (Lu et al., 2011).

Lead and cadmium are the most toxic and most abundant metals in food (Khan et al. 2009). The maximum permissible limit for mercury in food is 0.03 µg/g, for arsenic is 0.1 µg/g, for lead is 0.2 µg/g and for cadmium is 0.1 µg/g (Abbas et al., 2010). The permissible limits of heavy metals in Ayurvedic drugs with herbal ingredients as per World Health Organization (WHO) and Federal Drug Administration (FDA) are for arsenic 10 µg/g, mercury 1 µg/g cadmium 0.3 µg/g and lead 10 µg/g respectively (Kapoor, 2010).

Current provisional tolerable weekly intake (PTWI) for As, Cd, Hg, and Pb are 15 µg/kg of body weight, 7 µg/kg of body weight, 5 µg/kg of body weight and 25 µg/kg of body weight respectively (Kosalec et al., 2009; Llobet 2003, Smet 1999).

Most basic atomic absorption spectrophotometer (AAS) use a flame to atomize the sample. A solution of the sample is nebulized and aspirate into the flame. The elements to be analyzed are converted into atoms mostly remaining in the ground state. Monochromatic light from a hollow cathode lamp is passed through the flame, and the absorbance is measured. The instrument is first calibrated using a set of standards of the element of interest. There is an approximately linear relationship between the concentration of analyte and the absorbance. In the case of mercury, it is possible to convert mercury into its elemental form from any compound in solution by adding a reducing agent such as NaBH$_4$ or SnCl$_2$. By bubbling a stream of N$_2$ gas through the solution the mercury vapour is carried from the solution into gas stream (Pathirathna, 2007).
Inductive Coupled Plasma atomic emission spectroscopy (ICP-AES/ICP-OES) uses plasma to generate exited atoms. These atoms emit electromagnetic radiation at wavelength that is characteristic of a particular element. From a measure of intensity of this emission, one can quantify the concentration of the element present in a sample (Kapoor, 2010). ICP-AES is an analytical technique used for the detection of trace metals (Angadi, 2009).

Using ICP-AES mercury contamination of Yogaraja gugulu pills, Seetharama pills, Chandraprabha pills, Kaishor gugulu pills and Makaradwajaya powders were determined and reported to be 0.3 mg/kg, 47 mg/kg, 47 mg/kg 0.4/kg 2.3 mg/kg respectively (Amarasingha & Senevirathna, 2006).

In the case of Rasamanikya which includes orpiment as an ingredient, it was found that there was no elemental arsenic present in samples which is toxic in nature, but in the form of sulphide complex (Hariprasad et.al, 2011). In the case of Maha Yogaraja gugulu heavy metal content measurements indicated levels of 25.8 μg/g for lead 0.07 μg for mercury and 5.19 μg/g for arsenic. The test drug is well tolerated as no changes of a serious nature could be observed (Lavekar et.al, 2011).

2.11.2. Aflatoxin determination:

Aflatoxins are poisonous substances in the spores of fungi *Aspergillus flavus*, A. parasiticus, A. nomius, A. tamari. The fungus contaminates mainly stored nuts and cereals. In humans and animals, they may be implicated in high incidence of hepatocellular and lung carcinoma (Ehrlich et. al, 2010; Floyd and Bennett, 1981). The toxin is known to produce cancer in human beings living in warm and humid region of the world. Moisture acts as an important factor for the growth of fungi (Younis and Malik, 2008). The minimum moisture level for aflatoxin production at 30 °C by A. flavus is
equal to the moisture content of a product in equilibrium with 83% relative humidity or higher depending on the nature of substrate and duration of storage. For starchy cereal seeds such as maize and wheat the limiting moisture level for growth of *A. flavus* is about 18.5% whereas in oil seeds such as peanuts is 8 to 9% (Prabakaran & Dhanaphal, 2009; Balha and Lohnisky, 1990; Christine and Kaufmann, 1974).

Spices, peanuts and grains among the tropical crops need a special attention in drying and storage in order to prevent aflatoxin formation (Seenappa and Kempton, 1980).

With the development of extraction and concentration and thin layer chromatographic procedures, it was discovered that the toxins exhibit a florescence of characteristic colour under long wave UV light and could be separated into 4 components. These components were named as aflatoxins, indicating their generic origin. The individual components were designated as aflatoxin B₁, B₂, G₁ and G₂ denoting their fluorescence colour B-blue and G-green and relative chromatographic morbidity (Basappa, 2009).

The inhibitory effect of water soluble extract of garlic bulb, green garlic, green onions, hot peppers, ginger, Chinese parsley and basil on the growth of *Aspergillus niger* and *Aspergillus flavus* was examined. Garlic bulbs, green garlic and green onions showed an inhibitory effect, against fungi tested (Yin et al., 1998).

The effects of black pepper, cinnamon, peppermint, cumin, ginger and clove on growth and aflatoxin formation of *Aspergillus flavus* were studied in rice powder corn steep medium. The effects of these first five spices were judged that the inhibition of aflatoxin formation rather than mycelial growth. Cloves completely inhibit both mycelia growth and aflatoxin formation at a concentration above 0.1%. No aflatoxin was produced when cumin and mint levels of 5% and 10% were used. Black pepper and ginger levels of 10% decreased aflatoxin formation by 100% (Mabrouk et al. 1980).
Stored drugs samples are prone to attack by harmful mycotoxin producing fungi. Detection of mycotoxins is certainly a matter of great concern in stored drug of important medicinal plants. Eg. Fruits of Emblica officinalis (1.51 µg/g); Terminalia chebula (1.19 µg/g) (Shrikumar and Ravi, 2007).

Nonvolatile neem leaf constituents irreversibly and almost totally inhibit aflatoxin biosynthesis of A. parasiticus, but they do not affect fungal growth. Inhibition of aflatoxin biosynthesis appeared to occur in the early stages of the biosynthesis pathway. Neem leaf extracts might be used in controlling the pre harvest aflatoxin contamination of food and feed commodities (Bhatnagar and McCormick, 1988).

Eugenols extracted from powdered cloves completely inhibit the growth of A. flavus and A. vesicolor at concentration of 125 µg/mL (Hitokoto et al., 1980).

It is reported that the survey of oat samples for the presence of aflatoxins, initial chemical assays indicated that even though the fluorescing substances that behaved like aflatoxins on thin layer plates, but they did not have the toxicity as aflatoxins. Japanese workers have observed that a number of moulds in food fermentations produce nontoxic compounds, which resemble aflatoxin B₁ and G₁ in their chromatographic and fluorescent properties, but they were identified as the chemicals different to aflatoxins. TLC alone cannot confirm presences of aflatoxin further confirmatory test are essential (Shotwell et al., 1968). It may be pertinent to mention here that aflatoxin like fluorescent substances are present in many spices if sufficient care is not taken to remove these. They are likely to interfere with aflatoxin quantification (Madhyastha and. Bhat, 1984).

Only the 2002 European Regulatory sets maximum limits also in spices (Capsicum spp., Piper spp., Myristica fragrans, Zingiber officinale, Curcuma longa) AFB₁, 5 µg/kg;
total AFS 10 μg/kg. None of the aromatic herb, herbal tea and medicinal plant samples analyzed was contaminated even if they are from tropical countries. Aflatoxins analyses on spices are not simple because of highly coloured contaminating materials that are co-extracted with aflatoxins (Romagnoli et al., 2007).

The aflatoxin molecule contains a coumarin nucleus linked to a bifuran and either a pentose as in AFB₁ and dihydro derivative AFB₂ or a six-member lactone, as in AFG₁ and its corresponding derivative AFG₂. These four compounds are separated by the colour of their florescence under long wave ultraviolet illumination (B=blue; G=green) of the four B₁ is found in highest concentrations followed by G₁ and G₂. Aspergillus flavus only produce B₁ in higher concentrations and B₂ in lesser concentrations. Aspergillus parasiticus produce these same metabolites along with G₁ and G₂. However, due to the production of false positives, it is essential that positive results should be confirmed chemically by derivatisation with trifluoroacetic acid and by spraying with 50% sulfuric acid (Horn et al., 2009; Ruiqian et al., 2004; Dayananda, 1991; Roy and Churasia, 1989).

The European Union has legislated the maximum permitted level of 2 μg/Kg for aflatoxin B₁ and 4 μg/Kg for total aflatoxin (B₁, B₂, G₁ and G₂) in several products (Soboley and Dorner, 2002).

Accordingly, prevention of Food Adulteration act (1954) amended in 1986; U.S. Food and Drug Administration (FDA) and the Codex Alimentarius Commission (1989) have recommended a permissible limit of aflatoxins were 30 μg/kg, 20 μg/kg and 5 μg/kg, respectively.
Chapter 3

3. Materials and methods:

3.1. Selection of Samples:

Two effective and popular traditional preparations in pill form were selected from authentic Sri Lankan text Watika Prakaranaya.

1. Seetharama Watee - a herbo-mineral formulation.


Three samples from each formula were prepared (Kalsaria et al., 2010) to account for seasonal changes; raw drugs were collected in December - January, March - April and July - August months, prepared in the same period, used as control samples. They were prepared in the Department of Dravyaguna Vighana at Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya.

Ten commercial samples of the two preparations were purchased from Sri Lankan local market (i.e. Five different commercial samples from each preparation).

All herbal and mineral ingredients were purchased from the Sri Lankan local market and some fresh drugs were collected from the herbal gardens, a Professor, in the Department of Dravyaguna Vighana Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, authenticated the samples.

Analytical reagent grade hexane, dichloro methane, ethyl acetate, methanol, ethanol 95%, HPLC grade methanol, acetonitrile and pre coated silica gel 60F254 TLC aluminum plates (20x20 cm, 0.2 mm thick) were obtained from E. Merk Ltd, Germany. Chloroform, HCL, NaOH, acetone toluene, formic acid (90%) were purchased from BDH chemicals U.K.
De-mineralized water was prepared using Bharum Aqua DM 600 de-mineralized water plant at Department of Chemistry of University of Sri Jayewardenepura.

Most of the tests were done in the laboratory of the Department of the Food Science and Technology, and in the Department of Forestry University of Sri Jayewardenepura. Determination of disintegration, hardness and friability were done in the laboratory of Link Natural Products Mabima, Kapugoda. HPLC analysis was done in City analyst lab Colombo Municipal Council. Hg and As detection was done in SGS laboratory Colombo 2.

Aflatoxin was determined at the herbo-technology section, Industrial Technical Institute (ITI) laboratory.

3.2. Method of Sample Preparation:

3.2.1. Seetharama Watee:

Table 3.1. Herbal ingredient of Seetharama Watee.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Local Name</th>
<th>Common name</th>
<th>Part used</th>
<th>Weight of raw drug (g)</th>
<th>Weight of powdered drug (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuminum cymine</td>
<td>Suduru</td>
<td>Cumin</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Nigella sativa</td>
<td>Kaluduru</td>
<td>Black Cumin</td>
<td>Seed</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Foeniculum vulgare</td>
<td>Mahaduru</td>
<td>Fennel</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Trachyspermum ammi</td>
<td>Asamodagam</td>
<td>Ajowan</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Common Name</td>
<td>Scientific Name</td>
<td>Part</td>
<td>Quantity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------</td>
<td>----------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anethum graviolens</td>
<td>Shathapushpa</td>
<td>Dill Fruit</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Viyali inguru</td>
<td>Ginger Rhizome</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piper nigrum</td>
<td>Gammiris</td>
<td>Pepper Fruit</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piper longum</td>
<td>Tippili</td>
<td>Long Pepper Fruit</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristica fragrans</td>
<td>Sadikka</td>
<td>Nutmeg</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristica fragrans</td>
<td>Vasavasi</td>
<td>Mace Aril</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syzygium aromaticum</td>
<td>Karabu</td>
<td>Clove Flower bud</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aconitum palatum</td>
<td>Nerivisha</td>
<td>Root</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saussurea costus</td>
<td>Suvanda kottan</td>
<td>Costus Root</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>Welmi</td>
<td>Liquorice Stem</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coriandrum sativum</td>
<td>Kottamalli</td>
<td>Coriander Fruit</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holarrhena antidysenterica</td>
<td>Kelinda</td>
<td>-- Bark</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aconitum heteroppyllum</td>
<td>Atividayan</td>
<td>Atis root Root</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picrorhiza kurroa</td>
<td>Katukarosana</td>
<td>Picrorhiza Root</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocimum tenuiflorum</td>
<td>Maduruthala</td>
<td>Holy Basil Leaves</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitex nigundo</strong></td>
<td><strong>Nika</strong></td>
<td><strong>Indian privet</strong></td>
<td><strong>Leaves</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Toddalia asiatica</strong></td>
<td><strong>Kudumiris</strong></td>
<td><strong>Forest Pepper</strong></td>
<td><strong>Leaves</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Leucas zeylanica</strong></td>
<td><strong>Tumba dalu</strong></td>
<td><strong>--</strong></td>
<td><strong>Leaves</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Cleome gynandra</strong></td>
<td><strong>Wela</strong></td>
<td><strong>--</strong></td>
<td><strong>Whole plant</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Azadirachta indica</strong></td>
<td><strong>Kohomba</strong></td>
<td><strong>Neem</strong></td>
<td><strong>Leaves</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Allium sativum</strong></td>
<td><strong>Sudulunu</strong></td>
<td><strong>Garlic</strong></td>
<td><strong>Bulb</strong></td>
<td><strong>70</strong></td>
<td><strong>70</strong></td>
</tr>
<tr>
<td><strong>Pterocapus santalinus</strong></td>
<td><strong>Rath hadun</strong></td>
<td><strong>Red Sandalwood</strong></td>
<td><strong>Heart wood</strong></td>
<td><strong>60</strong></td>
<td><strong>32</strong></td>
</tr>
<tr>
<td><strong>Ferula assafoetida</strong></td>
<td><strong>Perunkayam</strong></td>
<td><strong>Asafoetida Oleogum resin</strong></td>
<td><strong>30</strong></td>
<td><strong>15</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Acorus calamus</strong></td>
<td><strong>Vada kaha</strong></td>
<td><strong>Cinnamon sedge</strong></td>
<td><strong>Rizome</strong></td>
<td><strong>20</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>
Table 3.2. Mineral ingredients of Seetharama Watee.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Sinhala name</th>
<th>Common name</th>
<th>Non purified form weight (g)</th>
<th>Purified form weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnCO₃</td>
<td>Redectuthan</td>
<td>Zinc ore</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>As₂S₂</td>
<td>Manoseela</td>
<td>Realgar</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>CuSO₄,5H₂O</td>
<td>Palmanikkan</td>
<td>Blue vitriol</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sahinda lunu</td>
<td>Rock salt</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Na₂B₄O₇.10H₂O</td>
<td>Pushkara</td>
<td>Borax</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>KAl(SO₄)₂.12H₂O</td>
<td>Seenakkaran</td>
<td>Alum</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>CaSO₄.7H₂O</td>
<td>Galmada</td>
<td>Gypsum</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>As₂S₃</td>
<td>Ranhiriyal</td>
<td>Orpiment</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>HgS</td>
<td>Sadilingan</td>
<td>Cinnabar</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

All herbal ingredients were washed, dried and pulverized separately except garlic and asafoetida and they were sieved through 100-mesh sieve and weighed as indicated in the Table-3.1

The mineral substances (Table 3.2) were purified using the traditional methods mentioned in the Watika Prakaranaya (Appendix II). After purification, they were also sieved through 100-mesh sieve. They were then all mixed together thoroughly to obtain a homogenous blend. Chopped garlic and asafoetida were then added and ground using juice of holy basil leaves, juice of neem leaves, juice of indian privet leaves, juice of sour orange and juice of fresh ginger respectively for first five days. On
the sixth day it was ground using the neem oil and on the seventh day by ghee in succession.

After grinding for seven days with those fluids and oils, the resultant soft fine paste was in a condition to be made into pills, of the size of raw green gram fruit and was dried under shade at temperature not exceeding 60 °C. It was packed in a tightly closed glass containers for further use (Alvis, 1879; Pattanaya et al., 2010).

3.2.2. Maha Varthikava Water:

Table 3.3. Herbal ingredients of Maha Varthikava Water.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Sinhala Name</th>
<th>Common name</th>
<th>Part used</th>
<th>Weight of raw drug (g)</th>
<th>Weight of powdered drug (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuminum</td>
<td>Suduru</td>
<td>Cumin</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Nigella sativa</td>
<td>Kaluduru</td>
<td>Black Cumin</td>
<td>Seed</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Allium sativum</td>
<td>Sudulunu</td>
<td>Garlic</td>
<td>Bulb</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Trachyspermum</td>
<td>Asamodagam</td>
<td>Ajowan</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Anethum graviolens</td>
<td>Shathapushpa</td>
<td>Dill</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Viyali inguru</td>
<td>Ginger</td>
<td>Rhizome</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Ferula assa-foetida</td>
<td>Perunkayam</td>
<td>Asafoetida</td>
<td>Exudates</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Piper nigrum</td>
<td>Gammiris</td>
<td>Pepper</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Common Name</td>
<td>Scientific Name</td>
<td>Part</td>
<td>Quantity</td>
<td>Price</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>---------</td>
<td>----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Piper longum</td>
<td>Tippili</td>
<td>Long pepper</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Solanum virginianum</td>
<td>Katuvelbatu</td>
<td>Yellow berried night shade</td>
<td>Root</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Terminalia chebula</td>
<td>Aralu</td>
<td>Chebulic Myrobalan</td>
<td>Pericarp</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Terminalia bellirica</td>
<td>Bulu</td>
<td>Bellaric Myrobalan</td>
<td>Pericarp</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Phyllanthus emblica</td>
<td>Nelli</td>
<td>Emblic Myrobalan</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Syzygium aromaticum</td>
<td>Karabu</td>
<td>Clove</td>
<td>Flower bud</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Myristica fragrans</td>
<td>Sadikka</td>
<td>Nutmeg</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Myristica fragrans</td>
<td>Vasavasi</td>
<td>Mace</td>
<td>Aril</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Fumaria parviflora</td>
<td>Pathpadagam</td>
<td>--</td>
<td>Whole plant</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Caesalpinia bonduc</td>
<td>Kumburu</td>
<td>Grey nicker</td>
<td>Seed</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Carum cavi</td>
<td>Devduru</td>
<td>Black Cumin</td>
<td>Fruit</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Cinnamonum verum</td>
<td>Kurundu</td>
<td>Cinnamon bark</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Acorus calamus</td>
<td>Vadakaha</td>
<td>Cinnamon sedge</td>
<td>Rhizome</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
All herbal ingredients were washed dried, pulverized separately and sieved through 100-mesh sieve and weighed as indicated in the Table-3.3 (Sugathadasa, et al, 2008) Then they were mixed together thoroughly to obtain a homogenous blend using juice of betel leaves, juice of Indian privet leaves, juice of fresh ginger and bees' honey respectively for four days. After grinding the mass properly to a fine soft paste, pills, of the size of a raw green gram fruit were prepared. They were then dried under shade at a temperature not exceeding 60 °C. It was packed in a tightly closed glass containers for further use.

### 3.3. Quality Assessment Methods for Standardization:

#### 3.3.1. Determination of Variation of Weight:

Randomly chosen 10 pills from each control and commercial samples from two preparations labeled as $1S_0$, $2S_0$, $3S_0$, $1V_0$, $2V_0$, $3V_0$ (Prepared samples) and $S_1$, $S_2$, $S_3$, $V_1$, $V_2$, $V_3$. 

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Part</th>
<th>Name</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Seasamus indicum</em></td>
<td>Thala</td>
<td>Gingerly Seed</td>
<td>10</td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>Aba</td>
<td>Mustard Seed</td>
<td>10</td>
</tr>
<tr>
<td><em>Embelica ribes</em></td>
<td>Valaghasal</td>
<td>Embelica Seed</td>
<td>10</td>
</tr>
<tr>
<td><em>Santalum album</em></td>
<td>Suduhandun</td>
<td>White Sandalwood Heart wood</td>
<td>10</td>
</tr>
<tr>
<td><em>Acasia chundra</em></td>
<td>Kaippu</td>
<td>Red cutch Solid extract</td>
<td>10</td>
</tr>
<tr>
<td><em>Mesua ferrea</em></td>
<td>Namal renu</td>
<td>Ceylon Iron Wood Stamen</td>
<td>10</td>
</tr>
<tr>
<td><em>Piper betel</em></td>
<td>Mala bulath</td>
<td>Betel Stem/ Leaves</td>
<td>10</td>
</tr>
<tr>
<td><em>Vitex nigundo</em></td>
<td>Nika</td>
<td>Indian privet Leaves</td>
<td>10</td>
</tr>
</tbody>
</table>
3.3.2. Determination of pH value:

The pH meter was calibrated using buffers of pH valued 4, 9 and 7. Control samples and commercial samples of Seetharama Watee and Maha Varthikava Watee were finely powdered separately using mortar and pestle. 1g from each sample, was weighed dissolved in 10 mL of de-mineralized water, and kept for 15 minutes (Meena et al., 2010 e). The electrode of the pH meter (WTW-PH 522) was immersed in the solution and the pH reading was taken (Anon, 2008; Soni et al., 2010). Repeated the test for three, four times for each sample.

3.3.3. Determination of Specific Gravity (Specific Gravity Bottle Method):

Clean, dry specific gravity bottles (50 mL) were used. Dry empty specific gravity bottles with lids were weighed to nearest 0.01 g. The bottle was filled with distilled water, closed with lid and stroked off excess from the top with straight edge. Then the bottles were wiped dried and weighed to nearest 0.01 g. Dry empty bottles and lids were weighed again. (Kalia, 2002). The dry SG bottle was filled with pure gingerly oil, closed, wiped, dried and weighed same as earlier. The bottles were washed thoroughly, dried and weighed. Ten pills of Seetharama Watee/ Maha Varthikava Watee from each prepared and commercial samples were selected randomly, put into the specific gravity bottle, then filled with gingerly oil, closed with the lid and weighed again. Readings were taken and calculated to obtain the final values.
3.3.4. Determination of Weight Loss on Drying (Oven-drying Method):

Prepared and commercial samples of Seetharama Water and Maha Varthikava Water were separately powdered using mortar and pestle.

A 10-g of each powdered preparation was weighed using four-digit electronic balance, (OHAUS-PA214), and was put into pre-weighed moisture disc and the lid was closed. It was dried at (105±2)°C in a hot air oven (Electron -1 -20067 Merlino (M I) Italy) for 5 hours and weighed using the same balance. The drying and weighing at one hour intervals was continued until the difference between two successive weighing corresponds to not more than 0.25%. Constant weight is determined when the difference between two constant weight after drying for 30 minutes and cooling for 30 minutes in a dessicator is not more than 0.01 g (Anon, 2008).

\[
\text{Moisture \% w/w} = \frac{\text{Weight loss} \times 100}{\text{Weight of Sample}} \\
\text{(Wet basis)}
\]

3.3.5. Determination of Total Ash Content:

Samples of control and commercial samples of Seetharama Water and Maha Varthikava Water were powdered using mortar and pestle.

Two-g was accurately weighed from each powdered drug, and was incinerated in a silica dish at temperature not exceeding 450 °C until free from carbon. It was then cooled in desiccators and weighed. If a carbon-free ash cannot be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on ash less filter paper, incinerated the residue and filter paper add the filtrate evaporate to dryness and ignited at a temperature not exceeding 450 °C in a Muffle Furnas (Heruaeus - Electric M 104 Netherlands). Calculated the percentage of ash with reference to air dried drug. This procedure was repeated for three times (Anon, 2008).
Ash % w/w = \( \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \)

3.3.6. Determination of Acid Insoluble Ash Content:

To the crucible containing total ash, added 25 mL diluted HCl (6N). The insoluble matter collected on an ash less filter paper, (Whatman 41) and washed with hot water until the filtrate is neutral. Transferred the filter paper containing the insoluble matter to original crucible dried on a hot plate and ignited at a temperature not exceeding 450 °C (Heruaeus -Electric M 104 Netherlands) to a constant weight. Allowed the residue to cool in a suitable dessicator for 30 minutes and weighed (Anon, 2008).

Acid insoluble ash % w/w = \( \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100 \)

3.3.7. Determination of Crude Fiber Content:

Weighed 2 g of powdered drug sample, accurately and extracted with petroleum ether (40–60) °C for de-fatting. Then 200 mL of 1.25% sulfuric acid was added to the extracted drug and the whole mixture was boiled for 30 minutes under reflux in 1 L Erlenmeyer flask. The mixture was then filtered through whatman 52 filter paper using a Buchner funnel and the residue was washed with boiling water, until free of acid. The entire residue was rinsed back into the flask with 200 mL of boiling 1.25% sodium hydroxide solution and again boiled under reflux for 30 minutes. The liquid is then quickly filtered through a rapid hardened filter paper and the residue on the filter paper was washed with boiling water until neutral, which was then dried at 110 °C in a hot air oven (Electron -I -20067 Merlino (M I) Italy) until it reached a constant
weight. Afterwards it was incinerated, until again it reached a constant weight. The difference between the weight of the dried residue and that of the incinerated residue represent the weight of the crude fiber. It was expressed as the percentage of the original weight of material.

Three controls and five commercial of the both preparations were analyzed for fiber by this method (Kalia, 2002; Ramasamy, 2000).

Crude fibre % w/w = \( \frac{\text{Loss in weight on incineration}}{\text{Weight of sample before de-fatting}} \times 100 \)

3.3.8. Determination of Disintegration Time:

One pill was introduced into each tube and added a disc to each tube. The assembly was suspended in water in a 1000- mL beaker. The volume of water was such that the wire mesh as its highest point is at least 25 mm below the surface of the water, and its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at (37\(\pm\)2) °C. Noted down the time required for all the pills to disintegrate and pass through wire mesh (Manesty disintegration tester MK 4) (Soni, 2010; Anon, 2008).

3.3.9. Determination of Hardness:

Manual hardness tester was used for the purpose. Six pills were selected at random from each sample. The pill was placed between spindle and anvil of the tester and the calibrated length adjusted to zero. The knob was then screwed to apply a diametric compression force on the tablet/ pill and the position of calibrated length at which the tablet just broke was recorded in \(\text{kg/cm}^2\) units (Shankar Monsanto type tablet hardness tester) (Ibezi et. al, 2008).
3.3.10. Determination of Friability:

Twenty pills from each sample of Seetharama Watee and Maha Varthikava Watee were taken and placed on a sieve no.1000; any loose dust was moved with the aid of a soft brush. The samples were separately weighed and the pills were placed in the drum of tablet friability apparatus. The drum was rotated 100 times and the tablets were removed. Any loose dust was removed from the pills as earlier. If no pills were cracked, split or broken, the tablets were weighed to the nearest milligram (Copley friability tester FR 2000). Generally, the test runs once. If the results were in drought, or if the mass loss is greater than 1% repeated the test twice and determined the mean of three tests. A maximum loss of 1% of the mass of the pills tested was considered to be accepted for most products.

The friability was expressed as the loss of mass and it was calculated as percentage of the initial mass (Anon, 2008).

3.3.11. Sequential Extractions:

3.3.11.1. Preparation of hexane extracts:

A 100- mL round bottom flask was placed in a dry air oven, cooled in a desiccator, and weighed. A 5- g of air dried coarsely powdered Seetharama / Maha Varthikava Watee was placed in a thimble made by filter paper (Whatman 02) and extraction carried out by Soxhlet apparatus using the pre weighed 100- mL round bottom flask with 70 mL of hexane for 3 hours. Heating mantel, controlled below to 60 °C was used for heating purpose and horizontal condenser for cooling of the Soxhlet apparatus. After three hours, the extract was taken out and evaporated the solvent by using a rotavaporator (Buchi R-114) to dryness. Then the extract was dried at 60 °C to a constant weight and weighed, the percentage of yield (w/w) was calculated (Shivhare et.al, 2010).
3.3.11.2. Preparation of dichloro methane extracts:
The thimble with the drug residue obtained after hexane extraction was dried in a hot air oven of 60 °C to remove any residual hexane. Another 100- mL round bottom flask were weighed after drying in a hot air oven and cooling in a desiccator. The same thimble was introduced into the Soxhlet apparatus and extracted with 70 mL of dichloromethane for three hours following the same procedure. After extraction, the solvent was evaporated using a rota-evaporator, and dried at 60 °C and weighed until it reached to a constant weight.

3.3.11.3. Preparation of ethyl acetate extracts:
Same thimble containing the drug residue after Dichloro methane extraction was dried in the hot air oven as before. (3.11.2.) Another new round bottom flask of 100 mL was weighed, dried and cooled. The dried thimble with the residue was introduced into Soxhlet apparatus and extracted using 70 mL of ethyl acetate for three hours. After extraction, the solvent was evaporated and then steps described earlier were followed. until it reached to a constant weight.

3.3.11.4. Preparation of methanol extracts:
The thimble with the drug residue remained after three solvent extractions was dried in the hot air oven as described before. Another new round bottom flask of 100 mL was weighed, dried and cooled. The same thimble was introduced to a Soxhlet apparatus, extracted using 70 mL of methanol for three hours, and followed the same steps mentioned in earlier extractions.

3.3.12. Thin Layer Chromatography (TLC):
Extractions of the main raw materials of these Seetharama and Maha Varthikava preparations, namely Allium sativum, Pteracopus santalinus, Ferula assa-foetida.
Acorus callumus, Ocimum sanctum, Azadirachta indica, Zingiber officinale, Piper betel and Citrus aurantium used for TLC finger prints. A 5-g from each ingredient sample were weighed and extracted with 70 mL of 95% ethanol in the Soxhlet apparatus for three hours as mentioned in 3.11.1.

The solvent ethanol was evaporated using rota-evaporator (Buchi R-114) at the temperature not exceeding 60 °C. The residue was weighed and dissolved again in 10 mL of 95% ethanol to make a solution with known concentration (Pattanaya et. al, 2010; Yuen & Lau-Cam, 1985).

Prepared and commercial samples of Seetharama Watee and Maha Varthikava Watee were powdered, weighed (5 g), put into thimbles and extracted using 70 mL of 95% ethanol in Soxhlet apparatus (ex 24/29) using the same procedures (Shah V.K. et. al, 2010). The solvent of extraction was evaporated and dissolved the residue in 10 mL of 95% ethanol.

For the TLC fingerprints of sequential extracts of the prepared and the commercial samples of Seetharama Watee and Maha Varthikava Watee, the hexane (3.3.11.1), dichloromethane (3.3.11.2), ethyl acetate (3.3.11.3.) and methanol (3.3.11.4) extracts were used. The all sixteen extract residues were dissolved in respective solvents and made up to the volume of 10 mL (Jeganathan, 2008; Harbourn, 1976).

The final 10 mL extracts was spotted on pre coated 20 x10 cm silica gel aluminum plate 60 F254 0.25 mm (E. Merck Germany) using 10 µL disposable micropipette (Wiretrol II-21-1155 USA). The silica on the plates were activated at 60 °C for 5 min. prior to spotting. The samples, in the form of spots diameter less than 1 mm, were spotted 15 mm from bottom edge, 10-15 mm apart and 20 mm away, from left and right edges of the plate. TLC plates were dried under heated air with the help of an air drier and
developed to a distance of about 8 cm in a tank that had been equilibrated with the solvent system for at least 30 minutes. Then the plates were developed with different solvent systems after the marking the solvent fronts, the plates were removed from the chromatographic tanks and allowed to air dry inside a hood. Spots were visualized under normal day light, short and long wavelength ultra violet lights or by exposing the plates to iodine vapors in a closed chamber or/ and sprayed using a suitable spray reagents, such as anysaldehyde sulfuric reagent and vanillin sulfuric acid . (Appendix III C). After spraying, the plates were heated at 105 °C until spots appeared (Meena et. al, 2010; Thirunavukkarasu et. al. 2010; Saraswatthy et.al . 2007). Rf value of each spot was calculated.

![Figure-3.1-TLC spotting](image1)

**Figure-3.1-TLC spotting**

**diagram of Seetharama**

*Seetharama-1S₀-S₅*

Rm- Raw material

![Figure -3.2 -TLC spotting](image2)

**Figure -3.2 -TLC spotting**

**diagram of Maha Varthikava**

*Maha Varthikava- 1V₀-V₅*

Rm- Raw material
Following solvent systems were attempted in the TLC analysis:

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Solvent in the mixture</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n-hexane: ethyl acetate</td>
<td>1:1 v/v</td>
</tr>
<tr>
<td>2.</td>
<td>toluene: ethyl acetate</td>
<td>9:1 v/v</td>
</tr>
<tr>
<td>3.</td>
<td>toluene: ethyl acetate: formic acid</td>
<td>(14:8:0.15/7:3:0.3) v/v</td>
</tr>
<tr>
<td>4.</td>
<td>n-butanol: propanol: acetic acid: water</td>
<td>(60:20:20:20) v/v</td>
</tr>
<tr>
<td>5.</td>
<td>benzene: acetone</td>
<td>(50:70) v/v</td>
</tr>
<tr>
<td>6.</td>
<td>n-hexane: acetone</td>
<td>(75:25) v/v</td>
</tr>
<tr>
<td>7.</td>
<td>benzene: ethyl acetate</td>
<td>(90:10) v/v</td>
</tr>
<tr>
<td>8.</td>
<td>ethyl acetate: water: acetic acid</td>
<td>(80:10:10) v/v</td>
</tr>
</tbody>
</table>

(Anon, 2008a; Rajpal, 2005, 2008; Guptha et al., 2003, 2005, 2006, 2008)

3.3.13. UV Spectrophotometry:

A 5-g sample of *Seetharama Watee/ Maha Varthikava Watee* was weighed out into a small thimble and the thimble was inserted into a small Soxhlet apparatus (ex 24/29). The lower end of the apparatus was connected to 100 mL round bottom flask containing 70 mL of 95% ethanol. A vertical double phase condenser was connected to the top end of the Soxhlet apparatus. The flask was placed on a heating mantel (Microsil India) and extraction carried out for three hours. The ethanol extracted sample was concentrated to 10 mL using the rota-evaporator. A volume of 0.5 mL was taken from this 10 mL ethanol extract and was diluted to 5 mL using the same solvent 95% ethanol. Same procedure was followed for extraction from raw materials.

Samples were scanned over range of 200-500 nm using UV mini1240 (Shimadzu) spectrophotometer using the quartz cuvettes of 10 mm path length. 95% ethanol was
used, as reference (Shaila et al., 2004). It is common in UV-Vis spectrophotometry to measure absorbance verses wavelength in nanometers (Roger, 1982).

3.3.14. High Performance Liquid Chromatography (HPLC):

A 0.5 g of *Seetharama Watee* / *Maha Varthikava Watee* were weighed and extracted using 95% ethanol in the Soxhlet apparatus. Extractions were done as in the procedures mentioned in TLC procedure (3.3.12). Solvents of the extracts were completely evaporated using rota-evaporator and the residues were dissolved in 10 mL HPLC grade methanol prior to HPLC analysis. The mixture was diluted with HPLC grade methanol and vortexed for 30 seconds. Before injecting in to the HPLC it was filtered through, 0.45 µm Teflon micro filters (Jia et al., 2006).

The details of the HPLC instrument and the operating adjustments:

Instrument - Simadzu HPLC

Pump - LC-10 AD

UV/vis. detector - SPD-10 AV

Printer - CR 6A Chromatopack

Mobile Phase - acetonitrile: water (2:3 v/v)

Column - C-18 Hypersil ODS column (250×4.6 µm ID, 5 µm particle) (Sigma Aldrich)

with a compatible guard column was used.

Flow rate - 1.0 mL/min

Injection Volume - 25 µL → 20 µL (Hamilton 1702 RNR 25 µl Syringe)

Wavelength - 254 nm

Temperature - Room temperature (Aeri et al., 2010; Chen et al., 2008; Wei et al. 2007)
Preparation of mobile phase solvent system:

Measured HPLC grade acetonitrile 200 mL, de-mineralized water 300 mL (Bharman Aqua DM 600 de-mineralized water plant) were mixed thoroughly to give acetonitrile : water (2:3 v/v) solution and filtered through 0.45 μL and 0.22 μL micro filters respectively. Then the solution was degassed using degasser (Branson 1210) for 30 minutes before using in the HPLC.

Meanwhile, methanol: water (2:3 v/v) mobile phase and wavelengths of 220, 270 and 330 nm were attempted to find out the best solvent system and the wavelength.

3.3.15. Heavy Metal Detection: (AOAC method 986.15 As, 977.15, 977.21 Hg, 999.10 Pb & Cd)

The controls and commercial samples of Seetharama Watee/ Maha Varthikava Watee were analyzed for the presence of Pb, Cd, As and Hg using atomic absorbance spectrophotometer (AAS).

3.3.15.1. Preparation of samples:

3.3.15.1.1. Dry-ashing technique:

0.5 g of powdered Seetharama Watee/ Maha Varthikava Watee sample was weighed and was put into pre weighed dry cooled crucible. It was gently put over an incinerator until the sample was charred. Then the crucible was tranferred to a muffle furnace not exceeding 550 °C and it was kept a side untill white or light grey colour appeared. If the residue was black in colour, it was moistened with a small amount of water, dried over a water bath and repeated the ashing procedure. The crucible was cooled in a desiccator and weighed until constant weight was obtained (Reilly, 1980; Korn et. al., 2008; Wickramasingha, 2009). Ten drops of conc. HCl were added into crucible, which was then stirred with a glass rod, and the sample was desolved in 10 mL of distilled water.
The sample was transferred into 50- mL volumetric flask and made up to the mark by distilled water. The sample was nebulized to AAS and the readings were taken.

3.3.15.1.2. Wet-ashing technique:

Wet digestion method includes sample decomposition by an acid or mixtures of acids. Acid for digestion was prepared by combining equal volumes of 70% H₂SO₄ and 70% HNO₃ acids. 0.5±0.05 g of sample was weighed into each tarred digestion tube on a balance (± 0.001 g). After charring for 10 minutes with 1 mL cone. Sulfuric acid, and 10 mL of digestion acid were added. Tubes were capped and placed on digestion turntable. A reagent blank was also prepared and digested for 45 minutes in the microwave oven (CEM MARS 240/250- ramp time was 15 min. ramp temperature 105 °C holding time 30 min) in digestion, tubes were at elevated pressure with microwave heating. After digestion tubes were cooled 10-15 minutes in a fume hood, uncapped and swirled until the vapor ceased. Added distilled water 20 mL to the digested solution, rinsed the inner wall and lid thoroughly. Samples and blank were quantitatively transferred by filtering Whatman paper No. 1 into 100- mL volumetric flasks, and diluted to volume with distilled water (Sapp and Davidson, 1991).

3.3.15.2. Preparation of the set of standard solutions:

Actual concentration of metal sample= ppm reading x Dilution factor

\[
\text{Dilution factor} = \frac{\text{Volume of digest used}}{\text{Weight of sample digested}} \quad \text{(Olaifa et. al, 2004)}
\]
Table 3.4. Measurements made for hollow cathode lamp

<table>
<thead>
<tr>
<th>Element</th>
<th>Slit width/ nm</th>
<th>Wave length/ nm</th>
<th>Reducing agent</th>
<th>Lamp curr. mA</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.7</td>
<td>283.3</td>
<td></td>
<td>5</td>
<td>2.5,0,15,20 µg/L</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.7</td>
<td>228.8</td>
<td></td>
<td>4</td>
<td>0.2,0.8,1.2,1. 8 µg/L</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.5</td>
<td>193.7</td>
<td>1.1 % w/v SnCl₂</td>
<td>10</td>
<td>5,10,20 µg/L</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.5</td>
<td>253.7</td>
<td>0.2 % w/v NaBH₄</td>
<td>4</td>
<td>10,20,40,80 µg/L</td>
</tr>
</tbody>
</table>

Cadmium and lead analysis were done by using dry ashing method followed by flame atomic absorption spectrophotometer with airacetylene fuel-oxidant combination which produce a flame temperature of 2400-2700 k (FASS) (GBC 906 AA). The Hg analysis was done by using microwave digester (CEM Mars 240/250) followed by hydride generation.

Atomic absorption spectrophotometer (HG-AAS) (Varian Spectra AA 220; VGA 77). As analysis was done by using Inductivity Couple Plasma Unit (Varian ICP-OES Spectrometer 720-ES).

Calculations-

\[
\text{Conc. of As,Cd, Pb,Hg in sample solution (µg/L) x sample volume (mL)}
\]

\[
\text{Sample weight (g) x 1000}
\]
3.3.16. Microbiological Analysis:

3.3.16.1. IMViC test:  (Microbiology lab manual - Experiment 25)

0.5 g of Seetharama Watee and Maha Varthikava Watee from each sample was weighed and dissolved in 10 mL of distilled water. They were labeled as 1S₀, 2S₀, 3S₀, S₁, S₂, S₃, S₄, S₅, 1V₀, 2V₀, 3V₀, V₁, V₂, V₃, V₄, V₅ as in the earlier steps.

Tryptophan broth was used as the medium.

3.3.16.1.1 Indole test:

A sample of 10 mL of tryptophan broth filled into 54 test tubes and sterilized in an autoclave 121 °C for 20 minutes. Before sterilization, the tubes were covered with pure cotton wool and aluminum foil. After taken out the tubes from the autoclave was kept until cooled, then was inoculated by an inoculation loop using all 16 samples. Three sample tubes were prepared from each preparation and three from controls. They were kept in an incubator at 37 °C for 48 hours. Following incubation, 5 drops of Kovac’s reagent was added to the culture broth.

After two minutes, a positive result was shown by the presence of a red or red-violet colour band at the junction of medium and reagent layer of the broth. A negative result appeared yellow (Sherman, 1998).

3.3.16.1.2. Methyl Red test:

Same as earlier step (3.3.16.1.1) 54 tubes were filled (10 mL) using MR-VP broth and sterilized as in earlier tests. They were inoculated by inoculation loop using all control and commercial samples. After incubation for 48 hours, the pH indicator methyl red was added to broth. Red colour of methyl red is red at pH below 4.4 taken as positive. An orange colour indicates an intermediate pH and was considered as negative (Sherman, 1998).
3.3.16.1.3 Vogues Proskauer test:

Same as in step 3.3.16.1., 54 tubes were filled (10 mL) using MR-VP broth and sterilized as in earlier tests. They were inoculated by inoculation loop using all control and commercial samples. After incubation for 48 hours, Barrit's reagent A and B (Appendix III) were added to each medium with shaking and after 15 minutes, the colour changes that occurred in each tube were observed and noted down. The positive reaction was showed a maroon colour band at the top of the broth in the tube, which was diffused over time into the rest of the media. The negative reaction no maroon colour band formed in tube after addition of Barrit's reagent A and B (Sherman, 1998).

3.3.16.1.4. Citrate test:

A 10- mL aliquot of Simmon's citrate agar medium was added in each of the 54 test tubes and autoclaved as in the previous steps. Then prepared slants from all the tubes and inoculated by stabbing using the loop to the base of the slant. Then after streaking the surface, kept the tubes at 37° C for 48 hours for incubation. If culture grows, blue colour was developed due to change of pH of medium, as it becomes alkaline following citrate utilization. The unutilized citrate indicates no colour changes due to any visible growth in the tube (Sherman, 1998).

3.3.16.2. Preparation method of dilution series of samples:

One g of each sample of powdered Seetharama Water/ Maha Varthikava Water was transferred into a tube containing 10 mL sterilized distilled water of pH 7 and was then mechanically homogenized at constant speed for 15 minutes on an electric stirrer (Microsil-India). The sample water suspension was allowed to stand for 10 minutes with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10)
were prepared and 1-mL aliquot of each dilution was used for pourplate technique. The procedure was repeated and made dilution series using sterilized distilled water, mixed with vortex mixture and labeled as $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilution series (Anon, 2008).

3.3.16.3. Preparation method of agar plates:

Forty g of glucose hydrate, 10 g of dried peptone and 15 g of agar were weighed to a conical flask and dissolved in sufficient distilled water to produce 1000 mL (Sabouraud glucose agar). The conical flask was closed with a cotton plug, covered with an aluminum foil and autoclaved at 121 °C for 15 minutes. To prevent the bacterial growth chloramphenicol 0.05 mg/mL was added (Bokhari and Aly, 2009; Anon, 2007) to the liquefied agar mixture. Used, washed and dried 9-10 cm diameter Petri dishes, were sterilized in a hot air oven (Herus) at 180°C for 45 minutes after wrapping with aluminum foil.

About 15 to 20 mL of liquefied agar was poured into Petri dishes after cooling in a desiccator. One mL aliquot of each dilution was added to these agar plates, which were not more than 45 °C. After closing the petri dishes, the sample was mixed thoroughly with agar by tilting or rotating the dishes, and allowed the contents to solidify at room temperature. Three replicates were prepared for each sample. The Petri dishes were inverted and incubated as $(28\pm2)$ °C for 7 days in an incubator (Deluxe automatic BOD incubator NSW 152 Microsil- India) kept upside down and examined daily. The total counts were recorded only after 4-5 days. Unless a reliable count was obtained selected the plates corresponding to one dilution and showing the highest number of colonies less than 100 colonies. Morphologically different mould colonies were individually sub cultured by hypha tip method on to Sabouraud glucose agar medium (Singh et. al, 2008;
Bugno et al., 2006; Anon, 1998; Coomaraswamy and Fonseka, 1981; Coomraswamy 1979).

3.3.16.4. Calculation of colony forming units:

After incubation, the plates were examined visually and under a microscope. The arithmetic average of counts were taken and calculated the number of colonies forming units (CFU) per gram.

\[
\text{CFU/g} = \frac{\text{CFU/plate} \times \text{dilution factor} \times \frac{1}{\text{aliquot}}}{n_1 + 0.1 \times n_2 \times d}
\]

\[N = \frac{\Sigma C}{n_1 + 0.1 \times n_2 \times d}\]

\(N = \text{Number of colonies per gram of product}\)

\(\Sigma C = \text{Sum of all colonies on all plates counted}\)

\(n_1 = \text{Number of plates in first dilution counted}\)

\(n_2 = \text{Number of plates in second dilution counted}\)

\(d = \text{Dilution from which first count were obtained} \quad (\text{Maturin and peeler, 1998})\)

Microbial analysis was carried for \textit{Water} as per procedures of Indian Pharmacopoeia 2007 and WHO guidelines.

3.3.16.5. Calculation of growth on solid media:

Two solid media Czapeck-Dox agar and malt extract agar plates were used. 20 mL of autoclaved solid media were poured into sterilized Petri plates. A 4 mm actively grown mycelia of selected fungi, a disc was cut using a cork borer and placed individually in the center of respective medium. Eight fungi were selected and they were labeled as A, B, C, D, E, F, G, and H. The inoculated plates were incubated at 28 °C for 7 days. Three replicates were maintained. The diameter of the fungal colony was measured (Senthamizhelselvan, 2010; Samson, et al, 2007).
3.3.16.6. Identification of fungi in Seetharama Watee and Maha Varthikava Watee:

The upper side and underside colours of these selected 8 fungi grown in the Czapeck Dox agar media and malt extract agar were notified (Jernjejc and Cimerman, 2001).

3.3.17. Detection of aflatoxin:

AOAC method (968.22, 978.15, and 975.37) was used for the detection of aflatoxin (Anon. 2008).

3.3.17.1. TLC Method:

3.3.17.1.1. Sample preparation:

The sample was powdered using mortar and pestle. Ten g of powdered sample was weighed into Stopped 100 mL Erlenmeyer flask. Chloroform 50 mL and 5 mL of deionized water were added into the flask, covered with cotton wool and aluminum foil and stirred well with magnetic stirrer at room temperature for 30 minutes. The solution was filtered and the filtrate was made up to 50 mL with chloroform, anhydrous sodium sulphate was added and left for 15 minutes. The filtered solution was used for clean up procedure.

3.3.17.1.2. Clean up procedure:

A glass column of 20 cm long and 1 cm internal diameter was used. The distal end of the column was plugged with a piece of cotton wool and packed with 3 g of anhydrous sodium sulphate. Then, the column was packed with activated silica gel 60-120 (E. Merck Germany) (drying 1 hour 105 °C and stored more than 15 hours in airtight container) using CHCl₃ as solvent. Five mL of extracted filtrate was introduced into the column and eluted. Then 30 mL of n-hexane and 30 mL of diethyl ether were passed successively and these fractions were discarded. Finally the 30 mL of solvent mixture of
methanol: chloroform (3:97 v/v) used for elution and this fraction was collected into round bottom flask and solvent was evaporated in a rota evaporator (Buchi type R-114) at 50 °C and used for TLC and HPLC analysis (Stubblefield et. al, 1982).

3.3.17.1.3. TLC fingerprints:
The residue was dissolved in 0.5 mL of chloroform and used for spotting. Spotting of 20 μL sample was done using a graduated capillary tube, (Wiretrol II micropipette) of lesser than 5 mm diameter. The sample and aflatoxin standards were spotted in different lines in one another. TLC plates were developed using the solvent system, water: acetone: chloroform (1.5:12:88 v/v) and observed under UV lamp 354 nm wavelength.

3.3.17.1.4. Solvent system for two-dimensional TLC

1) chloroform: acetone (90:10 v/v)

2) toluene: ethyl acetate: 90% formic acid (5:4:1 v/v)

1\textsuperscript{st} solvent system was run first then the 2\textsuperscript{nd} solvent was run in the right angle.

- Figure 3.3-Method of sample spotting in 2 dimensional TLC
3.3.17.1.5. Aflatoxin confirmatory method:

Thin layer plates with separated aflatoxin spots were sprayed with 25% sulfuric acid (Dashe et al. 1983; Velasco, 1981; Paranagama et al. 2003) as confirmatory test.

3.3.17.2. HPLC Method:

3.3.17.2.1. Sample derivatisation:

n- hexane 200 μL was added to the cleaned up dry extracted sample and was shaken in Vortex stirrer for 30 seconds. Secondly, 25 μL of trifluoro acetic acid was added and shaken for another 30 seconds using same stirrer. Finally mixture of deionized water: acetonitrile (9:1 v/v) 200 μL were added and shaken well and allowed to stand until the layers separated. The bottom layer was used for HPLC analysis.

3.3.17.2.2. Sample application:

The details of the HPLC instrument and operation adjustments are as follows.

Mobile phase- water: acetonitrile: methanol (280:80:30 v/v)

Detector - Fluorescence RF 10AXL

Excitation wavelength- 364 nm.

Emission wavelength- 445 nm.

Column - C18 μ bondapack

Flow rate- 1mL/min.

Inject volume - 20 μL

Printer- CR6A Chromatopack

Standards strengths- G₁- 1.100 ng/20 μL  
B₁- 0.930 ng/20 μL  
G₂- 0.280 ng/20 μL  
B₂- 0.301 ng/20 μL
Derivatised aflatoxin standard 20 µL (using Hamilton 705 NR 50 µl) was injected 3 times to the HPLC and separated peaks can be identified as G₁ (G₂a), B₁ (B₂a), G₂ and B₂ in order of retention time ranging from 3-30 minutes.

### 3.3.18. Data Analysis:

SPSS 15 package was used in analysis of data. One way ANOVA statistical technique was used for the analysis of data followed by Dunnett t test to compare with the formulated prepared samples. The prepared samples of Seetharama Watee (1S₀, 2S₀, 3S₀) and Maha Varthikava Watee (1V₀, 2V₀, 3V₀) were considered as one sample and labeled as S₀ and V₀ for the data analysis.

P value ≤ 0.05 was considered as the level of significance.

When the range of values was suggested at the 95% confident level, upper bound or lower bound values were considered for the prepared samples. When the commercial samples were considered in the range and that was the P value ≥ 0.05, the true values were taken.

Microsoft excel 2007 package was used for the chart preparations.
Chapter 4

4. Results and Discussion:

To date only a few research has been carried out on the herbo-mineral preparation, Seetharama Watee and no published literature on the poly herbal preparation Maha Varthikava Watce are available. In this study, physico-chemical analysis, phytochemical, spectrophotometric analysis, residue analysis, and microbiological screening were used as the parameters for quality assessment of these two medicinal preparations and thereby to establish procedures for standardization of these two drugs.

Seetharama Watee labeled as S_o (1S_o, 2S_o, 3S_o) for prepared samples and S_1- S_5 for commercial samples. Maha Varthikava Watce labeled as V_0 (1V_0, 2V_0, 3V_0) for Prepared Samples and V_1- V_5 for commercial samples.

4.1. Variation in Weight:

According to the guidelines given in Watika Prakaranaya the size or volume of the pill must have considered, in the preparation process whereas Sri Lankan Ayurveda pharmacopeia, emphasis on the the weight of the pill. However, the weight can be changed due to the diversity of the density of the raw materials and pills of different batches with the same volume may contain different doses. Therefore, it is necessary to find the best criterion for it, whether it is weight or the volume.
Table 4.1.1. Weight variation of authentically prepared (10 pills) and commercial samples (10 pills) of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (g)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>1.3204(α)</td>
<td>±0.043</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>1.4384(α)</td>
<td>±0.242</td>
<td>0.785</td>
</tr>
<tr>
<td>S₂</td>
<td>0.7282(β)</td>
<td>±0.004</td>
<td>0.000</td>
</tr>
<tr>
<td>S₃</td>
<td>0.7522(β)</td>
<td>±0.112</td>
<td>0.000</td>
</tr>
<tr>
<td>S₄</td>
<td>2.3495(β)</td>
<td>±0.004</td>
<td>0.000</td>
</tr>
<tr>
<td>S₅</td>
<td>1.5037(α)</td>
<td>±0.007</td>
<td>0.403</td>
</tr>
</tbody>
</table>

When compared the weight of the prepared sample of Seetharama Watee S₀ with those of the commercial samples, S₁ and S₅ are the same, while the S₂, S₃, S₄ are different at the significant level of 0.05. The weight of prepared as well as the commercial Seetharama pills (n=10) varies from 0.7282±0.004 and 2.3495±0.004 g.

Rodrigo and Samarathunga (2008) reported that the weights of all samples of tested Seetharama Watee were different from the weights of the samples produced according to Ayurveda pharmacopeia. Pills of equal size can be different in weight due to the

---

1 number of prepared samples
2 number of commercial samples
a,b,c significant different among columns denoted by different superscripts (p > 0.05)
Table 4.1.2. Weight variation of authentically prepared (10 pills) and commercial samples (10 pills) of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (g)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0</td>
<td>1.1879±0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>0.9334±0.008</td>
<td></td>
<td>0.034</td>
</tr>
<tr>
<td>V2</td>
<td>2.3339±0.005</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>V3</td>
<td>0.8104±0.030</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>V4</td>
<td>0.9436±0.027</td>
<td></td>
<td>0.043</td>
</tr>
<tr>
<td>V5</td>
<td>1.4344±0.018</td>
<td></td>
<td>0.041</td>
</tr>
</tbody>
</table>

All the V1, V2, V3, V4 and V5 samples are different from the formulated sample of Maha Varthikava Watee V0, at the significant level of 0.05. The weight of Maha Varthikava pills (n=10) varies between 0.8104±0.03 and 2.333±0.005 g.

There were no previous reports on weight comparison studies on Maha Varthikava Watee in the literature.

Savarikar et al., (2011) reported that the manually prepared tablets exhibited more variation than tablets prepared by direct compression.

According to the our results (Table 4.1.1 and 4.1.2), there are weight variation in three preparations in Seetharama Watee while only two preparations are equal and in the case of Maha Varthikava Watee all the commercial sample are different in weight compared the prepared preparations.
According to our results (Table 4.1.1 and 4.1.2), there are weight variation in three preparations in Seetharama Watee while only two preparations are equal and in the case of Maha Varthikava Watee all the commercial sample are different in weight compared the prepared preparations.

Moisture content, compactness of the materials and the density of the raw materials can be the causes for the observed variation of weight.

Therefore, according to our results, both the weight and the size of the pills must be used as the parameters for the standardization of both medicinal preparations.

At the 95% confidence level, weight range of a pill could be suggested as about 122-150 mg and about 104-133 mg for Seetharama and Maha Varthikava respectively.

Although the sizes of two pills are the same (size of green gram fruit), the weights are different due to the density variation. Herbo mineral preparation Seetharama is heavier than the poly herbal preparation Maha Varthikava.

4.2. pH value: (10% aqueous solutions)

The pH plays an important role in drug receptor site interactions and has gained considerable impetus. Research on this area is very important because acidic pH, inhibits microbial activity. The interrelation between pH, acid type, and acid concentration may contribute to either negative or positive effects of organic acids on microorganisms. The distinctive characteristics of different species of microorganisms may also be a factor that affects microbial growth and activity in acidic microenvironments (Cheung, 2008).
Table 4.2.1. pH values of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean pH&lt;sup&gt;c3&lt;/sup&gt;</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>5.37&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>±0.155</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>5.89&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>±0.179</td>
<td>0.252</td>
</tr>
<tr>
<td>S₂</td>
<td>4.76&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>±0.286</td>
<td>0.144</td>
</tr>
<tr>
<td>S₃</td>
<td>4.75&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>±0.27</td>
<td>0.131</td>
</tr>
<tr>
<td>S₄</td>
<td>4.59&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>±0.021</td>
<td>0.041</td>
</tr>
<tr>
<td>S₅</td>
<td>3.35&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>±0.054</td>
<td>0.000</td>
</tr>
</tbody>
</table>

n_<sub>p</sub> = 9  n_<sub>c</sub> = 3

A comparison study of commercial samples of Seetharama Watee shows no significant difference among S₁, S₂ and S₃ and the S₀ although S₄ and S₅ are different at the significant level of 0.05. The pH of Seetharama pill varies from 3.35±0.054 and 5.89±0.179.

<sup>c3</sup> - 10% aqueous solutions (w/v) used at room temperature

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Table 4.2.2. pH values of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean pHc</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>4.57 (a)</td>
<td>±0.074</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>4.14 (a)</td>
<td>±0.127</td>
<td>0.079</td>
</tr>
<tr>
<td>V₂</td>
<td>4.43 (a)</td>
<td>±0.02</td>
<td>0.916</td>
</tr>
<tr>
<td>V₃</td>
<td>4.27 (a)</td>
<td>±0.219</td>
<td>0.324</td>
</tr>
<tr>
<td>V₄</td>
<td>3.74 (b)</td>
<td>±0.055</td>
<td>0.000</td>
</tr>
<tr>
<td>V₅</td>
<td>3.94 (b)</td>
<td>±0.1</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The commercial samples V₁, V₂ and V₃ are not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₄ and V₅ samples are different. The pH of Maha Varthikava pill varies between 3.74±0.055 and 4.57±0.074.

All Seetharama Watee and Maha Varthikava Watee gives acidic pH. However, prepared samples of both drugs are in low acidic level when compared to the commercial samples.

Reference to the research findings both formulations are low acidic. This parameter can also be used for the standardization of these two preparations.

The pH range could be suggested as 4.75 - 5.89 and 4.14 - 4.74 for Seetharama and Maha Varthikava respectively.
4.3. Specific Gravity (SG):

The specific gravity is the ratio of the density (mass of a unit volume) of a substance to the density (mass of the same unit volume) of a reference substance. Specific gravity is commonly used as a simple means of obtaining information about the density. It helps in the determination of minerals in the preparation (Jerkings et al., 2008).

Table 4.3.1. Specific gravity of authentically prepared samples and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean $\text{SG}^{d4}$</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$</td>
<td>1.16$_{(a)}$</td>
<td>±0.010</td>
<td></td>
</tr>
<tr>
<td>$S_1$</td>
<td>1.14$_{(a)}$</td>
<td>±0.025</td>
<td>0.900</td>
</tr>
<tr>
<td>$S_2$</td>
<td>1.25$_{(b)}$</td>
<td>±0.172</td>
<td>0.009</td>
</tr>
<tr>
<td>$S_3$</td>
<td>1.06$_{(b)}$</td>
<td>±0.020</td>
<td>0.003</td>
</tr>
<tr>
<td>$S_4$</td>
<td>1.22$_{(b)}$</td>
<td>±0.029</td>
<td>0.000</td>
</tr>
<tr>
<td>$S_5$</td>
<td>1.09$_{(a)}$</td>
<td>±0.016</td>
<td>0.055</td>
</tr>
</tbody>
</table>

When comparing the prepared sample of Seetharama Watee $S_0$ with the commercial samples, $S_1$ and $S_3$ are not significantly different from the prepared sample $S_0$, while the $S_2$, $S_3$ and $S_4$ are different at the significant level of 0.05. The specific gravity of Seetharama pill varies between 1.06±0.02 and 1.22±0.029.

$d^4$ - measured with reference to distilled water.
Table 4.3.2. Specific gravity of authentically prepared and commercial samples of Maha Varthikava Watec.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean SG&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>1.24±0.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1.09±0.019</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.95±0.016</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.15±0.063</td>
<td>0.492</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.11±0.026</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;5&lt;/sub&gt;</td>
<td>1.11±0.049</td>
<td>0.161</td>
<td></td>
</tr>
</tbody>
</table>

n<sub>p</sub>= 9  n<sub>c</sub>= 3

The commercial samples V<sub>1</sub>, V<sub>3</sub>, V<sub>4</sub> and V<sub>5</sub>, are not significantly different from the prepared sample V<sub>0</sub>, at the significant level of 0.05, while the V<sub>2</sub> sample is different. The specific gravity of Maha Varthikava pill varies between 0.95±0.016 and 1.24±0.035.

Higher SG values indicate that the higher relative density of raw materials and vice versa. Therefore, SG value is a measurement of the usage of proper raw materials.

The SG value range could be suggested as 1.14-1.18 and 1.15-1.31 for Seetharama and Maha Varthikava respectively.

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4.4. Weight loss on Drying:

Weight loss on drying is mainly due to the moisture content, which directly leads to early decay of the product. The mold content of the product also depends on this factor.

Table 4.4.1 Weight loss on drying (% w/w) of authentically prepared and commercial samples of Seetharama Water.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S0</td>
<td>9.251 (a)</td>
<td>±0.970</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>7.193 (a)</td>
<td>±0.574</td>
<td>0.546</td>
</tr>
<tr>
<td>S2</td>
<td>10.85 (a)</td>
<td>±0.482</td>
<td>0.756</td>
</tr>
<tr>
<td>S3</td>
<td>9.203 (a)</td>
<td>±0.921</td>
<td>1.000</td>
</tr>
<tr>
<td>S4</td>
<td>9.306 (a)</td>
<td>±1.010</td>
<td>1.000</td>
</tr>
<tr>
<td>S5</td>
<td>11.11 (a)</td>
<td>±0.113</td>
<td>0.636</td>
</tr>
</tbody>
</table>

n_p = 9, n_c = 3

When comparing the prepared Seetharama Water sample S0 with the commercial samples, all S1, S2, S3, S4 and S5 are not significantly different from the prepared sample S0, at the significant level of 0.05. The weight loss on drying of Seetharama pill varies from 7.19±0.57% w/w to 11.11±0.11% w/w.
Table 4.4.2. Weight loss on drying (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Weight (g)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>12.3 ±0.478</td>
<td>±0.478</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>7.01 ±0.467</td>
<td>±0.467</td>
<td>0.003</td>
</tr>
<tr>
<td>V₂</td>
<td>7.63 ±1.080</td>
<td>±1.080</td>
<td>0.01</td>
</tr>
<tr>
<td>V₃</td>
<td>10.46 ±1.230</td>
<td>±1.230</td>
<td>0.568</td>
</tr>
<tr>
<td>V₄</td>
<td>10.04 ±1.039</td>
<td>±1.039</td>
<td>0.364</td>
</tr>
<tr>
<td>V₅</td>
<td>12.99 ±2.160</td>
<td>±2.160</td>
<td>0.984</td>
</tr>
</tbody>
</table>

n₀ = 9  nₑ = 3

Loss on drying of V₃, V₄ and V₅, samples are not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₁ and V₂ samples are different. The low values of the weight loss on drying of the commercial samples V₁ and V₂, may be due to excess drying in hot air ovens. The loss on drying of Maha Varthikava pill varies between 7.01±0.467% w/w and 12.99±2.16% w/w.

Savarikar et al. (2011) reported that the loss on drying values found to be more for tablets prepared by Ayurvedic methods, whereas the corresponding values were less for directly compressed tablets since all the ingredients were dry and water was not used during direct compression.

Loss on drying value is directly proportional to the weight of pills and directly interferes with the stability of the drugs.

Jayanetti (1987) suggested that loss on drying for Seetharama Watee as 7-7.5% in his unpublished data, which was closely related to the data of present study.
The loss on drying value range could be suggested as 7.2-11.5% w/w and as 10-13.4% w/w for Seetharama and Maha Varthikava respectively.

4.5. Total Ash:

Ash is the total inorganic residue from the incineration of the material. Ash content and its composition depend on the nature of the product (Kirk et al., 1991).

Table 4.5.1. Total ash content (% w/w) of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>10.09±0.587</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>12.66±0.013</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>S₂</td>
<td>16.21±0.585</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>12.35±0.187</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>9.79±0.207</td>
<td>0.997</td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>11.41±0.088</td>
<td>0.402</td>
<td></td>
</tr>
</tbody>
</table>

When comparing the ash content of the prepared sample S₀ with the ash content of commercial samples, there is no difference between S₄ and S₅, while the S₁, S₂ and S₃ are different at the significant level of 0.05. The total ash content of Seetharama pill varies from 9.79±0.207% w/w to 12.66±0.013% w/w.
Table 4.5.2. Total ash content (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>6.87 (a)</td>
<td>±0.166</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>7.42 (a)</td>
<td>±0.006</td>
<td>0.931</td>
</tr>
<tr>
<td>V₂</td>
<td>7.50 (a)</td>
<td>±0.100</td>
<td>0.887</td>
</tr>
<tr>
<td>V₃</td>
<td>3.70 (b)</td>
<td>±1.760</td>
<td>0.987</td>
</tr>
<tr>
<td>V₄</td>
<td>5.93 (a)</td>
<td>±1.760</td>
<td>0.931</td>
</tr>
<tr>
<td>V₅</td>
<td>5.43 (a)</td>
<td>±0.032</td>
<td>0.242</td>
</tr>
</tbody>
</table>

n₀= 9  n_c = 3

Ash values of V₁, V₂, V₄ and V₅, samples are not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₃ sample is different. The total ash content value of Maha Varthikava pill varies between 3.7±1.76% w/w and 7.42±0.006% w/w. Savarikar et al., (2011) reported that the total ash content was low when tablets are prepared by direct compression. Higher ash content level indicates that, the higher levels of inorganic matter. Adulteration of products using inappropriate raw materials can be found using the ash content values.

Jayanetti (1987) suggested that ash content for Seetharama Watee as 10.8-13% w/w in his unpublished data, which also closely related to this present data.

The ash value range could be suggested as 9.7-11.41% w/w and as 5.43-7.50% w/w for Seetharama and Maha Varthikava respectively.
Several minerals were used in the preparation of Seetharama pills. Therefore, it may more ash content than the Maha Varthikava, which was prepared without using any mineral substances.

4.6. Acid Insoluble Ash:

Acid insoluble ash usually consists mainly of silicates, which indicates contamination with earth material (Kirk et.al, 1991).

Table 4.6.1 Acid insoluble ash content (% w/w) of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean(% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>0.05 (a)</td>
<td>±0.029</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>0.03 (a)</td>
<td>±0.006</td>
<td>0.402</td>
</tr>
<tr>
<td>S2</td>
<td>0.03 (a)</td>
<td>±0.001</td>
<td>1.000</td>
</tr>
<tr>
<td>S3</td>
<td>0.07 (a)</td>
<td>±0.037</td>
<td>1.000</td>
</tr>
<tr>
<td>S4</td>
<td>0.05 (a)</td>
<td>±0.011</td>
<td>1.000</td>
</tr>
<tr>
<td>S5</td>
<td>0.31 (a)</td>
<td>±0.307</td>
<td>0.017</td>
</tr>
</tbody>
</table>

np = 9  nc = 3

When comparing the prepared S0 sample with the commercial samples, all S1, S2, S3, S4, and S5 are not significantly different from the prepared sample S0, at the significant level of 0.05. The acid insoluble ash of Seetharama pill varies from 0.03±0.006% w/w and 0.07±0.037% w/w.
Table 4.6.2. Acid insoluble ash content (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean(% w/w)</th>
<th>Standard Weight (g)</th>
<th>Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>0.09 (a)</td>
<td>±0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>0.09 (a)</td>
<td>±0.071</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>V₂</td>
<td>0.02 (a)</td>
<td>±0.008</td>
<td>0.526</td>
<td></td>
</tr>
<tr>
<td>V₃</td>
<td>0.03 (a)</td>
<td>±0.016</td>
<td>0.636</td>
<td></td>
</tr>
<tr>
<td>V₄</td>
<td>0.03 (a)</td>
<td>±0.020</td>
<td>0.685</td>
<td></td>
</tr>
<tr>
<td>V₅</td>
<td>0.01 (a)</td>
<td>±0.006</td>
<td>0.526</td>
<td></td>
</tr>
</tbody>
</table>

n₀ = 9  \ nₑ = 3

All V₁, V₂, V₃, V₄ and V₅, samples also are not significantly different from the prepared sample V₀, at the significant level of 0.05. The acid insoluble ash of Maha Varthikava pill varies between 0.01±0.006% w/w and 0.09±0.071% w/w.

Acid insoluble ash content is directly proportional to the insoluble minerals (e.g. silica) in the finished products.

Jayanetti (1987) suggested that acid insoluble ash content for Seetharama Watee as 1.4-2.2% w/w in his unpublished data, which has higher values when compared to the present data. According to these results, there are high insoluble minerals in the finished products. The acid insoluble ash value range could be suggested as 0.03-0.07% w/w and 0.01-0.09% w/w for Seetharama and Maha Varthikava respectively.

Several acid insoluble minerals are also present in Seetharama, because several mineral substances are used in the production. Therefore, it may contain more acid insoluble ash than Maha Varthikava, which does not contain any mineral substances.
4.7. Crude Fiber Content:

The determination of crude fiber is of considerable importance in the examination of certain drugs, since the commonly used adulterants consist of waste or refuse material derived from the drugs themselves or from other products. Frequently, this material is the outer cellular layer or protective coating, which contains a larger proportion of lignified tissues and consequently, more crude fiber than parts of superficial. (Trease and Evance, 1983)

Table 4.7.1 Crude fiber content (% w/w) of authentically prepared and commercial samples of Seetharama Water.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>7.59±1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>13.4±2.94</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>S₂</td>
<td>23.78±1.07</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>19.16±1.52</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>14.24±1.62</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>9.01±0.67</td>
<td>0.952</td>
<td></td>
</tr>
</tbody>
</table>

When comparing the prepared sample S₀ with the commercial samples, only the S₅ is not significantly different from the prepared sample, while the S₁, S₂, S₃, and S₄ are different at the significant level of 0.05. The crude fiber content of Seetharama pill varies between 7.59±1.01% w/w and 23.78±1.07% w/w.
Table 4.7.2. Crude fiber content (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean (% w/w)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0</td>
<td>8.42 (a)</td>
<td>±1.80</td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>11.40 (a)</td>
<td>±5.02</td>
<td>0.884</td>
</tr>
<tr>
<td>V2</td>
<td>14.46 (a)</td>
<td>±2.46</td>
<td>0.34</td>
</tr>
<tr>
<td>V3</td>
<td>10.25 (a)</td>
<td>±1.14</td>
<td>0.984</td>
</tr>
<tr>
<td>V4</td>
<td>11.40 (a)</td>
<td>±1.21</td>
<td>0.888</td>
</tr>
<tr>
<td>V5</td>
<td>8.87 (a)</td>
<td>±2.02</td>
<td>1.000</td>
</tr>
</tbody>
</table>

n0 = 9   nc = 3

Crude fiber content of all V1, V2, V3, V4 and V5, samples are not significantly different from the control sample V0, at the significant level of 0.05. The crude fiber of Maha Varthikava pill varies between 8.42±1.8% w/w and 14.46±2.46% w/w.

More crude fiber indicates the excess woody material of the products, and may be adulterants.

The crude fiber value range could be suggested as 5.2-9.01% w/w and 4.28-14.46% w/w for Seetharama and Maha Varthikava respectively.

In the preparation of Seetharama both herbal and mineral substance are used while Maha Varthikava used only the herbal material. Therefore, Maha Varthikava contains more fiber content than the Seetharama.
4.8. Disintegration Time:

Disintegration test determines whether the tablets/ pills disintegrate within prescribed time at 37 °C body temperature. According to modern drug pharmacopeias, after swallowing the tablet/ pill it should be dissolved inside the body, within its disintegration time, otherwise the action of the drug can not be expected.

Table 4.8.1 Disintegration time of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Time (min)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>21.78 (a)</td>
<td>±3.61</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>39.33 (a)</td>
<td>±5.21</td>
<td>0.174</td>
</tr>
<tr>
<td>S₂</td>
<td>60.00 (b)</td>
<td>±10.02</td>
<td>0.001</td>
</tr>
<tr>
<td>S₃</td>
<td>32.00 (a)</td>
<td>±3.79</td>
<td>0.659</td>
</tr>
<tr>
<td>S₄</td>
<td>6.00 (a)</td>
<td>±0.58</td>
<td>0.255</td>
</tr>
<tr>
<td>S₅</td>
<td>48.00 (b)</td>
<td>±11.67</td>
<td>0.018</td>
</tr>
</tbody>
</table>

nₚ= 9    nₑ= 3

When comparing the prepared sample S₀ with the commercial samples, S₁, S₃ and S₄ are not significantly different from prepared sample S₀, while the S₂ and S₅ are different at the significant level of 0.05. Although the commercial sample S₄ is not significantly different, it has a very short mean disintegration time. So it may be due to lose compactness of the pill. The disintegration time of Seetharama pill varies from 6±0.577 min. to 60±10.02 min.
Table 4.8.2. Disintegration time of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Time (min)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>30.56 (a)</td>
<td>±2.814</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>20.67 (a)</td>
<td>±2.185</td>
<td>0.177</td>
</tr>
<tr>
<td>V₂</td>
<td>160.00 (b)</td>
<td>±2.88</td>
<td>0.000</td>
</tr>
<tr>
<td>V₃</td>
<td>17.33 (b)</td>
<td>±1.453</td>
<td>0.042</td>
</tr>
<tr>
<td>V₄</td>
<td>7.66 (b)</td>
<td>±1.2</td>
<td>0.000</td>
</tr>
<tr>
<td>V₅</td>
<td>60.33 (b)</td>
<td>±5.281</td>
<td>0.000</td>
</tr>
</tbody>
</table>

nₚ = 9  nₑ = 3

V₁ sample is not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₂, V₃, V₄ and V₅ samples are different. V₂ sample has very long time while the V₄ has a very short time, longer time may be due to hard compactness and the short time may be due to lose compactness. The disintegration time of Maha Varthikava pill varies between 7.66 ±1.2 min. and 60.33 ±5.281 min.

Savarikar et al. (2011) reported that the Triphala guggul kalpa tablets prepared by Ayurvedic methods are found have long disintegration time as compared to Triphala guggul kalpa tablets prepared by direct compression. Disintegration times of directly compressed Triphala guggulu kalpa tablets were found to be satisfactory.

Most of the herbal drugs are not recommended to swallow directly, but after dissolving in a vehicle like decoction, juice extract or bee’s honey etc. Therefore, disintegration time factor is not more important in the therapeutic use but it may be essential in the transporting and storage. The compactness and compression are directly proportional to

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the disintegration time. However, if the disintegration time is very high it is difficult to
dissolve and if the disintegration time is very low, most pills are broken.

The disintegration values range could be suggested as 6-39 minutes and 21-37 minutes
for Seetharama and Maha Varthikava respectively.

4.9. Friability:

Friability refers to the ability of the compressed tablets to avoid fracture and breaking
apart during the transport. Surface hardness is another term for the friability.

Table 4.9.1. Friability (% w/w) of authentically prepared and commercial samples
of Seetharama Watce.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean % w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>0.77 (a)</td>
<td>±0.106</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>0.27 (a)</td>
<td>±0.123</td>
<td>0.081</td>
</tr>
<tr>
<td>S₂</td>
<td>0.29 (a)</td>
<td>±0.90</td>
<td>0.102</td>
</tr>
<tr>
<td>S₃</td>
<td>0.52 (a)</td>
<td>±0.098</td>
<td>0.638</td>
</tr>
<tr>
<td>S₄</td>
<td>1.48 (b)</td>
<td>±0.185</td>
<td>0.009</td>
</tr>
<tr>
<td>S₅</td>
<td>1.62 (b)</td>
<td>±0.229</td>
<td>0.002</td>
</tr>
</tbody>
</table>

When compared the prepared S₀ sample with the commercial samples, S₁, S₂ and S₃ are
not significantly different from the prepared sample S₀, while the S₄ and S₅ are different
at the significant level of 0.05. The friability of Seetharama pill varies from
0.27±0.123% w/w and 1.62±0.229% w/w.
Table 4.9.2. Friability (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean % w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>1.10 (a)</td>
<td>±0.085</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>0.05 (b)</td>
<td>±0.012</td>
<td>0.000</td>
</tr>
<tr>
<td>V₂</td>
<td>0.09 (b)</td>
<td>±0.01</td>
<td>0.000</td>
</tr>
<tr>
<td>V₃</td>
<td>0.31 (b)</td>
<td>±0.07</td>
<td>0.000</td>
</tr>
<tr>
<td>V₄</td>
<td>0.76 (a)</td>
<td>±0.04</td>
<td>0.052</td>
</tr>
<tr>
<td>V₅</td>
<td>0.22 (b)</td>
<td>±0.003</td>
<td>0.000</td>
</tr>
</tbody>
</table>

nᵢ = 9  nₑ = 3

V₄ sample is not significantly different from the prepared sample V₀, while V₁, V₂, V₃ and V₅ are different at the significant level of 0.05. The friability of Maha Varthikava pill varies between 0.09±0.01% w/w and 1.1±0.085% w/w.

Ahamed and Shaikh (1994) reported that the friability depends on the particular size distribution, moisture content and the component formulation.

The friability values range could be suggested as 0.27-1% w/w and 0.8-1.3% w/w for Seetharama and Maha Varthikava respectively.

4.10. Hardness:

Hardness has been regarded as an important quality characteristic of a tablet or a pill. Appropriate hardness of a tablet or a pill is an important indicator to reflect the quality of the product.
Table 4.10.1. Hardness of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Hardness kg/cm²</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>1.33 (a) ±0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>1.67 (a) ±0.166</td>
<td>0.314</td>
<td></td>
</tr>
<tr>
<td>S₂</td>
<td>1.83 (a) ±0.166</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>1.17 (a) ±0.166</td>
<td>0.870</td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>1.33 (a) ±0.166</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>1.33 (a) ±0.166</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

nₚ = 9  nₑ = 3

When comparing the prepared sample S₀ with the commercial samples, All S₁, S₂, S₃, S₄ and S₅ are not significantly different, at the significant level of 0.05. The hardness of Seetharama pill varies from 1.17±0.166 kg/cm² to 1.83±0.166 kg/cm².
Table 4.10.2. Hardness of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Hardness kg/cm²</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>0.78 (a)</td>
<td>±0.121</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>1.66 (b)</td>
<td>±0.166</td>
<td>0.003</td>
</tr>
<tr>
<td>V₂</td>
<td>1.17 (a)</td>
<td>±0.166</td>
<td>0.332</td>
</tr>
<tr>
<td>V₃</td>
<td>1.17 (a)</td>
<td>±0.166</td>
<td>0.332</td>
</tr>
<tr>
<td>V₄</td>
<td>0.67 (a)</td>
<td>±0.166</td>
<td>0.987</td>
</tr>
<tr>
<td>V₅</td>
<td>1.17 (a)</td>
<td>±0.166</td>
<td>0.332</td>
</tr>
</tbody>
</table>

n₀= 9  nₖ= 3

V₂, V₃, V₄, and V₅ samples are not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₁, sample is different. The hardness of Maha Varthikava pill varies between 0.67±0.055 kg/cm² and 1.66±0.166 kg/cm².

Savarikar et al. (2011) reported that when comparing, the Triphala guggul kalpa tablets prepared by Ayurvedic methods are harder than compressed triphala guggul kalpa tablets prepared by direct compression. Hardness of directly compressed triphala guggul kalpa tablets was found to be more satisfactory.

The hardness of the pill/tablet may also be influenced by moisture content and higher humidity content. The hardness value range could be suggested as 1.1-1.8 kg/cm² and 0.5-1.2 kg/cm² for Seetharama and Maha Varthikava respectively.
4.11. Sequential Extractions:

4.11.1. Hexane extractions:

Table 4.11.1.1. Hexane extracts values (% w/w) of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean % w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>26.54(₁₆)</td>
<td>±1.431</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>13.04(₁₆)</td>
<td>±1.321</td>
<td>000</td>
</tr>
<tr>
<td>S₂</td>
<td>6.73(₁₆)</td>
<td>±0.048</td>
<td>000</td>
</tr>
<tr>
<td>S₃</td>
<td>12.22(₁₆)</td>
<td>±0.45</td>
<td>000</td>
</tr>
<tr>
<td>S₄</td>
<td>5.80(₁₆)</td>
<td>±0.135</td>
<td>000</td>
</tr>
<tr>
<td>S₅</td>
<td>13.08(₁₆)</td>
<td>±0.283</td>
<td>000</td>
</tr>
</tbody>
</table>

nₚ= 9  nₑ= 3

When comparing the prepared sample S₀ with the commercial samples, all S₁, S₂, S₃, S₄, and S₅ samples are different at the significant level of 0.05. The hexane extract of Seetharama pill varies from 5.80±0.135% w/w and 26.54±1.431% w/w.
Table 4.11.1.2. Hexane extracts values (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean %w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>6.12±0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>5.83±1.57</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₂</td>
<td>10.96±0.643</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>V₃</td>
<td>4.64±0.5</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>V₄</td>
<td>6.31±2</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₅</td>
<td>10.12±1.39</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

nₚ=9 nₜ=3

V₁, V₃, V₄ and V₅, samples are not significantly different from the prepared sample V₀, at the significant level of 0.05 while the V₂ sample is different. The hexane extract of Maha Varthikava pill varies between 4.64±0.5% w/w and 10.96±0.64% w/w.

Hexane is non-polar solvent and in the extraction, oily matters are extracted. High hexane extract values indicate the presence of more oily matter content.

The hexane extractive value range could be suggested as 23.3-29.8% w/w for Seetharama and Maha Varthikava respectively.
4.11.4. Methanol extractions:

Table 4.11.4.1. Methanol extracts values (% w/w) of authentically prepared and commercial samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean % w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$</td>
<td>8.45 (a)</td>
<td>±1.47</td>
<td></td>
</tr>
<tr>
<td>$S_1$</td>
<td>12.10 (a)</td>
<td>±3.16</td>
<td>0.553</td>
</tr>
<tr>
<td>$S_2$</td>
<td>15.20 (a)</td>
<td>±0.738</td>
<td>0.072</td>
</tr>
<tr>
<td>$S_3$</td>
<td>11.34 (a)</td>
<td>±1.90</td>
<td>0.750</td>
</tr>
<tr>
<td>$S_4$</td>
<td>12.21 (a)</td>
<td>±0.627</td>
<td>0.525</td>
</tr>
<tr>
<td>$S_5$</td>
<td>15.55 (a)</td>
<td>±1.81</td>
<td>0.055</td>
</tr>
</tbody>
</table>

$n_p = 9$, $n_c = 3$

When comparing the prepared sample $S_0$ with the commercial samples, all $S_1$, $S_2$, $S_3$, $S_4$ and $S_5$ are not significantly different, at the level of 0.05. The methanol extraction of Seetharama pill varies from $8.45 \pm 1.47\%$ w/w and $15.55 \pm 1.81\%$ w/w.
Table 4.11.4.2. Methanol extracts values (% w/w) of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean % w/w</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>15.29 (±4.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>5.78 (±1.03)</td>
<td></td>
<td>0.497</td>
</tr>
<tr>
<td>V₂</td>
<td>13.16 (±0.55)</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>V₃</td>
<td>2.99 (±0.399)</td>
<td></td>
<td>0.257</td>
</tr>
<tr>
<td>V₄</td>
<td>9.86 (±4.53)</td>
<td></td>
<td>0.894</td>
</tr>
<tr>
<td>V₅</td>
<td>10.52 (±3.87)</td>
<td></td>
<td>0.934</td>
</tr>
</tbody>
</table>

n₀= 9  nₑ= 3

All V₁, V₂, V₃, V₄ and V₅ samples are not significantly different from the prepared sample V₀, at the significant level of 0.05. Although none of the commercial samples is significantly different, V₁ and V₃ samples have low mean extract values, so it may be due to poor raw material conditions. The methanol extract of Maha Varthikava pill varies between 2.99 ±0.399% w/w and 15.29 ±4.36% w/w.

Methanol is a polar solvent and extracts the polar constituents of the two preparations. Jayanetti (1987) suggested that methanol extracted value for Seetharama Watee as 13% in his unpublished data, which is closely related to this present data.

The methanol extractive value range could be suggested as 5-15.5% w/w and 5.3-25.3% w/w for Seetharama and Maha Varthikava respectively.
4.12. Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is used to separate components of mixtures of substances. It is also a simple, low-cost, versatile and specific method that can be used in the identification of compounds in herbal medicines.

Thin layer chromatography can be used to:

- Monitor the progress of a reaction
- Identify compounds present in a given mixture
- Determine the purity of a substance

In this study, TLC fingerprints were used for the identification of the compounds in the preparations Seetharama and Maha Varthikava. The \( R_f \) values of the major compounds in the prepared samples were compared with the \( R_f \) values of the main raw materials used in the preparations.

Seetharama Water labeled as \( S_0 \) (1\( S_0 \), 2\( S_0 \), 3\( S_0 \)) for prepared samples and \( S_1-S_5 \) for commercial samples. Maha Varthikava Water labeled as \( V_0 \) (1\( V_0 \), 2\( V_0 \), 3\( V_0 \)) for prepared samples and \( V_1-V_5 \) for commercial samples.
Figure 4.12.1.1. Comparison of $R_f$ values of the authentically prepared samples, with the commercial samples of Seetharama Watee and red sandalwood samples.

(SW and RSW are extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light of 254 nm.)

While 6 spots namely B, C, D, F, J and K ($R_f$ 0.06, 0.08, 0.11, 0.16, 0.38 and 0.42) are present in Sri Lankan red sandalwood samples, 5 spots namely B, C, D, F and K (0.06, 0.08, 0.11, 0.16 and 0.42) are present in Indian red sandalwood samples. Spots B, D and F (0.06, 0.11, and 0.38) are present in all prepared and commercial Seetharama Watee samples. Spot C (0.08) is present in all the prepared and commercial samples S₁, S₂, S₄ and S₅.

In order to detect red sandalwood in samples of Seetharama Watee, spots B, C, D and F (0.06, 0.08, 0.11 and 0.38) (Figure 4.12.1.1.) can be considered which were obtained by using ethanol extract, running with toluene: ethyl acetate (9:1 v/v) solvent system.

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5 sri lankan red sandalwood
6 Indian red sandalwood
and examining under shortwave UV light (254 nm). No considerable variation was seen between Sri Lankan and Indian red sandalwood samples.

Figure 4.12.1.2. Comparison of R_f values of the authentically prepared samples, with the commercial samples of Seetharama Watee and red sandalwood samples. (SW and RSW extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) detected in iodine vapour.)

Sri Lankan red sandalwood and Indian red sandalwood samples contain 6 spots namely B_1, D, H, J, K_1 and N (R_f 0.07, 0.11, 0.27, 0.38, 0.43 and 0.83). Spot N (R_f 0.83) is present in all prepared and commercial Seetharama Watee samples. Spot K_1 (R_f 0.44/0.43) is present in all commercial samples and sample 1S_0. Spot J (0.38) is present in S_1, S_2, S_3 commercial samples.

In order to detect red sandalwood in samples, of Seetharama Watee, spot N (0.83) (Figure 4.12.1.2) can be considered which was obtained by using ethanol extract,
running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.

Figure 4.12.1.3. Comparison of Rf values of the authentically prepared samples, with the commercial samples of Seetharama Watee and red sandalwood sample. (SW and RSW extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected with vanillin sulfuric acid reagent.)

While the Sri Lankan red sandalwood sample contains 6 spots namely A, B1, E, J, K, and M2 (Rf 0.05, 0.08, 0.13, 0.37, 0.42 and 0.74). Indian red sandalwood sample contains 7 spots A, B1, E, J, K, M2, and L1 (Rf 0.05, 0.08, 0.13, 0.37, 0.42, 0.74 and 0.77). Spot K (0.42) is present in all the prepared and commercial Seetharama Watee samples. Spot A (0.05) was present in all prepared samples and S1, S2, S3 and S5 commercial samples.
In order to detect red sandalwood in samples, of Seetharama Watee, spots A and K (0.05 and 0.42) (Figure 4.12.1.3) can be considered which was obtained by using ethanol extract, running with toluene: ethyl acetate (9:1 v/v) solvent system. Then spraying with vanillin sulfuric acid reagent heated at 105 °C for 10 minutes.

Figure 4.12.1.4. Comparison of Rf values of the authentically prepared samples, with the commercial samples of Seetharama Watee and red sandal wood sample. (SW and RSW extracted by ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Red sandalwood contains 3 spots namely BB, FF and HH (Rf 0.25, 0.51 and 0.63). Spots BB and HH (Rf 0.25 and 0.63) are present in all prepared and commercial Seetharama Watee samples. Spot FF (0.51) is present in all the prepared and commercial samples except S1.
In order to detect red sandalwood in samples, of *Seetharama Watee*, spot BB, FF and HH (0.25, 0.51 and 0.63) (Figure 4.12.1.4) can be considered which were obtained by using ethanol extract running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

![Graph](image)

Figure 4.12.1.5. Comparison of $R_f$ values of the authentically prepared samples, with the commercial samples of *Seetharama Watee* and holy basil sample. (SW and H Basil extracted with ethanol and developed using toluene: ethyl acetate (9:1v/v) solvent system, detected under shortwave UV light of 254 nm.)

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7 Holy basil
Holy basil sample contains 5 spots namely G, J, L, M and N (R_f 0.22, 0.38, 0.55, 0.61 and 0.83). Spot L (0.55) was present in all prepared and commercial Seetharama Watee samples. Spot G (0.22) is present in S_0, S_3, S_4 commercial samples (Figure 4.12.1.5-6).

In order to detect holy basil in samples, of Seetharama Watee, spot L (0.55) can be considered which was obtained by using ethanol extraction running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave of UV light 254 nm.

Figure 4.12.1.6. TLC fingerprints of Seetharama Watee and holy basil (Tulasi) samples detected under shortwave UV light (254 nm).

S_0 - ^8  S_1-S_5 - ^9  T - ^10

^8 Autentically prepared samples
^9 Commercial samples
^10 Tulasi
Figure 4.12.1.7. Comparison of $R_f$ values of the authentically prepared samples, with the commercial samples of Seetharama Watee and holy basil samples. (SW and H. basil extracted by ethanol, developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected in iodine vapour.)

Holy basil contains 7 spots namely A, G, I, K₁, L, L₁ and O ($R_f$ 0.05, 0.22, 0.33, 0.44, 0.55, 0.77 and 0.87). Spot A, G and L (0.05, 0.22 and 0.55) are present in all the prepared and the commercial Seetharama Watee samples. Spot I (0.33) is present in all the commercial and 2S₀, 3S₀ prepared samples. K₁ spot (0.44/ 0.43) is present in all commercial and 1S₀ prepared samples.

In order to detect holy basil in samples, of Seetharama Watee, spots A, G, L and I (0.05, 0.22, 0.55 and 0.33) (Figure 4.12.1.7-8) can be considered which was obtained by using ethanol extraction running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.8. TLC fingerprints of Seetharama Watee and holy basil (Tulasi) samples detected in iodine vapour.
Figure 4.12.1.9. Comparison of $R_f$ values of the authentically prepared samples, with the commercial samples of Seetharama Watee and holy basil sample. (SW and H. basil extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system detected with vanillin sulfuric acid reagent.)

Holy basil contains 8 spots namely F, G, I, K, L, M₀, M and N ($R_f$ 0.16, 0.21, 0.33, 0.42, 0.55, 0.58, 0.61 and 0.83). Spots K and M₀ (0.42 and 0.58) are present in all the prepared and the commercial Seetharama Watee samples. Spot N (0.83) is present in all the prepared samples and commercial sample $S_1$. Spot F (0.16) is present in all the prepared samples and $S_1$ and $S_2$ commercial samples.

In order to detect holy basil in samples, of Seetharama Watee, spots F, K, M₀ and N (0.16, 0.42, 0.58 and 0.83) (Figure 4.12.1.9-10) can be considered which was obtained by using ethanol extract running with toluene: ethyl acetate (9:1 v/v) solvent system and spraying with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Figure 4.12.1.10. TLC fingerprints of Seetharama Watee and holy basil (Tulasi) samples after spraying vanillin sulphate.
Figure 4.12.1.11. Comparison of $R_f$ values of the authentically prepared samples, with the commercial samples of Seetharama Watee and holy basil sample. (SW and holy basil extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Holy basil contains 7 spots namely $AA_1$, $AA_2$, $AA$, $DD$, $DD_1$, $GG_1$ and $HH_1$ ($R_f$ 0.12, 0.17, 0.18, 0.36, 0.4, 0.67 and 0.81). Spot AA (0.18) is present in all prepared and commercial Seetharama Watee samples.

In order to detect holy basil in samples, of Seetharama Watee, spot AA (0.18) (Figure 4.12.1.11) can be considered which are obtained using ethanol extraction and running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Indian privet contain 5 spots namely A, F, H, I and K ($R_f$ 0.05, 0.16, 0.27, 0.33 and 0.42). Spot F (0.16) was present in all prepared and commercial Seetharama Watee samples. Spot H (0.27) was present in 2S₀, 3S₀, S₁, S₂ and S₃ samples. Spot I (0.33) was present in 1S₀ and S₂ samples.

In order to detect holy basil in samples, of Seetharama Watee, spots F and H (0.16 and 0.27) (Figure 4.12.1.12.) can be considered which was obtained by using ethanol extraction and run with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave UV light 254 nm.

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11 Indian privet
Figure 4.12.1.13. Comparison of R_f values of authentically prepared samples, commercial samples of Seetharama Watee and Indian privet samples. (SW and Indian privet extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system detected in iodine vapour.)

Indian privet contains 4 spots namely A, F, H and N (R_f 0.05, 0.16, 0.27 and 0.83). Spot A and N (0.05 and 0.83) were present in all prepared and commercial Seetharama Watee samples. Spot F (0.16) was present in all prepared samples.

In order to detect Indian privet in samples, of Seetharama Watee, spots A, F and N (0.05, 0.16 and 0.83) (Figure 4.12.1.13) can be considered which was obtained by using ethanol extract, running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.14. Comparison of $R_f$ values of prepared authentically prepared samples, commercial samples of Seetharama Watee and Indian privet sample. (SW and Indian privet extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system detected with vanillin sulfuric acid reagent.)

Indian privet sample contains 7 spots namely A, D, F, I, K, L and N ($R_f$ 0.05, 0.11, 0.15, 0.33, 0.42, 0.55 and 0.83). Spot K (0.42) was present in all prepared and commercial Seetharama Watee samples. Spot A (0.05) was present in all prepared and $S_1$, $S_2$, $S_3$ and $S_5$ commercial samples. Spot N (0.83) was present in all prepared and commercial sample $S_1$. Spot F (0.16) was present in all prepared and $S_1$ and $S_2$ commercial samples.

In order to detect Indian privet in samples, of Seetharama Watee, spots A, F, K and N (0.05, 0.16, 0.42 and 0.83) (Figure 4.12.1.14) can be considered which was obtained by using ethanol extract and running with toluene: ethyl acetate (9:1 v/v) solvent system and sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Figure 4.12.1.15. Comparison of $R_f$ values of authentically prepared samples, commercial samples of Seetharama Watee and Indian privet sample. (SW and Indian privet extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Indian privet contains 9 spots namely AA1, AA, BB, CC1, DD, DD1, FF, GG0 and HH0 ($R_f$ 0.15, 0.17, 0.24, 0.28, 0.36, 0.4, 0.47, 0.65 and 0.73). Spots AA, BB and FF (0.65, 0.47 and 0.25) were present in all prepared and commercial Seetharama Watee samples. Spot DD (0.36) was present in 1S0 and 2S0, prepared and S2, S4 and S5 commercial samples. Spot EE (0.43) was present in 3S0 prepared and all commercial samples.
In order to detect Indian privet in samples, of *Seetharama Watee*, spots AA, BB and DD (0.65, 0.47, 0.36) (Figure 4.12.1.15) can be considered which was obtained by using ethanol extract, and running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

Figure 4.12.1.16. Comparison of R_f values of authentically prepared sample, with commercial samples of Seetharama Watee and ginger sample. (SW and ginger extracted with ethanol, developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light of 254 nm.)

Ginger contains 4 spots E, F, L and N namely (R_f 0.14, 0.16, 0.55 and 0.83). While the spots F and L (0.16 and 0.55) were present in all prepared and commercial Seetharama Watee samples.

In order to detect ginger in samples, of *Seetharama Watee*, spots F (0.16) and L (0.55) (Figure 4.12.1.16) can be considered which was obtained by using ethanol extract,
running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave UV light of 254 nm.

Figure 4.12.1.17. Comparison of Rf values of authentically prepared samples, commercial samples of Seetharama Watee and ginger sample. (SW and ginger extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected in iodine vapour.)

Ginger contains 4 spots E, F, K and N namely (Rf 0.14, 0.16, 0.42 and 0.84). Spot N (0.83) was present in all prepared and commercial samples. Spot F (0.16) was present in all prepared Seetharama Watee samples.

In order to detect ginger in samples, of Seetharama Watee, spots N and F (0.83 and 0.16) (Figure 4.12.1.17.) can be considered which was obtained by using ethanol extraction and run with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.18. Comparison of R$_f$ values of authentically prepared samples, with commercial samples of Seetharama Watee and ginger sample. (SW and ginger extracted with ethanol and developed using, toluene: ethyl acetate (9:1 v/v) solvent system detected with vanillin sulfuric acid reagent.)

Ginger contains 6 spots namely A, F, K, L, M$_0$ and M$_2$ (R$_f$ 0.05, 0.16, 0.42, 0.55, 0.58 and 0.81). Spots K and M$_0$ (0.42, 0.58) were present in all prepared and commercial Seetharama Watee samples. Spot A (0.05) was present in all prepared samples and S$_1$, S$_2$, S$_3$ and S$_5$ samples. Spot F (0.16) was present in all prepared samples and S$_1$ and S$_2$ commercial samples.

In order to detect holy basil in samples, of Seetharama Watee, spots A, F, K and M$_0$ (0.05, 0.16, 0.42 and 0.58) (Figure 4.12.1.18) can be considered which was obtained by using ethanol extraction and run with toluene: ethyl acetate (9:1 v/v) solvent system and sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Ginger contains 4 spots namely AA, CC, EE and HH\(_1\) (R\(_f\) 0.18, 0.31, 0.44 and 0.81) Spot AA, (0.18) was present in all prepared and commercial **Seetharama Watee** samples. CC (0.31) was present in 2S\(_0\), 3S\(_0\) and S\(_1\) samples. EE (0.44) was present in 3S\(_0\) and all commercial samples. HH\(_1\) (0.81) was present in 1S\(_0\), 2S\(_0\), S\(_2\), S\(_3\) and S\(_4\) samples.

In order to detect ginger in samples, of **Seetharama Watee**, spots AA, CC and HH\(_1\) (0.18, 0.31, 0.81) (Figure 4.12.1.19) can be considered which was obtained by using

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**Figure 4.12.1.19.** Comparison of R\(_f\) values of authentically prepared samples, with commercial samples of Seetharama Watee and ginger sample. (SW and ginger extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)
ethanol extract running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

CIN-SEDGE\textsuperscript{12}

Figure 4.12.1.20. Comparison of \( R_f \) values of authentically prepared samples, with the commercial samples of Seetharama Watee and cinnamon sedge sample. (SW and cinnamon sedge extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light of 254 nm.)

Cinnamon sedge contains 2 spots namely F and L (\( R_f \) 0.16 and 0.55) were present in all prepared and commercial Seetharama Watee samples. In order to detect cinnamon sedge in samples, of Seetharama Watee, spots F and L (0.16 and 0.55) (Figure 4.12.1.20.) can be considered which was obtained by using ethanol extract running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave UV light of 254 nm.

\textsuperscript{12} Cinnamon sedge
Figure 4.12.1.21. Comparison of Rf values of authentically prepared samples, with the commercial samples of Seetharama Watee and cinnamon sedge sample. (SW and cinnamon sedge extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system detected in iodine vapour.)

Cinnamon sedge contains 2 spots, namely H and L (Rf 0.27 and 0.55). Spot L (0.55) was present in all prepared and commercial Seetharama Watee samples.

In order to detect Cinnamon sedge in samples of Seetharama Watee, spot L (0.55) (Figure 4.12.1.21.) can be considered, which was obtained by using ethanol extract, running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.22. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Seetharama Watee and cinnamon sedge sample. (SW and cinnamon sedge extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected with vanillin sulfuric acid reagent.)

Cinnamon sedge contains 2 spots namely A and L₁ ($R_f$ 0.05 and 0.77). Spot A (0.05) was present in all prepared samples and S₁, S₂, S₃ and S₅ commercial Seetharama Watee samples.

In order to detect Cinnamon sedge in samples, of Seetharama Watee, spots A (0.05) (Figure 4.12.1.22.) can be considered, which was obtained by using ethanol extract, running with toluene: ethyl acetate (9:1 v/v) solvent system, sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Figure 4.12.1.23. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Seetharama Watee and cinnamon sedge sample. (SW and cinnamon sedge extracted with ethanol and developed benzene: acetone solvent system (5:7 v/v) detected under shortwave UV light of 254 nm.)

Cinnamon sedge contains spot namely C ($R_f$ 0.72) was present in all prepared and commercial Seetharama Watee samples.

In order to detect cinnamon sedge in samples, of Seetharama Watee, spots C (0.72) (Figure 4.12.1.23) can be considered, which was obtained by using ethanol extract, running with benzene: acetone (50:70 v/v) solvent system and examining under shortwave UV light of 254 nm.
Figure 4.12.1.24. Comparison of $R_f$ values of authentically prepared samples commercial samples of Seetharama Watee and cinnamon sedge sample. (Extracted with ethanol and developed using benzene: acetone (5:7 v/v) solvent system, detected in iodine vapour).

Cinnamon sedge contains spot C namely ($R_f$ 0.72) was present in all prepared and commercial Seetharama Watee samples.

In order to detect cinnamon sedge in samples, of Seetharama Watee, spot C (0.72) (Figure 4.12.1.24.) can be considered, which was obtained using ethanol extract, running with benzene: acetone (50:70 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.25. Comparison of $R_f$ values of authentically prepared samples with the commercial samples of Seetharama Watee and cinnamon sedge samples. (SW and cinnamon sedge extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Cinnamon sedge contains 2 spots namely FF and JJ ($R_f$ 0.5 and 0.75). Spot FF (0.5) was present in all prepared and S2, S3, S4 and S5 commercial samples. Spot JJ (0.75) was present in all prepared and commercial Seetharama Watee samples except S5.

In order to detect cinnamon sedge in samples, of Seetharama Watee, spots FF and JJ (0.5 and 0.75) (Figure 4.12.1.25.) can be considered which was obtained by using ethanol extract, running with n-hexane: acetone (75:25 v/v) solvent system and sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Figure 4.12.1.26. Comparison of *R*<sub>f</sub> values of authentically prepared samples, with the commercial samples of Seetharama Watee and garlic sample. (SW and garlic extracted with ethanol and developed using n butanol: propanol: acetic acid: water (6:2:2:2 v/v) solvent system detected under shortwave UV light of 254 nm.)

Garlic contains 4 spots namely C<sub>1</sub>, E<sub>1</sub>, G<sub>1</sub>, H<sub>1</sub> (*R*<sub>f</sub> 0.11, 0.17, 0.25, and 0.43). Spots C<sub>1</sub> and E<sub>1</sub> (0.11 and 0.17) were present in all prepared and commercial Seetharama Watee samples. Spot G<sub>1</sub> (0.25) was present in 2S<sub>0</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> samples. H<sub>1</sub> (0.25) was present in 3S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> samples.

In order to detect garlic in samples, of Seetharama Watee, spots C<sub>1</sub> and E<sub>1</sub> (0.11 and 0.17) (Figure 4.12.1.26) can be considered which was obtained by using ethanol extraction and running with n butanol: propanol: acetic acid: water (6: 2:2:2 v/v) solvent system and examining under shortwave UV light of 254 nm.
Figure 4.12.1.27. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Seetharama Watee and garlic sample. (SW and garlic extracted with ethanol and developed using n butanol: propanol: acetic acid: water (6:2:2:2 v/v) solvent system detected in iodine vapour.)

Garlic sample contains 6 spots namely B$_2$, C$_2$, D$_2$, E$_2$, F$_2$ and G$_2$ ($R_f$ 0.2, 0.27, 0.35, 0.37, 0.5 and 0.75). Spot G$_2$ (0.75) was present in all prepared and commercial Seetharama Watee samples. E$_2$ (0.06) spot was present in all prepared and S$_1$ and S$_2$ commercial samples.

In order to detect garlic in samples, of Seetharama Watee, spot G$_2$ and E$_2$ (0.75 and 0.06) (Figure 4.12.1.27) can be considered which was obtained by using ethanol extract, running with n butanol: propanol: acetic acid: water (6: 2:2:2 v/v) solvent system and examining under iodine vapour.
Figure 4.12.1.28. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Seetharama Watee and garlic sample. (SW and garlic extracted with ethanol and developed using n butanol: propanol: acetic acid: water (6:2:2:2 v/v) solvent system, detected with ninhydrin reagent.)

Garlic contains 6 spots namely A$_3$, B$_3$, C$_3$, D$_3$, E$_3$ and F$_3$ ($R_f$ 0.09, 0.31, 0.37, 0.61, 0.68 and 0.81). Spot B$_3$ and C$_3$ (0.31 and 0.37) were present in all prepared and commercial Seetharama Watee samples.

In order to detect garlic in samples, of Seetharama Watee, spots B$_3$ and C$_3$ (0.31 was 0.37) (Figure 4.12.1.28) can be considered which was obtained by using ethanol extract, and running with n butanol: propanol: acetic acid: water (6:2:2:2 v/v) solvent system and sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Garlic contains 2 spots namely BB and GG ($R_f$ 0.25 and 0.56). Spot BB ($R_f$ 0.25) was present in all prepared and commercial Seetharama Watee samples.

In order to detect garlic in samples, of Seetharama Watee, spot BB (0.25) (Figure 4.12.1.29) can be considered which was obtained by using ethanol extract and running with n-hexane: acetone (75:25 v/v) solvent system and sprayed with anisaldehyde sulfuric acid reagent heated at 110 °C for 10 minutes and observed.
Figure 4.12.1.30. Comparison of Rf values of authentically prepared samples with the commercial samples of Seetharama Watee and neem sample. (SW and neem extracted with ethanol and developed using n-hexane: ethyl acetate (5:5 v/v) solvent system detected under shortwave UV light of 254 nm.)

Neem contains 9 spots namely A, B, C, D, E, F, G, H and I (Rf 0.05, 0.11, 0.22, 0.42, 0.52, 0.55, 0.69, 0.8 and 0.86). Spots A, B, D, E and H (0.05, 0.11, 0.42, 0.52 and 0.8) were present in all prepared and commercial Seetharama Watee samples.

In order to detect neem in samples, of Seetharama Watee, spots A, B, D, E and H (0.05, 0.11, 0.42, 0.52 and 0.8) (Figure 4.13.1.30) can be considered which was obtained by using ethanol extract and running with n-hexane: ethyl acetate (50:50 v/v) solvent system and examining under shortwave UV light of 254 nm.

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Figure 4.12.1.31. Comparison of Rf values of authentically prepared samples, with the commercial samples of Seetharama Watee and neem sample. (SW and neem extracted with ethanol and developed using n-hexane: ethyl acetate (5:5 v/v) solvent system detected with vanillin sulfuric acid regent.)

Neem contains 7 spots namely A, C, C₁, D₁, E₁, F₁ and I (Rf 0.05, 0.22, 0.29, 0.58, 0.72, 0.8 and 0.86). Spots A and D₁ (Rf 0.05 and 0.58) were present in all prepared and commercial Seetharama Watee samples.

In order to detect neem in samples, of Seetharama Watee, spots A, and D₁ (0.05 and 0.58) (Figure 4.12.1.31.) can be considered which was obtained by using ethanol extract and running with n-hexane: ethyl acetate (50:50 v/v) solvent system and sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.
Figure 4.12.1.32. Comparison of R<sub>f</sub> values of authentically prepared samples, with the commercial samples of Seetharama Watee and neem sample. (SW and neem extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Neem contains 10 spots namely AA<sub>0</sub>, AA<sub>1</sub>, AA, BB, BB<sub>1</sub>, DD, DD<sub>1</sub>, EE, GG<sub>0</sub> and HH<sub>0</sub> (R<sub>f</sub> 0.1, 0.15, 0.17, 0.24, 0.28, 0.36, 0.4, 0.43, 0.65 and 0.73). Spot AA and BB (0.18 and 0.25) were present in all prepared and commercial Seetharama Watee samples.

In order to detect neem in samples of Seetharama Watee, spot AA and BB (0.18, 0.25) (Figure 4.12.1.32.) can be considered which was obtained by using ethanol extract and running with n-hexane: acetone (75:25 v/v) solvent system and sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Figure 4.12.1.33. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Seetharama Watee and asafoetida (resin) sample. (SW and asafoetida extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Asafoetida contains 6 spots namely BB, CC, EE, GG, HH and JJ ($R_f$ 0.25, 0.31, 0.43, 0.56, 0.63 and 0.76). Spot BB and HH (0.25 and 0.63) were present in all prepared and commercial Seetharama Watee samples. Spot CC (0.31) was present in all prepared samples and $S_2$, $S_3$, $S_4$ and $S_5$ commercial samples. Spot JJ (0.76) was present in $1S_0$, $2S_0$, $S_2$, $S_3$, $S_4$ and $S_5$ samples.

In order to detect asafoetida in samples, of Seetharama Watee, spots BB, CC and HH (0.25, 0.31 and 0.63) (Figure 4.12.1.33) can be considered which was obtained by using ethanol extract and running with n-hexane: acetone (75:25 v/v) solvent system and
sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

Figure 4.12.1.34. Comparison of R_f values of authentically prepared samples with the commercial samples of Seetharama Watee and asafoetida sample. (SW and asafoetida extracted with ethanol and developed using toluene: ethyl acetate: formic acid (70:30:3 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

Asafoetida contains 7 spots namely A, D, E, G, H, I and J (R_f 0.05, 0.31, 0.36, 0.54, 0.62, 0.72 and 0.92). Spots E and G (R_f 0.36 and 0.54) were present in all prepared and commercial Seetharama Watee samples.

In order to detect asafoetida in samples of Seetharama Watee, spots E and G (0.36 and 0.54) (Figure 4.12.1.34) can be considered which was obtained by using ethanol extract and running with toluene: ethyl acetate: formic acid (70:30:3 v/v) solvent system and...
sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

Figure 4.12.1.35. TLC profiles/patterns of the n-hexane extracts of Seetharama Watee developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light (254 nm), long wave UV light (366 nm) and sprayed with anisaldehyde sulfuric acid reagent.

13 Autentically Prerared samples
14 Commercial samples.
Figure 4.12.1.36. TLC profiles/ patterns of the dichloromethane extract of Seetharama Watee developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light (254 nm), long wave UV light (366 nm) and sprayed with anisaldehyde sulfuric acid reagent.

Figure 4.12.1.37. TLC profiles/ patterns of the ethyl acetate extract of Seetharama Watee developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light (254 nm), long wave UV light (366 nm) and sprayed with anisaldehyde sulfuric acid reagent.
Figure 4.12.1.38. TLC profiles / patterns of the methanol extract of Seetharama Watee developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light (254 nm), long wave UV light (366 nm) and sprayed with anisaldehyde sulfuric acid reagent.

Karunanayaka et al. (1971) developed two dimensional thin layer chromatographic techniques for analyzing methanol and chloroform extracts (10:1 v/v) of Seetharama Watee.

Five spots were observed, under ultra violet light (254 nm) in the standard sample and lesser number of spots in commercial samples when using xylene: chloroform (1:1 v/v) as 1st and 2nd solvent systems.

When TLC plates of Seetharama Watee extracts and the selected raw materials' ethanol extract were developed in toluene: ethyl acetate (90:10 v/v) solvent system and visualised under short wave UV light (254 nm), 4 spots in red sandal wood ($R_f$ 0.06, 0.08, 0.11 and 0.38) one spot in holy basil ($R_f$ 0.55), 2 spots in indian privet ($R_f$ 0.16 and 0.27), 2 spots in ginger ($R_f$ 0.16 and 0.55) 2 spots in cinnamon Sedge ($R_f$ 0.16 and 0.55) sample were detected and have equal $R_f$ values to those of the Seetharama Watee (Figure 7.v.vi, 7.v.ix).
When the same samples detect under iodine vapour one spot for red sandalwood (R_f 0.83) 4 spots for holy basil (R_f 0.05, 0.22, 0.33 and 0.55), 3 spots for indian privet (R_f 0.05, 0.16 and 0.83), 2 spots for ginger (R_f 0.16 and 0.83) and, one spot for cinnamon sedge (0.55) sample were detected and have equal R_f values to those of Seetharama Watee (Figure 7.vi.vii, 7.v.x).

When same samples detect after spraying vanillin sulfuric acid and heating to 105 °C, 2 spots were detected in red sandalwood (R_f 0.05 and 0.42), 4 spots in holy basil, (R_f 0.16, 0.42, 0.58 and 0.83), 4 spots in indian Privet (R_f 0.05, 0.16, 0.42 and 0.83), 4 spots in ginger (R_f 0.05, 0.16, 0.42 and 0.58), and one in cinnamon sedge (0.05) were detected and have equal R_f values to those of the Seetharama Watee (Figure 7.vi.vii, 7.v.x).

When the TLC of Seetharama Watee extract and the selected raw materials' etanol extracts developed in n-hexane: acetone (7.5: 2.5 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 105 °C for 10 minutes and examined, 3 spots in red sandalwood (R_f 0.25, 0.51 and 0.63), one in holy basil (R_f 0.18), 3 in indian privet (R_f 0.36, 0.47 and 0.65), 3 in asafoetida (R_f 0.25, 0.31 and 0.63), 3 in ginger (R_f 0.18, 0.31 and 0.81), 2 in neem (R_f 0.18 and 0.25), one in garlic (R_f 0.25) and 2 in cinnamon sedge (R_f 0.05 and 0.75) were detected and have equal R_f values to those of the Seetharama Watee (Figure 7.vi.xxiv).

When TLC plates of Seetharama Watee extracts and the selected raw materials' ethanol extracts were developed in n butanol: propanol: acetic acid: water (60:20:20:20 v/v) solvent system and visualized under short wave UV light (254 nm), 2 spots were detected in garlic (R_f 0.11 and 0.17) and have the equal R_f value of the Seetharama Watee.
When TLC plates of Seetharama Watee and the selected raw materials’ ethanol extracts were developed in n-hexane: ethyl acetate (50:50 v/v) solvent system and visualised under short wave UV light (254 nm), 5 spots in (Rf 0.05, 0.11, 0.42, 0.52 and 0.8) neem samples were observed. When the same TLC plate was exposed to iodine vapour, 2 spots (Rf 0.06 and 0.75) were detected. When detected after spraying the ninhydrin and heating up to 105 °C, 2 spots were detected (Rf 0.31 and 0.37) and have the equal Rf value of the Seetharama Watee.

When TLC plates of Seetharama Watee extracts and the selected raw materials’ ethanol extracts were developed in toluene: ethyl acetate: formic acid (70:30:3 v/v) solvent system after spraying anisaldehyde sulfuric acid and heating to 105 °C, 2 spots were in asafoetida (Rf 0.36 and 0.55) were detected and have the equal Rf value of the Seetharama Watee. When Seetharama Watee and the cinnamon sedge sample extracted by ethanol and developed in the solvent system benzene: acetone (50:50 v/v) visualized under short wave UV light (254 nm) and iodine vapour, one spot (Rf 0.72) was detected and have the equal Rf value of the Seetharama Watee.

Toluene: ethyl acetate (9:1 v/v) and n-hexane: acetone (75:25 v/v) solvent systems can be used for TLC analysis of Seetharama Watee and its raw materials, red sandalwood, holy basil, indian privet, ginger and cinnamon sedge samples.

n-Hexane: acetone (75:25 v/v) and n-butanol: propanol: acetic acid: water (6:2:2:2v/v) can be used for TLC analysis of Seetharama Watee and garlic samples.

n-Hexane: acetone (75:25 v/v) and toluene: ethyl acetate: formic acid (70:30:3 v/v) can be used to TLC analysis of Seetharama Watee and asafoetida samples.

Benzene: acetone (50:70 v/v) solvent system also can be used for TLC analysis of Seetharama Watee and cinnamon sedge samples.
These spots can be taken for the standardization, using the various solvent systems under the above mentioned conditions.

![Figure 4.12.2.1. Comparison of R_f values of the authentically prepared samples, with the commercial samples of Maha Varthikava Watee, and betel samples (MV and betel were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, and detected under shortwave UV light of 254 nm.)](image)

Betel sample has 6 spots namely A, E, F, G_1, H and I (R_f 0.05, 0.22, 0.27, 0.38, 0.44 and 0.5). Spots A, E, F and G_1 (0.05, 0.22, 0.27 and 0.38) were present in all prepared and commercial Maha Varthikava Watee samples. Spot I (0.5) was present in all prepared and V_1, V_2 and V_5 commercial samples.
In order to detect betel in samples, of *Maha Varthikava Watee*, spots A, E, F, G₁ and I (0.05, 0.22, 0.27, and 0.38) (Figure 4.12.2.1) can be considered which was obtained by using ethanol extract of betel and running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave UV light 254 nm.

![Graph showing Rf values of samples](image)

**Figure 4.12.2.2.** Comparison of Rf values of authentically prepared sample, with the commercial samples of Maha Varthikava Watee, and betel sample. (MV and betel was extracted with ethanol and developed using solvent system toluene: ethyl acetate (9:1 v/v) solvent system, detected in iodine vapour.

Betel sample includes 7 spots namely B, C, C₁, G₄, J, K, K₁ and L (Rf 0.08, 0.11, 0.19, 0.35, 0.52, 0.55, 0.6 and 0.78). Spot C, J, and K (0.11, 0.52, and 0.55) were present in all prepared and commercial *Maha Varthikava Watee* samples. Spot G₄ (0.35) was present in 1V₀, 2V₀, prepared and in V₁, V₂, V₄ and V₅ commercial samples.
In order to detect betel in samples, of Maha Varthikava Watee, spots C, J, K and G₄ (0.11, 0.52, 0.55 and 0.35) (Figure 4.12.2.2.) can be considered which was obtained by using ethanol extract of betel and running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.

Figure 4.12.2.3. Comparison of \( R_f \) values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee, and betel sample. (MV and betel were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system and detected with vanillin sulfuric acid reagent.)

While betel sample includes 6 spots namely E, F, G₁, H, I and M₁ (\( R_f \) 0.22, 0.27, 0.38, 0.44, 0.5 and 0.86). Spot G₁ (0.38) was present in all prepared and commercial Maha Varthikava Watee samples; H and I (0.44 and 0.5) were present in all prepared and in
V₁, V₂, V₄ and V₅ commercial samples; M₁ (0.86) was present in all prepared and commercial samples V₁ and V₂.

In order to detect betel in samples, of Maha Varthikava Watee spots G₁, H, I and M₁ (0.38, 0.44, 0.5 and 0.86) (Figure 4.12.2.3) can be considered which was obtained by using ethanol extract of betel and running with toluene: ethyl acetate (9:1 v/v) solvent system and sprayed with vanillin sulfuric acid reagent, heated at 105°C for 10 minutes and observed.

Figure 4.12.2.4. Comparison of Rᵢ values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee, and betel sample. (MV and betel were extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system and detected with anisaldehyde sulfuric acid reagent.)
Betel sample contains 9 spots namely AA, BB, DD, EE, FF, GG, HH, JJ, and LL (R<sub>f</sub> 0.12, 0.18, 0.25, 0.31, 0.37, 0.43, 0.5, 0.63 and 0.75). Spot BB (0.18) was present in all prepared and in commercial *Maha Varthikava Watee* samples V<sub>1</sub>, V<sub>2</sub>, and V<sub>3</sub> and V<sub>5</sub>. Spot FF (0.37) was present in all prepared and commercial samples. Spot GG (0.43) was present in 2V<sub>0</sub> and 3V<sub>0</sub> prepared samples and V<sub>1</sub>, V<sub>2</sub>, V<sub>3</sub> and V<sub>5</sub> commercial samples.

In order to detect betel in samples, of *Maha Varthikava Watee* spots BB, FF and GG (0.18, 0.37 and 0.43) (Figure 4.12.2.4.) can be considered which was obtained by using ethanol extract of betel, running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Figure 4.12.2.5. Comparison of R\textsubscript{f} values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and Indian privet sample. (MV and Indian privet were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under shortwave UV light of 254 nm.)

Indian privet sample showed 8 spots namely A, B, C, E, F, G\textsubscript{1}, H\textsubscript{1} and I (R\textsubscript{f} 0.05, 0.08, 0.11, 0.22, 0.27, 0.38, 0.47 and 0.5). Spot A, E, F and G\textsubscript{1} (0.05, 0.22, 0.27, 0.38) were present in all prepared and commercial Maha Varthikava Watee samples. Spot I (0.5) was present in all prepared and commercial sample V\textsubscript{1}, V\textsubscript{2} and V\textsubscript{5}. Spot C (0.11) was present in all prepared and V\textsubscript{1} and V\textsubscript{2} commercial samples.

In order to detect betel in samples, of Maha Varthikava Watee, spots A, C, E, F, G\textsubscript{1} and I (0.05, 0.11, 0.22, 0.27 and 0.38) (Figure 4.12.2.5.) can be considered which was
obtained by using the ethanol extract of betel, running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under shortwave UV light of 254 nm.

Figure 4.12.2.6. Comparison of Rf values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and Indian privet sample. (MV and Indian privet were extracted with ethanol and developed using solvent system toluene: ethyl acetate (9:1 v/v), detected under iodine vapour.)

Indian privet sample gave 5 spots namely A, C, E₁, G₂ and M (Rf 0.05, 0.11, 0.25, 0.3 and 0.8). Spot A and C (0.05 and 0.11) were present in all prepared and commercial Maha Varthikava Watee samples; E₁ (0.25) was present in all prepared and commercial samples V₁, V₂, V₄ and V₅.

In order to detect betel in samples, of Maha Varthikava Watee, spots A, C, and E₁ (0.05, 0.11 and 0.25) (Figure 4.12.2.6.) can be considered which was obtained by using
ethanol extract of betel and running with toluene: ethyl acetate (9:1 v/v) solvent system, and examining under iodine vapour.

Figure 4.12.2.7. Comparison of Rf values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and Indian privet sample. (MV and Indian privet were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system detected with vanillin sulfuric acid regent.)

Indian privet sample contained 12 spots namely A, C, C1, E1, F, F1, G3, G1, H, I, L, and M1 (Rf 0.05, 0.11, 0.19, 0.22, 0.25, 0.27, 0.33, 0.38, 0.44, 0.5, 0.77 and 0.86). Spot A (0.05) was present in all prepared and commercial Maha Varthikava Watee samples V1, V2, V3 and V4; C (0.11) was present in all prepared and commercial sample V5; G1 and H (0.38 and 0.44) were present in all prepared and commercial samples. L (0.77)
was present in all prepared and commercial samples $V_1$, $V_2$, $V_4$ and $V_5$. $M_1$ (0.86) was present in all prepared and commercial sample $V_2$.

In order to detect Indian privet in samples, of *Maha Varthikava Watee* spots A, C, G_1, H, L and M_1 (0.05, 0.11, 0.38, 0.44, 0.77 and 0.86) (Figure 4.12.2.7) can be considered which was obtained by using ethanol extract of Indian privet and running with toluene: ethyl acetate (9: 1 v/v) solvent system, sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.

![Figure 4.12.2.8. Comparison of Rf values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee, and Indian privet sample. (MV and Indian privet were extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent).](image-url)

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While Indian privet sample includes 9 spots namely FA, BB, CC, EE, FF, GG, HH, JJ and LL (Rf 0.12, 0.18, 0.22, 0.31, 0.37, 0.43, 0.56, 0.62 and 0.75) BB (0.18) was present in all prepared and commercial *Maha Varthikava Watee* samples V2, V3 and V5, CC (0.22) was present in all prepared and commercial samples V3, V4 and V5. FF (0.37) was present in all prepared and commercial samples, GG (0.43) was present in prepared samples V0, V3 and commercial samples V1, V2, V3 and V5.

In order to detect Indian privet in samples, of *Maha Varthikava Watee* spots BB, CC, FF and GG (0.18, 0.22, 0.37 and 0.43) (Figure 4.12.8) can be considered which was obtained by using ethanol extract of Indian privet and running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Figure 4.12.2.9. Comparison of Rf values of the authentically prepared sample, with the commercial samples of Maha Varthikava Watee, and sour orange sample. (MV and sour orange were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under iodine vapour.)

Sour orange sample gave a spot namely C (Rf 0.11). It was present in all prepared and commercial Maha Varthikava Watee samples.

In order to detect sour orange in samples, of Maha Varthikava Watee, spot C (0.11) (Figure 4.12.2.9.) can be considered which was obtained by using ethanol extract of sour orange running with toluene: ethyl acetate (9:1 v/v) solvent system and examining under iodine vapour.
Figure 4.12.2.10. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee, and sour orange sample. (MV and sour orange were extracted with ethanol and developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected with vanillin sulfuric acid reagent.)

Sour orange sample contained 12 spots namely A, B, D$_1$, E, E$_1$, F, G$_3$, G$_1$, H, I, L and M$_1$ ($R_f$ 0.05, 0.08, 0.13, 0.22, 0.25, 0.27, 0.33, 0.38, 0.44, 0.5, 0.77 and 0.86). Spots A and D$_1$ (0.05 and 0.13) were present in all prepared and commercial Maha Varthikava Watee samples $V_1$, $V_2$, $V_3$ and $V_4$, G$_3$ (0.27) was present in all prepared and commercial samples $V_2$ and $V_5$; spots G$_1$, H and I (0.38, 0.44 and 0.5) were present in all prepared and commercial samples. L and M$_1$ (0.77 and 0.86) were present in all prepared and commercial sample $V_2$. 

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In order to detect sour orange in samples, of Maha Varthikava Watee spots A, D₁, G₁, G₃, H, I, L and M₁ (Rᵣ 0.05, 0.13, 0.38, 0.27, 0.44, 0.5, 0.77 and 0.86) (Figure 4.12.2.10.) can be considered which was obtained by using ethanol extract of sour orange, running with toluene: ethyl acetate (9:1 v/v) solvent system, sprayed with vanillin sulfuric acid reagent, heated at 105 °C for 10 minutes and observed.

![Graph showing Rf values of samples](image)

**Figure 4.12.2.11.** Comparison of Rᵣ values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and fresh ginger and dry ginger. (MV and gingers were extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent.)

While fresh ginger sample contained 4 spots namely AA, BB, DD and EE, (Rᵣ 0.12, 0.18, 0.25 and 0.31) dry ginger contained 7 spots namely DD, EE, FF, GG, HH, II, KK.
and MM (R_{f} 0.25, 0.31, 0.37, 0.43, 0.50, 0.68 and 0.81). Spot DD (0.25) was present in all prepared and commercial *Maha Varthikava Watee* samples V_{1}, V_{2}, and V_{3}. Spot EE (0.31) was present in all prepared and commercial samples V_{1}, V_{3}, V_{4}, V_{5}; Spot FF (0.37) was present in all prepared and commercial samples V_{3}, V_{4}, V_{5}; Spot II was present in all prepared and commercial samples V_{1}, V_{3}; Spot MM (0.81) was present in all prepared, and commercial sample V_{1}.

In order to detect ginger in samples, of *Maha Varthikava Watee* spots DD, EE, FF, II and MM (0.25, 0.31, 0.37, 0.5 and 0.81) (Figure 4.12.2.11) can be considered which was obtained by using ethanol extract of ginger, running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.
Figure 4.12.2.12. Comparison of $R_f$ values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and asafoetida samples. (MV and asafoetida extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system detected with anisaldehyde sulfuric acid reagent).

Asafoetida sample includes 6 spots namely DD, EE, GG, HH, MM and NN ($R_f$ 0.25, 0.31, 0.43, 0.50, 0.81 and 0.87). Spot DD (0.25) was present in all prepared and commercial Maha Varthikava Watee sample $V_1$, $V_2$, and $V_3$. Spot EE (0.31) was present in all prepared and $V_1$, $V_3$, $V_4$ and $V_5$ commercial samples. Spot GG (0.43) was present in all prepared and commercial samples $V_2$, $V_3$, $V_4$ and $V_5$. Spot HH (0.5) was present in all prepared sample, and commercial sample $V_1$.

In order to detect asafoetida in samples, of Maha Varthikava Watee spots DD, EE, FF, II and MM (0.25, 0.31, 0.37, 0.5 and 0.81) (Figure 4.12.2.12) can be considered
which was obtained by using ethanol extract of asafoetida, running with n-hexane: acetone (75:25 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

Chemicals in asafoetida samples may be destroy in the large scale manufacturing process, procedures, storage and storing period.

![Graph showing Rf values](image)

Figure 4.12.2.13. Comparison of Rf values of authentically prepared samples, with the commercial samples of Maha Varthikava Watee and iron wood flower stigma samples. (MV and iron wood flower stigma were extracted with ethanol and developed using n-hexane: acetone (3:1 v/v) solvent system, detected with anisaldehyde sulfuric acid reagent).

Iron wood flower stigma sample includes 9 spots namely AA, BB, DD, FF, FF1, GG, II, JJ and NN (Rf 0.12, 0.18, 0.25, 0.37, 0.4, 0.43, 0.56, 0.63 and 0.86). Spot DD (0.25) was
present in all prepared and \( V_1, V_2 \) and \( V_3 \) commercial Maha Varthikava Watee samples. Spot FF (0.37) was present in all prepared and \( V_3, V_4 \) and \( V_5 \) commercial samples. Spot GG (0.43) was present in all prepared and \( V_2, V_3 \) and \( V_4 \) commercial samples.

In order to detect iron wood in samples of Maha Varthikava Watee spots DD, FF and GG (0.25, 0.37 and 0.43) (Figure 4.12.2.13) can be considered which was obtained by using ethanol extract of iron wood stigma, running with n-hexane: acetone (75:25 v/v) solvent system and sprayed with anisaldehyde sulfuric acid reagent, heated at 110 °C for 10 minutes and observed.

Pleasant smell of iron wood flower stigmas may be due to the various volatile chemicals. It might be destroyed in the large-scale manufacturing process, procedures, storage and storing period. Therefore, the \( R_f \) values can be used as standardization procedures of the Maha Varthikava Watee.

![TLC profile of Maha Varthikava Watee extract of n-hexane developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under UV light 254 nm and 366 nm and sprayed with anisaldehyde sulfuric acid reagent.](image)

\[ Ab1 \text{ (UV254)} \quad Bb2 \text{ (UV 366)} \quad \text{Cb3 (After sprayed)} \]

\[ 1V_0 - 3V_0^{15} \quad V_1 - V_5^{16}. \]

**Figure 4.12.2.14.** TLC profile of Maha Varthikava Watee extract of n-hexane developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under UV light 254 nm and 366 nm and sprayed with anisaldehyde sulfuric acid reagent.

\(^{15}\) Authentically prepared samples
\(^{16}\) commercial samples
Figure 4.12.2.15. TLC profile of Maha Varthikava Watee, extract of dichloromethane developed using toluene: ethyl acetate (9:1 v/v) solvent system, detected under UV light 254 nm and 366 nm and sprayed with anisaldehyde sulfuric acid reagent.

Figure 4.12.2.16. TLC profile of Maha Varthikava Watee extract of ethyl acetate developed using solvent system toluene: ethyl acetate (9:1 v/v) solvent system, detected under UV light 254 nm and 366 nm and sprayed with anisaldehyde sulfuric acid reagent.
**Figure 4.12.2.17.** TLC profile of Maha Varthikava Watee extracted of methanol developed using toluene: ethyl acetate (9:1 v/v) solvent system, detect under UV light 254 nm and 366 nm and sprayed with anisaldehyde sulfuric acid reagent.

Maha Varthikava Watee and the selected raw materials, betel and indian privet were extracted by ethanol, spotted on a TLC plate developed in toluene: ethyl acetate (90:10 v/v) solvent system and detected under 254 nm UV light. Five spots (R_f 0.05, 0.22, 0.27, 0.38 and 0.5) in betel and indian privet samples were detected that had the equal R_f values to Maha Varthikava Watee. (Figure 7.v.xi, 7.v.xiii)

When the same TLC plates were detected under iodine vapour, 4 spots (R_f 0.11, 0.35, 0.52 and 0.55) for betel sample, 3 spots (R_f 0.05, 0.11 and 0.25) for indian privet sample, were detected. Those R_f values were equal to the Maha Varthikava Watee (Figure 8.v.xii, 8.v.xiii).

Again the same plates were sprayed with vanillin sulfuric acid and heated to 105 °C, 4 spots (R_f 0.05, 0.11, 0.44 and 0.86) were detected in betel sample, 6 spots (R_f 0.05, 0.11, 0.38, 0.44, 0.77 and 0.86) in indian privet sample, were detected. The R_f values of these raw materials were equal to the R_f values of the Maha Varthikava Watee (Figure 7.v.xii, 7.v.xiii).
When TLC of the Maha Varthikava Watee and ethanol extract of the selected raw materials of that preparation were developed in n-hexane: acetone (7.5:2.5 v/v) solvent system, sprayed with anisaldehyde sulfuric acid reagent and heated to 105 °C for 10 minutes 3 spots in (R_f 0.18, 0.37 and 0.43) were observed in betel sample, 4 spots in Indian privet (R_f 0.18, 0.22, 0.37 and 0.43) 5 in ginger (R_f 0.25, 0.31, 0.37, 0.56 and 0.81) 4 in asafoetida (R_f 0.25, 0.31, 0.43 and 0.5), and 3 in iron wood stigma (R_f 0.25, 0.37 and 0.43). The R_f values of these raw materials were equal to the R_f values of the Maha Varthikava Watee (Figure 7 'xxv).

Toluene: ethyl acetate (9:1 v/v) solvent system can be used for TLC analysis of Maha Varthikava Watee betel and Indian privet.

n-Hexane: acetone (75:25 v/v) solvent system can be used for TLC analysis of Maha Varthikava Watee for ginger and iron wood flower stigma.

Both the toluene: ethyl acetate (9:1 v/v) and the n-hexane: acetone (75:25 v/v) solvent system can be used for TLC analysis of Maha Varthikava Watee for Indian privet.

Under similar conditions, one solvent system should have similar pattern of separation. Therefore, compounds with same R_f should be the same compound in different raw materials and as such, the content of similar compound (as per zone) should be accumulated in the final drug. If not, it should have formed a different compound, which can be an answer for synergistic effect of these herbal and herbo mineral preparations.
4.13. UV Spectrophotometric studies:

The word spectroscopy is used as a collective term for all the analytical techniques based on the interaction of light and matter. Spectrophotometry is one of the branches of spectroscopy where the absorption of light by molecules that are in gas or in vapour state or dissolved molecules or ions is measured. Spectrophotometry investigates the absorption of the different substances between the wavelength limits 190 nm and 780 nm. Lambda max ($\lambda_{\text{max}}$) is just the wavelength of light that the molecules have the maximum absorbance.

**Table 4.13.1. $\lambda_{\text{max}}$ values of the authentically prepared and commercial samples of Seetharama Watee.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>$\lambda_{\text{max}},$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S0</td>
<td>0.51, 0.73</td>
<td>287</td>
</tr>
<tr>
<td>2S0</td>
<td>0.48, 0.45</td>
<td>287</td>
</tr>
<tr>
<td>3S0</td>
<td>0.62, 0.42</td>
<td>287</td>
</tr>
<tr>
<td>S1</td>
<td>1.4, 0.70</td>
<td>287</td>
</tr>
<tr>
<td>S2</td>
<td>1.2, 0.68</td>
<td>290</td>
</tr>
<tr>
<td>S3</td>
<td>0.64, 0.56</td>
<td>287</td>
</tr>
<tr>
<td>S4</td>
<td>1.0, 0.62</td>
<td>290</td>
</tr>
<tr>
<td>S5</td>
<td>0.86, 0.57</td>
<td>287</td>
</tr>
</tbody>
</table>

It can be seen from the spectrophotometric measurements; all prepared samples and commercial samples $S_1$, $S_3$ and $S_5$ have a $\lambda_{\text{max}}$ of 287 nm and the $S_2$ and $S_4$ samples have a $\lambda_{\text{max}}$ 290 nm. The chemical compounds of all prepared and the most of the commercial samples of $\lambda_{\text{max}}$ value is around 287-290 nm. All the samples have almost
equal $\lambda_{\text{max}}$ values, so the chemical compounds are almost same in all formulations. The actions of all samples may have a close relationship.

Table 4.13.2. $\lambda_{\text{max}}$ values of authentically prepared and commercial samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>$\lambda_{\text{max}}$, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>0.58, 0.54</td>
<td>287</td>
</tr>
<tr>
<td>$V_2$</td>
<td>0.92, 0.58</td>
<td>287</td>
</tr>
<tr>
<td>$V_3$</td>
<td>1.2, 0.83</td>
<td>290</td>
</tr>
<tr>
<td>$V_1$</td>
<td>0.42, 0.82, 1.6</td>
<td>345, 340</td>
</tr>
<tr>
<td>$V_2$</td>
<td>0.95, 0.35, 1.3</td>
<td>287, 290</td>
</tr>
<tr>
<td>$V_3$</td>
<td>1.0, 1.6</td>
<td>345, 340</td>
</tr>
<tr>
<td>$V_4$</td>
<td>1.0, 1.24</td>
<td>345, 340</td>
</tr>
<tr>
<td>$V_5$</td>
<td>1.29, 0.5</td>
<td>287</td>
</tr>
</tbody>
</table>

It is clear that all the prepared samples and the commercial samples $V_2$ and $V_5$ have a $\lambda_{\text{max}}$ 287-290 nm while the $V_1$, $V_2$ and $V_4$ samples were in $\lambda_{\text{max}}$ 340-345 nm. The chemical compounds related to $\lambda_{\text{max}}$ value are around 287-290 nm in all prepared and two commercial samples and another chemical compounds in $\lambda_{\text{max}}$ value are around 340-345 nm in other three commercial samples. Therefore the prepared and $V_2$ and $V_5$ commercial samples have the same chemical compounds, while the other three commercial samples have different. The actions of all the prepared samples and the two commercial samples may have a close relationship while in the other three commercial samples the actions may be different.
Table 4.13.3. $\lambda_{\text{max}}$ Values of Garlic, Asafoetida, Red sandal wood, Ginger, Cinnamon sedge, Holy basil, Indian privet, Neem, Sour orange, Betel, Bee honey, Ghee and Margosa oil.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance</th>
<th>$\lambda_{\text{max}}, \text{nm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>0.77</td>
<td>287</td>
</tr>
<tr>
<td>Asafoetida i</td>
<td>0.4, 0.38</td>
<td>287, 320</td>
</tr>
<tr>
<td>Red sandal wood(i S/L)</td>
<td>0.39, 0.36</td>
<td>287, 320</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.38</td>
<td>285</td>
</tr>
<tr>
<td>cinnamon sedge</td>
<td>0.66</td>
<td>295</td>
</tr>
<tr>
<td>Holy basil</td>
<td>0.66</td>
<td>287</td>
</tr>
<tr>
<td>Indian privet</td>
<td>0.67, 0.72</td>
<td>285, 330</td>
</tr>
<tr>
<td>Neem</td>
<td>0.53</td>
<td>287</td>
</tr>
<tr>
<td>Sour orange</td>
<td>0.66</td>
<td>287</td>
</tr>
<tr>
<td>Betel</td>
<td>0.73</td>
<td>287</td>
</tr>
<tr>
<td>Asafoetida ii</td>
<td>0.38, 0.48</td>
<td>287, 320</td>
</tr>
<tr>
<td>Bees honey</td>
<td>0.98</td>
<td>293</td>
</tr>
<tr>
<td>Red sandal wood (ii Indian)</td>
<td>0.68</td>
<td>287</td>
</tr>
<tr>
<td>Ghee</td>
<td>0.25</td>
<td>287</td>
</tr>
<tr>
<td>Margosa oil</td>
<td>1.62, 1.69</td>
<td>293</td>
</tr>
</tbody>
</table>
Ethanol extracts of the raw materials garlic, asafoetida, Indian and Lanka red sandalwood, holy basil, neem, sour orange, betel, bee’s honey show \( \lambda_{\text{max}} \) value of 287 nm, asafoetida and red sandalwood show \( \lambda_{\text{max}} \) value of 320 nm. The \( \lambda_{\text{max}} \) value of ethanol extracts of Indian privet and ginger samples is 285 nm, whilst ethanol extract of Indian privet has a \( \lambda_{\text{max}} \) value of 330 nm.


The chromatographic fingerprints can successfully demonstrate both similarities and differences between various samples. The authentication and identification of herbal medicines can therefore be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicines.

4.14.1.1. HPLC analysis of the sample 1S0:

Six peaks were identified and they were labeled as A, B, C, C2, D and E. Peak E (12.9 mg/mL) had the largest area, which implies that, the activity of the compound corresponding to E is the highest in Seetharama Watee (Figure 4.14.1).

Figure 4.14.1. HPL chromatogram of the Sample 1S0 (acetonitrile: water, 20:30 v/v)
Six main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the IS₀ sample of the drug (Table 4.14.1.).

Table 4.14.1. HPLC analysis of sample IS₀ (254 nm acetonitrile: water 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time ,min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.49</td>
<td>81973</td>
<td>4.507</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>14.37</td>
<td>35538</td>
<td>1.954</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>26.23</td>
<td>112146</td>
<td>6.166</td>
</tr>
<tr>
<td>4</td>
<td>C₂</td>
<td>35.15</td>
<td>73982</td>
<td>4.068</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>37.73</td>
<td>76605</td>
<td>4.212</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>42.49</td>
<td>235021</td>
<td>12.923</td>
</tr>
</tbody>
</table>

4.14.1.2. HPLC analysis of the sample 2S₀:

Seven peaks were identified, and they were labeled as A, B, C, C₂, D and E. Peak C (12.54 mg/mL) had the largest area, which implies that, the activity of the compound corresponding to C is the highest in Seetharama Watee (Figure 4.14.2).
Figure 4.14.2. HPL chromatogram of sample 2S₀ (acetonitrile: water, 20:30 v/v)

Seven main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the 2S₀ sample of the drug (Table 4.14.2.).

Table 4.14.2. HPLC analysis of sample 2S₀ (254 nm acetonitrile: water 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.64</td>
<td>43104</td>
<td>6.170</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>12.93</td>
<td>36047</td>
<td>5.171</td>
</tr>
<tr>
<td>3</td>
<td>B₁</td>
<td>23.20</td>
<td>34282</td>
<td>4.918</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>29.26</td>
<td>87384</td>
<td>12.535</td>
</tr>
<tr>
<td>5</td>
<td>C₂</td>
<td>31.63</td>
<td>32796</td>
<td>4.704</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>36.16</td>
<td>22626</td>
<td>3.246</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>41.83</td>
<td>8079</td>
<td>1.159</td>
</tr>
</tbody>
</table>
4.14.1.3. HPLC analysis of the sample 3S₀:

Eight peaks were identified and they were labeled as A, A₁, B, C, C₂, D, E and F. Peak E (5.9 mg/mL) had indicated the largest area, which implies that, the activity of the compound corresponding to E is the highest in Seetharama Watee (Figure 4.14.3).

![HPL chromatogram of sample 3S₀ (acetonitrile: water, 20:30 v/v)](image)

Eight main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the 3S₀ sample of the drug (Table 4.14.3.).
Table 4.14.3 HPLC analysis of sample 3S₀ (254 nm acetonitrile: water 20:30 v/v)

<table>
<thead>
<tr>
<th>No</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.823</td>
<td>91919</td>
<td>4.544</td>
</tr>
<tr>
<td>2</td>
<td>A1</td>
<td>10.312</td>
<td>27640</td>
<td>1.366</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>15.24</td>
<td>98201</td>
<td>4.855</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>28.65</td>
<td>88422</td>
<td>4.371</td>
</tr>
<tr>
<td>5</td>
<td>C2</td>
<td>35.49</td>
<td>22523</td>
<td>1.114</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>39.4</td>
<td>114171</td>
<td>5.644</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>42.22</td>
<td>120263</td>
<td>5.946</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>47.68</td>
<td>60191</td>
<td>2.976</td>
</tr>
</tbody>
</table>

4.14.1.4. HPLC analysis of the sample 5₁:

Eight peaks were identified that they were labeled as A, A₁, B, C, C₂, D, E and F. Peak F (11.6 mg/mL) had indicated the largest area which implies that the activity of the compound corresponding to F is the highest in Seetharama Water (Figure 4.14.4).

Figure 4.14.4 HPL chromatogram of sample 5₁ (acetonitrile: water, 20:30 v/v)

Eight main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the 5₁ sample of the drug (Table 4.14.1.).
Table 4.14.4 HPLC analysis of S₁ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.89</td>
<td>138105</td>
<td>4.362</td>
</tr>
<tr>
<td>2</td>
<td>A₁</td>
<td>10.25</td>
<td>4773</td>
<td>0.151</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>13.79</td>
<td>7862</td>
<td>0.248</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>28.93</td>
<td>70527</td>
<td>2.228</td>
</tr>
<tr>
<td>5</td>
<td>C₂</td>
<td>35.13</td>
<td>13037</td>
<td>0.412</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>39.77</td>
<td>85643</td>
<td>2.705</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>42.64</td>
<td>93753</td>
<td>2.961</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>48.54</td>
<td>367529</td>
<td>11.608</td>
</tr>
</tbody>
</table>

4.14.1.5. HPLC analysis of the sample S₂:

Nine peaks were identified that they were labeled as A, A₁, B, C, C₂, D, D₁, E and F. Peak E (12.9 mg/mL) had indicated the largest area which implies that the activity of the compound corresponding to E is the highest in Seetharama Watee (Figure 4.14.5).

![Figure 4.14.5 HPL chromatogram of sample S₂ (acetonitrile: water, (20:30 v/v)](image)

Nine main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the S₂ sample of the drug (Table 4.14.5.).
Table 4.14.5. HPLC analysis of sample S_2 (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.73</td>
<td>131310</td>
<td>5.423</td>
</tr>
<tr>
<td>2</td>
<td>A1</td>
<td>10.12</td>
<td>108915</td>
<td>4.498</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>15.04</td>
<td>18020</td>
<td>0.744</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>28.33</td>
<td>26408</td>
<td>1.091</td>
</tr>
<tr>
<td>5</td>
<td>C2</td>
<td>34.59</td>
<td>16713</td>
<td>0.690</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>38.1</td>
<td>63734</td>
<td>2.632</td>
</tr>
<tr>
<td>7</td>
<td>D1</td>
<td>39.03</td>
<td>134255</td>
<td>5.544</td>
</tr>
<tr>
<td>8</td>
<td>E</td>
<td>41.92</td>
<td>314476</td>
<td>12.986</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>46.6</td>
<td>126795</td>
<td>5.236</td>
</tr>
</tbody>
</table>

4.14.1.6. HPLC analysis of sample S_3:

Seven peaks were identified that they were labeled as A, A_1, B, C, D, E, and F. Peak A (4.1 mg/mL) had indicated the largest area that implies that the activity of the compound corresponding to E is the highest in Seetharama Watee (Figure 4.14.6).
Figure 4.14.6. HPL chromatogram of S₃ (acetonitrile: water, 20:30 v/v)

Seven main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the S₃ sample of the drug (Table 4.14.6.).

Table 4.14.6. HPLC analysis of sample S₃ (254 nm acetonitrile: water 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.96</td>
<td>50977</td>
<td>4.108</td>
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<tr>
<td>2</td>
<td>A₁</td>
<td>10.60</td>
<td>13152</td>
<td>1.059</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>15.54</td>
<td>4193</td>
<td>0.337</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>29.21</td>
<td>17406</td>
<td>1.402</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>40.20</td>
<td>32342</td>
<td>2.606</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>43.08</td>
<td>32.963</td>
<td>2.656</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>49.46</td>
<td>3732</td>
<td>0.301</td>
</tr>
</tbody>
</table>
4.14.1.7. HPLC analysis of the sample S₄:

Eleven peaks were identified that they were labeled as A, A₁, A₂, B, B₁, B₂, C, C₂, D, E and F. Peak F (4.7 mg/mL) had indicated the largest area which implies that the activity of the compound corresponding to F is the highest in Seetharama Watee, while the peak E (4.3 mg/mL) also had the larger area value (Figure 4.14.7).

![HPL chromatogram of sample S₄ (acetonitrile: water, 20:30 v/v)](image)

Figure 4.14.7. HPL chromatogram of sample S₄ (acetonitrile: water, 20:30 v/v)

Eleven main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the S₄ sample of the drug (Table 4.14.7.).
Table 4.14.7 HPLC analysis of sample $S_4$ (254 nm: acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No</th>
<th>Label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>7.75</td>
<td>83460</td>
<td>4.591</td>
</tr>
<tr>
<td>2</td>
<td>A1</td>
<td>10.16</td>
<td>52862</td>
<td>2.907</td>
</tr>
<tr>
<td>3</td>
<td>A2</td>
<td>13.51</td>
<td>30039</td>
<td>1.652</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>14.63</td>
<td>25731</td>
<td>1.415</td>
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<tr>
<td>5</td>
<td>B1</td>
<td>24.27</td>
<td>17557</td>
<td>0.965</td>
</tr>
<tr>
<td>6</td>
<td>B2</td>
<td>25.63</td>
<td>62279</td>
<td>3.425</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>28.36</td>
<td>38922</td>
<td>2.141</td>
</tr>
<tr>
<td>8</td>
<td>C2</td>
<td>34.46</td>
<td>17440</td>
<td>0.959</td>
</tr>
<tr>
<td>9</td>
<td>D</td>
<td>39.04</td>
<td>76406</td>
<td>4.202</td>
</tr>
<tr>
<td>10</td>
<td>E</td>
<td>41.84</td>
<td>78588</td>
<td>4.323</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>46.70</td>
<td>85570</td>
<td>4.707</td>
</tr>
</tbody>
</table>

4.14.1.8. HPLC analysis of the sample $S_5$:

Five peaks were identified that they were labeled as A, B, C, D and E. Peak E (6.6 mg/mL) had indicated the largest area, which implies that, the activity of the compound corresponding to E is the highest in *Seetharama Watee* (Figure 4.14.8).
Five main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the $S_5$ sample of the drug (Table 4.14.8.).

**Table 4.14.8. HPLC analysis of sample $S_5$ (254 nm acetonitrile: water 20:30 v/v)**

<table>
<thead>
<tr>
<th>No</th>
<th>Peak label</th>
<th>Time, min.</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
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<td>69944</td>
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</tr>
<tr>
<td>2</td>
<td>B</td>
<td>14.17</td>
<td>14028</td>
<td>0.916</td>
</tr>
<tr>
<td>3</td>
<td>C2</td>
<td>35.02</td>
<td>96667</td>
<td>6.312</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>37.64</td>
<td>80511</td>
<td>5.257</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>42.48</td>
<td>101168</td>
<td>6.606</td>
</tr>
</tbody>
</table>

When comparing the even the authentically prepared samples, some extra peaks ($C_1=28.45$ min., $C_2=35.145$ min.) could be identified. That may be due to the seasonal effects of the fresh raw materials.
Table 4.14.9. Concentration of chemical compounds in Seetharama Watee in HPLC analysis.

<table>
<thead>
<tr>
<th></th>
<th>IS₀</th>
<th>2S₀</th>
<th>3S₀</th>
<th>S₁</th>
<th>S₂</th>
<th>S₃</th>
<th>S₄</th>
<th>S₅</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
<td>mg/mL</td>
</tr>
<tr>
<td>A1</td>
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<td></td>
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<td>4.497</td>
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<td></td>
<td>2.907</td>
<td></td>
</tr>
<tr>
<td>A2</td>
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<td></td>
<td></td>
<td></td>
<td>0.652</td>
<td></td>
</tr>
<tr>
<td>B</td>
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<td>1.872</td>
<td>4.854</td>
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<td>0.744</td>
<td>0.337</td>
<td>1.415</td>
<td>0.916</td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.425</td>
<td></td>
</tr>
<tr>
<td>C</td>
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<td>1.090</td>
<td>1.403</td>
<td>2.141</td>
<td></td>
</tr>
<tr>
<td>C1</td>
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<td></td>
<td>6.312</td>
<td></td>
</tr>
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<td>4.068</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>4.212</td>
<td>2.403</td>
<td>5.644</td>
<td>2.704</td>
<td>2.631</td>
<td>2.606</td>
<td>4.202</td>
<td>5.257</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
<td>5.544</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>12.923</td>
<td>2.430</td>
<td>5.945</td>
<td>2.961</td>
<td>12.986</td>
<td>2.656</td>
<td>4.323</td>
<td>6.606</td>
</tr>
<tr>
<td>F</td>
<td>1.936</td>
<td>2.975</td>
<td>11.607</td>
<td>5.236</td>
<td>0.301</td>
<td>4.707</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chemical compound A, B, D and E are present in all prepared and commercial samples. Although the chemical compound C and F are present in all prepared sample and commercial samples except commercial sample S₅.

The retention times of these chemical compounds (A=7-8, B=14-17, C=26-29, D=37-40, E=41-43 min.) could be taken as the measurements for the standardization of Seetharama Watee using the solvent system acetonitrile: water (20:30 v/v) at 254 nm.
Figure 4.14.9. Chemical Compound Concentrations of Prepared Samples of Seetharama - HPL Chromatogram.
Figure 4.14.10. Chemical Compound Concentrations of Commercial Samples of Seetharama- HPL Chromatogram

4.14.2.1. HPLC analysis of the sample 1V₀:

Eleven peaks were identified that they were labeled as A, B, C, G, H, I, K, M, N, O and S. Peak K (21.4 mg/mL) indicates the largest area which implies that the activity of the compounds corresponding to K is the highest in Maha Varthikava Watee (Figure 4.14.11).
Eleven main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the sample of the drug (Table 4.14.10.).

Table 4.14.10. HPLC analysis of sample $1V_0$ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time min.</th>
<th>Area,mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>5.04</td>
<td>13347</td>
<td>1.349</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>5.50</td>
<td>19382</td>
<td>1.959</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>6.85</td>
<td>7019</td>
<td>0.709</td>
</tr>
<tr>
<td>4</td>
<td>G</td>
<td>13.31</td>
<td>4819</td>
<td>0.487</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>14.85</td>
<td>3687</td>
<td>0.372</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>19.74</td>
<td>11164</td>
<td>1.218</td>
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<tr>
<td>7</td>
<td>K</td>
<td>22.15</td>
<td>211892</td>
<td>21.417</td>
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<tr>
<td>8</td>
<td>M</td>
<td>25.32</td>
<td>40937</td>
<td>4.137</td>
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<td>9</td>
<td>N</td>
<td>27.62</td>
<td>37186</td>
<td>3.758</td>
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<tr>
<td>10</td>
<td>O</td>
<td>29.22</td>
<td>4053</td>
<td>0.409</td>
</tr>
<tr>
<td>11</td>
<td>S</td>
<td>37.25</td>
<td>7580</td>
<td>0.766</td>
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</tbody>
</table>
4.14.2.2. HPLC analysis of the sample 2V₀:

Seventeen peaks were identified that they were labeled as A, B, C, E, F, G, H, I, J, K, L, M, N, P, Q, R and T. Peak M (6.3 mg/mL) indicated the largest area which implies that the activity of the compound, corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.12).

Figure 4.14.12. HPL chromatogram of the sample 2V₀ (acetonitrile: water, 20:30 v/v)

Seventeen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the 2V₀ sample of the drug (Table 4.14.1.).
Table 4.14.11. HPLC analysis of sample of $2V_0$ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>A</td>
<td>5.12</td>
<td>84457</td>
<td>1.206</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>5.69</td>
<td>115160</td>
<td>1.644</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>6.56</td>
<td>13299</td>
<td>0.189</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>8.60</td>
<td>28981</td>
<td>0.413</td>
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<tr>
<td>5</td>
<td>F</td>
<td>12.55</td>
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<td>G</td>
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<td>H</td>
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<td>I</td>
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<td>10631</td>
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<td>J</td>
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<td>0.151</td>
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<tr>
<td>10</td>
<td>K</td>
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<td>270091</td>
<td>3.856</td>
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<tr>
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<td>L</td>
<td>23.89</td>
<td>31887</td>
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</tr>
<tr>
<td>12</td>
<td>M</td>
<td>26.75</td>
<td>441867</td>
<td>6.309</td>
</tr>
<tr>
<td>13</td>
<td>N</td>
<td>29.17</td>
<td>112263</td>
<td>1.603</td>
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<tr>
<td>14</td>
<td>P</td>
<td>32.33</td>
<td>54679</td>
<td>0.780</td>
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<tr>
<td>15</td>
<td>Q</td>
<td>33.20</td>
<td>76850</td>
<td>1.097</td>
</tr>
<tr>
<td>16</td>
<td>R</td>
<td>35.44</td>
<td>41392</td>
<td>0.591</td>
</tr>
<tr>
<td>17</td>
<td>T</td>
<td>39.26</td>
<td>15698</td>
<td>0.224</td>
</tr>
</tbody>
</table>
4.14.2.3. HPLC analysis of the sample 3V₀:

Fifteen peaks were identified that they were labeled as A, B, C, D, E, F, H, I, J, K, M, N, P, Q, and T. Peak M (8.85 mg/mL) indicated the largest area which implies that the activity of the compound, corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.13).

![HPL Chromatogram of 3V₀ (acetonitrile: water, 20:30 v/v)](image)

Figure 4.14.13. HPL Chromatogram of 3V₀ (acetonitrile: water, 20:30 v/v)

Fifteen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the 3V₀ sample of the drug (Table 4.14.12.).
Table 4.14.12. HPLC analysis of sample 3V₀ (254 nm acetone: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>5.02</td>
<td>5049</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>5.59</td>
<td>18004</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>6.68</td>
<td>4282</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>7.08</td>
<td>370</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>8.46</td>
<td>8342</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>12.4</td>
<td>33987</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>16.86</td>
<td>30624</td>
</tr>
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<td>8</td>
<td>I</td>
<td>19.51</td>
<td>20061</td>
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<td>J</td>
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<td>K</td>
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<tr>
<td>15</td>
<td>T</td>
<td>39.10</td>
<td>10368</td>
</tr>
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</table>
4.14.2.4. HPLC analysis of the sample $V_1$:

Eighteen peaks were identified that they were labeled as A, B, C, E, E1, F, G, H, I0, I, J, K, L, M, N, O, P and R. Peak M (67.2 mg/mL) indicated the largest area which implies that the activity of the compound, corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.14).

![HPL Chromatogram of $V_1$ (acetonitrile: water, 20:30 v/v)](image)

Figure 4.14.14. HPL Chromatogram of $V_1$ (acetonitrile: water, 20:30 v/v)

Eighteen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the $V_1$ sample of the drug (Table 4.14.13.).
Table 4.14.13. HPLC analysis of sample V₁ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>C</td>
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</tr>
<tr>
<td>7</td>
<td>G</td>
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<td>O</td>
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<td>P</td>
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<tr>
<td>18</td>
<td>R</td>
<td>34.12</td>
<td>6357</td>
<td>0.053</td>
</tr>
</tbody>
</table>
4.14.2.5. HPLC analysis of the sample V₂:

Fourteen peaks were identified that they were labeled as A, B, C, E, F, G, I, J, K, L, M, N, P, and S. Peak M (7.8 mg/mL) indicates the largest area which implies that the activity of the compound corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.15).

![HPL chromatogram of the sample V₂](image)

Figure 4.14.15. HPL chromatogram of the sample V₂ (acetonitrile: water, 20:30 v/v)

Fourteen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the V₂ sample of the drug (Table 4.14.14.).
Table. 4.14.14 HPLC analysis of sample V₂ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
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<td>C</td>
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<td>66078</td>
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<td>4</td>
<td>E</td>
<td>8.67</td>
<td>31596</td>
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<td>F</td>
<td>12.08</td>
<td>18362</td>
</tr>
<tr>
<td>6</td>
<td>G</td>
<td>13.45</td>
<td>3536</td>
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<td>I₀</td>
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<td>18002</td>
</tr>
<tr>
<td>8</td>
<td>I</td>
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<td>42590</td>
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<tr>
<td>9</td>
<td>K</td>
<td>21.30</td>
<td>143787</td>
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<td>111159</td>
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<td>M</td>
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<td>14970</td>
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</table>

4.14.2.6. HPLC analysis of the sample V₃:

Eighteen peaks were identified that they were labeled as A, B, C, D, E, E₁, F, G, H, I₀, I, J, L, M, N, P, R and S. Peak M (63.5 mg/mL) indicates the largest area which implies that the activity of the compound corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.16).
Eighteen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the V3 sample of the drug (Table 4.14.15.).
Table 4.14.15. HPLC analysis of sample V₃ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
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<th>Time, min</th>
<th>Area,mAU</th>
<th>Concentration mg/mL</th>
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</thead>
<tbody>
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<td>C</td>
<td>6.43</td>
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<td>D</td>
<td>7.67</td>
<td>55472</td>
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<td>8.62</td>
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<td>I</td>
<td>19.67</td>
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<td>J</td>
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<td>S</td>
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4.14.2.7. HPLC analysis of the sample $V_4$:

Twenty peaks were identified that they were labeled as A, B, C, E, E$_1$, F, G, H, I$_0$, I, J, K, L, M, N, O, P, R, S and T. Peak M (46.8 mg/mL) indicates the largest area which implies that the activity of the compound corresponding to M is the highest in Maha Varthikava Watee (Figure 4.14.17).

![HPL chromatogram of the sample $V_4$](image)

Figure 4.14.17. HPL chromatogram of the sample $V_4$ (acetonitrile: water, 20:30 v/v)

Seventeen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the $V_4$ sample of the drug (Table 4.14.16.).
Table 4.14.16. HPLC analysis of sample V₄ (254 nm acetonitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
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</tr>
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<td>C</td>
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<tr>
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<td>E</td>
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<tr>
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<td>E1</td>
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<td>6</td>
<td>F</td>
<td>12.12</td>
<td>84116</td>
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<tr>
<td>7</td>
<td>G</td>
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<td>33035</td>
<td>0.265</td>
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<tr>
<td>8</td>
<td>H</td>
<td>15.12</td>
<td>194069</td>
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<tr>
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<td>I0</td>
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</tr>
<tr>
<td>10</td>
<td>I</td>
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<td>J</td>
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<td>20</td>
<td>T</td>
<td>39.73</td>
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</table>
4.14.2.8. HPLC analysis of the sample V₅:

Thirteen peaks were identified that they were labeled as B, C, E₁, E₂, H, K, L, M, N, O, P and T. Peak M (5.6 mg/mL) indicates the largest area which implies that the activity of the compound corresponding to M is the highest in *Maha Varthikava Watee* (Figure 4.15.18).

![HPL chromatogram of the sample V₅](image-url)

**Figure 4.14.18. HPL chromatogram of the sample V₅ (acetonitrile: water, 20:30 v/v)**

Thirteen main chemical components were identified in different concentrations and some of them may be involved in the efficacy of the V₅ sample of the drug.
Table 4.14.17. HPLC analysis of sample V₅ (254 nm acetoneitrile: water, 20:30 v/v)

<table>
<thead>
<tr>
<th>No</th>
<th>Peak label</th>
<th>Time, min</th>
<th>Area, mAU</th>
<th>Concentration mg/mL</th>
</tr>
</thead>
<tbody>
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<td>102177</td>
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<tr>
<td>5</td>
<td>E₂</td>
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<tr>
<td>6</td>
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</tbody>
</table>

When comparing even the authentically prepared samples, some extra peaks were identified. In the sample 2V₀ there were 18 peaks while the 1V₀ and 3V₀ there were 11 and 14 peaks respectively. Those differences may be due to the seasonal effects of the fresh raw materials. Therefore, only the main peaks have to be considered.
Table 4.14.18. Concentration of chemical compounds in Maha Varthikava Watee in HPLC analysis.

<table>
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<th></th>
<th>1V_0 mg/mL</th>
<th>2V_0 mg/mL</th>
<th>3V_0 mg/mL</th>
<th>V_1 mg/mL</th>
<th>V_2 mg/mL</th>
<th>V_3 mg/mL</th>
<th>V_4 mg/mL</th>
<th>V_5 mg/mL</th>
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</thead>
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<td>1.965</td>
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</tr>
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<td>0.820</td>
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<td></td>
<td></td>
</tr>
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<td>0.692</td>
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<td>4.137</td>
<td>6.309</td>
<td>8.858</td>
<td>67.226</td>
<td>7.820</td>
<td>63.546</td>
<td>46.867</td>
<td>5.662</td>
</tr>
<tr>
<td>N</td>
<td>3.758</td>
<td>1.603</td>
<td>3.750</td>
<td>5.112</td>
<td>1.888</td>
<td>5.032</td>
<td>5.060</td>
<td>5.136</td>
</tr>
<tr>
<td>O</td>
<td>0.409</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.780</td>
<td>0.416</td>
<td>0.231</td>
<td>1.426</td>
<td>0.178</td>
<td>0.199</td>
<td>0.863</td>
<td></td>
</tr>
</tbody>
</table>

243
Chemical compounds B, C, I, M and N were present in all prepared and commercial samples. Chemical compound H is present in all prepared samples and all commercial samples except sample V_2. In the case of compound K it is not present in sample V_3 and V_5. Higher concentration of chemical compound M was found in V_1 (67.22) V_3 (63.55) and V_4 (46.87). Those higher amount of chemical M might be due to the recipe changes and it may contain higher amount of the specific raw material.

The retention times of these compounds (B = 5-6, C = 6-7, G = 13-14, H = 15-17, I = 19-20, K = 21-23, M = 25-28, N = 28-30 min.) could be taken as the measurements for the standardization of the *Maha Varthikava Watee* using the solvent system acetonitrile: water (20:30 v/v) at 254 nm.

<table>
<thead>
<tr>
<th></th>
<th>1V_0 mg/mL</th>
<th>2V_0 mg/mL</th>
<th>3V_0 mg/mL</th>
<th>V_1 mg/mL</th>
<th>V_2 mg/mL</th>
<th>V_3 mg/mL</th>
<th>V_4 mg/mL</th>
<th>V_5 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>1.097</td>
<td>0.496</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.663</td>
</tr>
<tr>
<td>R</td>
<td>0.591</td>
<td></td>
<td>0.053</td>
<td></td>
<td></td>
<td>0.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.766</td>
<td></td>
<td></td>
<td>0.243</td>
<td>0.031</td>
<td></td>
<td>1.148</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.224</td>
<td>0.666</td>
<td></td>
<td></td>
<td></td>
<td>0.035</td>
<td>0.169</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.14.19. Chemical Compound Concentrations of Prepared Samples of Maha Varthikava-HPL Chromatogram.
Figure 4.14.20. Chemical Compound Concentrations of Commercial Samples of Maha Varthikava-HPL Chromatogram.
4.15. Heavy metals: Using Atomic Absorption Spectroscopy (AAS)

Herbo-mineral and herbal medicinal preparations may contain heavy metals. Specially in herbo-mineral formulations which contain heavy metal based raw materials. Determination of heavy metal content may help to standardize the products, SW and MV. Mainly mercury, arsenic, lead and cadmium amounts were determined as heavy metals.

4.15.1. Mercury (Hg):

Table 4.15.1.1. Mercury content of the authentically prepared and the commercial samples of Seetharama Water.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Hg Content ppm</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>3736.36</td>
<td>±580.37</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>8026.00</td>
<td>±992.67</td>
<td>0.026</td>
</tr>
<tr>
<td>S2</td>
<td>12814.50</td>
<td>±1205.61</td>
<td>0.000</td>
</tr>
<tr>
<td>S3</td>
<td>2695.25</td>
<td>±1049.80</td>
<td>0.965</td>
</tr>
<tr>
<td>S4</td>
<td>9211.50</td>
<td>±1828.90</td>
<td>0.004</td>
</tr>
<tr>
<td>S5</td>
<td>7793.75</td>
<td>±1686.68</td>
<td>0.038</td>
</tr>
</tbody>
</table>

n_ρ=9  n_c=3

When compared the mercury levels; prepared samples contained 3736 ±580 ppm; S2 sample contained the highest level of 12815±1205 ppm while the S3 contained the lowest level of 2695±1050 ppm.

Senevirathna and Amarasinghe (2006) reported that ayurvedic medicines contain Hg at toxic levels and the Seetharama Water contains, 47 ppm mercury. Maduwanthi et. al,
(2010) have reported that the commercial samples of *Seetharama Watee* contain 2350±150 ppm and 2880±3300 ppm of Hg present in the prepared sample.

According to the studies done by Fernando using AAS, (1986) the cinnabar contains 712800-745300 ppm Hg, and the purified cinnabar, which was one of the component used in the preparation of *Seetharama Watee*, contains 454900-489800 ppm Hg. *Buddharaja kalka* is an indigenous medicinal preparation, which also contains purified cinnabar as a component (Alvis, 1879). Fernando (1986) reported that this kalka should have the mercury level of 12,000 -13,000 ppm.

Table 4.15.1.2. Mercury Content of Authentically Prepared and Commercial Samples of Maha Varthikava Watee

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Hg Content ppm</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_0)</td>
<td>0.77 ±0.235</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_1)</td>
<td>6.92 ±2.163</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>(V_2)</td>
<td>0.67 ±0.093</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>(V_3)</td>
<td>0.49 ±0.328</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>(V_4)</td>
<td>1.78 ±0.701</td>
<td></td>
<td>0.818</td>
</tr>
<tr>
<td>(V_5)</td>
<td>0.64 ±0.332</td>
<td></td>
<td>1.000</td>
</tr>
</tbody>
</table>

\(n_p=9\) \(n_c=3\)

Analysis of the first prepared *Maha Varthikava* sample for Hg content indicated the presence of a large quantity of Hg. *Maha Varthikava* is a poly herbal preparation and it should not contain any quantity of mercury. A sample prepared at first time, contained 39.72 ppm Hg. Therefore, a new batch was prepared under same conditions with in the same period and used for the AAS analysis. The sample prepared at first time was
removed from the analysis procedure. However, the raw materials that were used for the preparation of Maha Varthikava were analyzed for mercury content using the AAS.

Hg level of 0.82 ppm found in one cinnamon sample, which has been used in the preparation of MV. Then another three cinnamon samples were analyzed for the Hg content but there was no Hg present in those three samples. This high content of Hg, in the firstly prepared sample may be due to, the contaminated raw materials or/ and the processing procedures. When processing the herbal medicines with herbo mineral substanceses, using of the same utensils, even after practicing the proper cleaning methods, should be avoided, because that there may be some cross contaminations. It might happen not only in the processing methods but also in the drying procedures. Therefore, the preparation of herbal and poly-herbal preparations should be done separately from the herbo-mineral preparations. The cinnamons samples containing, mercury, were purchased from a drug store and it may be due to contamination, by mal practicing of Good Manufacturing Procedures (GMP).

Commercial sample V₁ contained the highest level of 6.92±2.2 ppm mercury while the commercial sample V₅ contained lowest level of 0.64±0.33 ppm. Mean value of 0.77±0.24 ppm contained in the prepared sample.

Small quantity of Hg present in most of the samples may be due the environmental contaminations and it was within the range of WHO recommended dosage (1 ppm) (Anon, 2004).

The Hg content could be suggested as $1.937 \times 10^{-1} - 5.287 \times 10^{-1}$% for Seetharama.
4.15.2. Arsenic (As):

Table 4.15.2.1. Arsenic Content of the Authentically prepared and the Commercial Samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean AS Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>13385.64 ±1551.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>5139.25 ±447.74</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>S₂</td>
<td>21054.5 ±3404.94</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>2353.75 ±1707.1</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>8928.5 ±112.84</td>
<td>0.311</td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>14530.5 ±246.44</td>
<td>0.992</td>
<td></td>
</tr>
</tbody>
</table>

When compared the mean values, the As content prepared samples contains 13385±1551 ppm, S₂ sample contain the highest value of 21054.5±3404 ppm while the S₃ contains the lowest value of 2353±1707 ppm.

Maduwanthi et al. (2010) revealed that the commercial samples of Seetharama Watee contained 4190±540 ppm of As and 7040±60 ppm in the sample prepared for the study. Fernando (1986) reported that the realgar (As₂S₂) and orpiment (As₂S₃) can be contained 360600-433100 and 356500-457200 ppm levels respectively. The purified realgar and orpiment were two components used in the preparation of Seetharama Watee for the study and they contained As level of 203500-304300 and 332400-461300 ppm respectively. Buddharaja kalka is an indigenous medicinal preparation, which was also reported to contain purified realgar and purified orpiment as a source of As.
forms (Alvis, 1879). Fernando (1986) reported that this kalka should have the arsenic content of 8,000 -9,000 ppm.

Table 4.15.2.2. Arsenic Content of the Authentically Prepared and the Commercial Samples of Maha Varthikava Watec.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean As Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>0.00 ±0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>0.11 ±0.106</td>
<td>0.441</td>
<td></td>
</tr>
<tr>
<td>V₂</td>
<td>0.00 ±0.001</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₃</td>
<td>0.11 ±0.108</td>
<td>0.372</td>
<td></td>
</tr>
<tr>
<td>V₄</td>
<td>0.00 ±0.001</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₅</td>
<td>0.00 ±0.001</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Maha Varthikava is a poly herbal preparation and it cannot contain any arsenic. However, firstly prepared sample contained 0.81 ppm of As. Then in the same period of the following year, a new batch was prepared under the same conditions and used for the AAS analysis. The contamination in the first prepared sample may be due to the poor processing procedures and using of the same utensils, which were used in the processing of herbo mineral preparations. Contamination was probably due to improper drying procedures. It can be recommended that the herbal preparations should be prepared using separate utensils, a place completely separated from the mineral preparations. Small quantity of As present in commercial samples V₁ and V₃, may be due to the environmental contaminations, and it was within the permissible range (1 ppm) recommended by WHO (Anon, 2004).
The As content could be suggested as $8.946 \times 10^{-1} - 1.7907\%$ for Seetharama.

4.15.3. Cadmium (Cd):

Table 4.15.3.1 Cadmium Content of the Authentically Prepared and Commercial Samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Cd Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>0.000</td>
<td>±0.000</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>4.895</td>
<td>±0.445</td>
<td>0.000</td>
</tr>
<tr>
<td>S2</td>
<td>0.925</td>
<td>±0.093</td>
<td>0.991</td>
</tr>
<tr>
<td>S3</td>
<td>0.195</td>
<td>±0.195</td>
<td>0.826</td>
</tr>
<tr>
<td>S4</td>
<td>0.000</td>
<td>±0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>S5</td>
<td>0.000</td>
<td>±0.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Cadmium is not directly used for the preparation, but sometimes it may contain in ingredients used. When compared the cadmium mean values, prepared samples contained no cadmium. Commercial sample S1 contained the highest Cd content of 4.8±0.445 ppm, and S2 and S3 samples contained 0.925±0.093 and 0.195±0.195 ppm of Cd respectively.

Small quantity of Cd present in S3 sample may be due the environmental contaminations and it was under the range of WHO recommended dosage (0.3 ppm) (Anon, 2004). Samples S1 and S2 contained high amount of Cd and this may be due to the contaminated raw materials used for the preparations.
Table 4.15.3.2. Cadmium Content of the authentically prepared and the Commercial Samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Cd Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>0.07 ±0.067</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>7.98 ±2.915</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>V₂</td>
<td>0.16 ±0.156</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₃</td>
<td>0.23 ±0.122</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₄</td>
<td>0.07 ±0.067</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V₅</td>
<td>0.07 ±0.067</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

When compared the cadmium levels, prepared sample contained 0.07 ±0.067 ppm Cd level; V₁ sample contained the highest Cd level of 7.98±3 ppm while sample V₄ and V₅ contained the lowest Cd level of 0.07±0.06 ppm respectively.

Small quantity of Cd present in the most of the samples may be due the environmental contaminations and it was within the range of WHO recommended dosage (10 ppm) (Anon, 2004) but in the sample V₁ contained higher level of Cd concentration and it may be due to environmental and raw material contaminations.
4.15.4. Lead (Pb):

Table 4.15.4.1. Lead Content of the Authentically Prepared and the Commercial Samples of Seetharama Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Pb Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>8.454 ±1.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>13.168 ±0.754</td>
<td>0.315</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>116.677 ±9.11</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>9.047 ±2.395</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>7.125 ±2.90</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>15.027 ±3.76</td>
<td>0.148</td>
<td></td>
</tr>
</tbody>
</table>

n_p=9  n_c=3

Maduwanthi et al, (2010) revealed that the commercial samples and the prepared samples contain 710±0.10 and 790±0.00 ppm lead respectively. The prepared samples contained 8.454±1.47 ppm and commercial sample S2 contained the highest lead level of 116.67 ppm while the S4 contains the lowest level of 7.125 ppm. Lead is not a component in the raw materials. However, when using the mineral substances lead can be present as a contaminant of the mineral materials. Raw materials blue vitriol, gypsum and alum contained lead in the levels of 67.1, 15.14 and 17.8 ppm respectively. Therefore, contaminations can be mainly due to these mineral materials and sometimes due to herbal materials.

When compared the lead values; prepared samples contained 8.5±1.47 ppm; S2 sample contained the highest content of 116±9 ppm while the S3 contained the lowest level of 7.12±2.9 ppm. However, lead was not used for the drug preparations, it may contain as
an additional compound of the minerals used. Most of them were within the range of WHO permitted level (10 ppb) (Anon, 2004). Higher Pb concentrations in some commercial preparations also may be due to environmental conditions or may be due to other contaminated mineral raw materials.

Table 4.15.4.2. Lead Content of the Authentically Prepared and the Commercial Samples of Maha Varthikava Watee.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mean Pb Content (ppm)</th>
<th>Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0</td>
<td>1.57 ±0.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>1.66 ±0.804</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>V2</td>
<td>3.15 ±1.524</td>
<td>0.942</td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>5.93 ±3.438</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>V4</td>
<td>0.48 ±0.484</td>
<td>0.979</td>
<td></td>
</tr>
<tr>
<td>V5</td>
<td>1.02 ±0.659</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

n_p=9     n_c=3

As Maha Varthikava Watee is a poly herbal formulation lead should not be present in the final product. However, the prepared sample contained 1.57±0.542 ppm level and the highest level of 5.93±3.44 ppm was present in the commercial sample V3 and the lowest of 0.48±0.48 ppm was in the commercial sample V4. All the sample means were within the WHO recommended range (10 ppm) (Anon, 2004).
4.16. Microbiological Assessments:

4.16.1. IMViC test:

The IMViC tests are a group of individual tests used in microbiology testing to identify an organism in the Coliform group of microbes, which includes such organisms as *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli*. Coliform bacteria are gram negative, aerobic, or facultative aerobic microorganisms, which produce gas from lactose within 48 hours. The presence of some Coliforms indicates fecal contamination.

Table 4.16.1. Indole test results of the authentically prepared samples, commercial samples of Seetharama Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>S₁</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S₂</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S₃</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S₅</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ n_{p^{17}} = 18 \quad n_{com^{18}} = 6 \quad n_{com^{19}} = 6 \]

\(^{17}\) Authentically prepared samples
\(^{18}\) Commercial samples
\(^{19}\) controls
Negative results indicate that there was no *E. coli* found. Reference to indole test in all prepared and commercial *Seetharama* samples indicated that there were no *E. coli* contaminations.

Table 4.16.2. Indole test results of the authentically prepared samples, commercial samples of Maha Varthikava Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>V₁</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₂</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₃</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₅</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>


$n_p = 18$ $n_{com} = 6$ $n_{con} = 6$

Negative results indicate that there was no *E. coli* found. Negative results indicate that there was no *E. coli* found. Reference to indole test in all prepared and commercial *Maha Varthikava* samples indicated that there were no *E. coli* contaminations.
Table 4.16.3. Methyl Red test results of the authentically prepared samples, commercial samples of Seetharama Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>S₁</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>S₂</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>S₃</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>S₄</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>S₅</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\( n_p = 18 \quad n_{com} = 6 \quad n_{con} = 6 \)

Negative results indicate that there was no *E. coli* found. Positive methyl red test indicates that there may be an *E. coli* contamination. Four prepared samples and seven commercial samples indicated positive results. Positive methyl red test indicates that there may be a *Coliform* contamination. However, if there was any other reducing agent it also indicates that positive colour change.
Table 4.16.4. Methyl Red test results of the authentically prepared samples, commercial samples of Maha Varthikava Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>V₁</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>V₂</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>V₃</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₅</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

\( n_p = 18 \) \( n_{con} = 6 \) \( n_{con} = 6 \)

Negative results indicated that there was no \( E. \) \( coli \) found. Two prepared samples and five commercial samples gave positive results. Positive methyl red test indicates that there may be an \( E. \) \( coli \) contamination. However, any other reducing agent also can indicate positive colour changes.
Table 4.16.5. Voges-Proskauer test results of the authentically prepared samples, commercial samples of Seetharama Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\( n_p = 18 \hspace{1em} n_{com} = 6 \hspace{1em} n_{con} = 6 \)

Negative results on Voges-Proskauer test indicate the absence of *Enterobacter aerogenes*. As there was no positive Voges-Proskauer test results found it can be stated that there were no *Enterobacter aerogenes* contamination in Seetharama preparations.
Table 4.16.6. Voges- Proskauer test results of the authentically prepared samples, commercial samples of Maha Varthikava Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>V₁</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₂</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₃</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₅</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ n_p = 18 \quad n_{com} = 6 \quad n_{con} = 6 \]

Similarly, as there were no positive results found on Voges-Proskaur test. It can be mentioned that there were no *Enterobacter aerogenes* contamination in Maha Varthikava preparations.
Table 4.16.7. Citrate utilization test results of the authentically prepared samples, commercial samples ofSeetharama Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>S₁</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>S₂</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>S₃</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>S₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S₅</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

\( n_p = 18 \quad n_{com} = 6 \quad n_{com} = 6 \)

Negative results on citrate utilization test indicate the absence of *Enterobacter aerogenes*.

One prepared and five commercial samples indicate the positive results. Positive results indicate that it may be contaminated by *Enterobacter aerogenes*. However, other reducing agents also can indicate the same colour change.
Table 4.16.8. Citrate utilization test results of the authentically prepared samples, commercial samples of Maha Varthikava Watee and the control media.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Negative Values</th>
<th>Positive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₀</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>V₁</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₂</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>V₃</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₄</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V₅</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

\( n_p = 18 \quad n_{com} = 6 \quad n_{con} = 6 \)

Negative results indicate that there was no *Enterobacter aerogenes* found. One prepared and five commercial samples indicate that positive results. Positive results indicate that there may be *Enterobacter aerogenes* contamination. However, other reducing agent also can indicate the same colour change.

Both indol and the methyl red tests have to be given positive results to conform the *E. coli* contaminations and the both Voges-Proskauer and Citrate utilization tests have to be given the positive results to confirm the *Enterobacter aerogenes*.

In this study, all the indol tests were negative while the several samples were positive in methyl red test that proves there was no *E. coli* contamination. Furthermore, all Voges-Proskauer tests were negative while the several samples were positive in citrate utilization test that also indicate there was no *Enterobacter aerogenes* contaminations in
the samples. There were no E coli and Enterobacter aerogenes present in Seetharama Watee and Maha Varthikava Watee.

4.16.2. Colony Forming Units: (CFU)

The numbers of microbes, which are capable of growing on specific media, are referred as colony forming units (CFU). Calculations of CFU help to count the maximum bacteria / mould load per gram.

Table 4.16.9. Colony forming units of the authentically prepared and the commercial samples of Seetharama Watee in Sabouraud glucose agar medium.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Colony forming units</th>
<th>CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S0</td>
<td>90, 91, 20</td>
<td>6X10^2</td>
</tr>
<tr>
<td>2S0</td>
<td>11, 10, 1</td>
<td>1X10^2</td>
</tr>
<tr>
<td>3S0</td>
<td>2, 2, 1</td>
<td>0.2X10^2</td>
</tr>
<tr>
<td>S1</td>
<td>over growth</td>
<td>6x10^3</td>
</tr>
<tr>
<td>S2</td>
<td>1, 1, 1</td>
<td>0.09x10^2</td>
</tr>
<tr>
<td>S3</td>
<td>1, 2, 1</td>
<td>0.12x10^2</td>
</tr>
<tr>
<td>S4</td>
<td>no growth</td>
<td>0</td>
</tr>
<tr>
<td>S5</td>
<td>2, 2, 2</td>
<td>1.82x10^2</td>
</tr>
</tbody>
</table>

Nageeb (2009) reported that the colony forming units in Seetharama Watee was 1x10^0 to 1.5x 10^3/g. Among these fungal growth Penicillium spp. Aspergillus spp. Mucor spp. Rhizopus spp. and Fusarium spp. were identified. Poorly practiced Good Manufacturing
Procedures were given as the reason for this high fungi count. According to the WHO guidelines, fungi count should not exceed $10^3$ /g in non-boiled herbal medicines.

**Table 4.16.10.** Colony forming units of the authentically prepared and the commercial samples of Maha Varthikava Watee in Sabouraud glucose agar medium.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Colony forming units</th>
<th>CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>over growth: 18, 28, 36, 3, 2, 3, no growth</td>
<td>$2.727 \times 10^5$</td>
</tr>
<tr>
<td>2V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>28, 10, 3: 4, 5, 3, no growth, no growth</td>
<td>$1.16 \times 10^2$</td>
</tr>
<tr>
<td>3V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>12, 28, 15: 3, 4, 2, no growth, no growth</td>
<td>$1.94 \times 10^2$</td>
</tr>
<tr>
<td>4V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>2, 0, 1: 0, 0, 0, no growth, no growth</td>
<td>$0.09 \times 10^2$</td>
</tr>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0, 2, 0: no growth, no growth, no growth</td>
<td>$0.06 \times 10^2$</td>
</tr>
<tr>
<td>V&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2, 0, 2: 2, 1, 0, no growth, no growth</td>
<td>$0.21 \times 10^2$</td>
</tr>
<tr>
<td>V&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0, 10, 1: 1, 10, 1, no growth, no growth</td>
<td>$0.67 \times 10^2$</td>
</tr>
<tr>
<td>V&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1, 2, 0: 1, 1, 0, No growth, No growth</td>
<td>$0.15 \times 10^2$</td>
</tr>
<tr>
<td>V&lt;sub&gt;5&lt;/sub&gt;</td>
<td>5, 32, 33: 6, 1, 6, No growth, No growth</td>
<td>$2.36 \times 10^2$</td>
</tr>
</tbody>
</table>

With the reference to the WHO standards, microbial contamination limits for the herbal materials used for internally, were given as follows.

Aerobic bacteria maximum $10^3$ /g Yeast and moulds maximum $10^3$ /g  *E.coli*, maximum $10^1$ /g.
Table 4.16.11. Fungi isolated from Seetharama Watee and Maha Varthikava Watee.

<table>
<thead>
<tr>
<th></th>
<th>Aspergillus a</th>
<th>Aspergillus b</th>
<th>Penicillium</th>
<th>Mucor</th>
<th>Curvularia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S&lt;sub&gt;0&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3S&lt;sub&gt;0&lt;/sub&gt;</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;5&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1V&lt;sub&gt;0&lt;/sub&gt;</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;5&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When all the Seetharama Watee samples were checked under the light microscope, two Aspergillus species (a and b) one Penicillium species and one Mucor species were identified in the two prepared and one commercial samples.

When all the Maha Varthikava Watee samples were checked under the light microscope two Aspergillus species (a and b), one Penicillium species and one Curvularia species were identified in the two prepared and three commercial samples.

Figure 4.16.1 Aspergillus (1S<sub>0</sub>)  
Figure 4.16.2 Penicillium (1V<sub>0</sub>)  
Figure 4.16.3 Curvularia (V<sub>1</sub>)

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These fungi were identified in the commercial samples as well as in some of the prepared samples. These fungi contaminations were under the recommended values ($<10^3/g$). These contaminations may be due to the contaminated raw materials. Therefore, more attention has to be paid when choosing the raw materials.

4.16.3. Calculations of the growth and colour changes in the selected media:

Various fungi have the various growth rates and colour changes in different period of time and growth media.
Table 4.16.12. Colony diameter of the selected fungi found in Seetharama Watee in Czapeck dox agar medium.

<table>
<thead>
<tr>
<th></th>
<th>Day 1/ mm</th>
<th>Day 2/ mm</th>
<th>Day 3/ mm</th>
<th>Day 4/ mm</th>
<th>Day 5/ mm</th>
<th>Day 6/ mm</th>
<th>Day 7/ mm</th>
<th>Day 8/ mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$</td>
<td>7.2±1.7</td>
<td>16.3±1</td>
<td>17±82</td>
<td>21.6±.62</td>
<td>24.1±.08</td>
<td>26.3±1</td>
<td>28.6±1.1</td>
<td>31.3±0.8</td>
</tr>
<tr>
<td>$1S_{0a}$</td>
<td>8.3±.56</td>
<td>18.3±1</td>
<td>21±1.4</td>
<td>21.2±1.3</td>
<td>21.2±1.3</td>
<td>21.3±1.2</td>
<td>21.3±1.2</td>
<td>21.3±1.2</td>
</tr>
<tr>
<td>$S_5$</td>
<td>7±1</td>
<td>21±1</td>
<td>29.3±0.4</td>
<td>38.8±0.9</td>
<td>51.3±1.2</td>
<td>61.8±2.7</td>
<td>77.3±3</td>
<td>83.3±2.2</td>
</tr>
<tr>
<td>$1S_{0b}$</td>
<td>7.7±.33</td>
<td>18±1</td>
<td>27.8±3.3</td>
<td>33±4.8</td>
<td>32.6±6.4</td>
<td>36.6±8.8</td>
<td>40±10.3</td>
<td>43.3±12</td>
</tr>
</tbody>
</table>

$n^{20} = 6$ $^{21}$ $^{22}$

Final colony diameters of $1S_{0a}$ and $1S_{0b}$ were 21.3±1.2 and 43.3±1.2 mm, $S_1$ was 31.3±0.8 and the $S_5$ was 83.3±2.2 mm.

Figure 4.16.8. Comparison of colony diameter of the fungi in Seetharama Watee in Czapeck dox agar medium.

---

$^{20}$ Sample size  
$^{21}$ 1st replicate  
$^{22}$ 2nd replicate
Fungi in commercial sample S₅ had the highest growth rate and the fungi in prepared sample 1S₀ₐ had the lowest in Czapeck dox agar medium.

**Table-4.16.13. Colony colours of Seetharama Watee samples in Czapeck dox agar medium.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Upside colour</th>
<th>Under side colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Middle</td>
</tr>
<tr>
<td>S₁</td>
<td>green</td>
<td>white</td>
</tr>
<tr>
<td>1S₀ₐ</td>
<td>green</td>
<td>light yellow</td>
</tr>
<tr>
<td>S₅</td>
<td>black</td>
<td>white</td>
</tr>
<tr>
<td>1S₀ₐ</td>
<td>dark green</td>
<td>yellow green</td>
</tr>
</tbody>
</table>

Fungus isolated from the sample 1S₀ₐ colony diameter was 21.3±1.2 mm upper surface colour was green to yellow from the centre, lower surface was yellow in colour when used Czapeck dox agar medium plate.

Fungus isolated from the sample 1S₀ₐ colony diameter was 43.3±1.2 mm upper surface colour was dark green to green from centre, lower surface was creamy in colour when used Czapeck dox agar medium plate.

Fungus isolated from sample S₁ colony diameter was 31.3±0.8 mm upper surface colour was green to white from centre, while the lower surface was pink in colour when used Czapeck dox agar medium plate.

Fungus isolated from sample S₅ colony diameter was 83.3±2.2 mm upper surface was black to white from centre, while the lower surface was white in colour when used Czapeck dox agar medium plate.
Table 4.16.14. Colony diameters of fungi in Maha Varthikava Watee in Czapeck dox agar medium.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
</tr>
<tr>
<td>V5a</td>
<td>7.3±0.8</td>
<td>17±1</td>
<td>25.8±0.8</td>
<td>30.8±0.8</td>
<td>38.3±0.7</td>
<td>44.8±1.7</td>
<td>55.3±2.4</td>
<td>66.3±1.2</td>
</tr>
<tr>
<td>2V0a</td>
<td>8.3±1</td>
<td>25.2±3.3</td>
<td>32.8±3.1</td>
<td>39.5±2.2</td>
<td>47.3±1.9</td>
<td>55.2±2.4</td>
<td>63.3±3.4</td>
<td>75.5±3.6</td>
</tr>
<tr>
<td>V5b</td>
<td>3.5±0.3</td>
<td>5.8±0.5</td>
<td>10.7±0.3</td>
<td>15±1.3</td>
<td>20.5±2.8</td>
<td>26.3±5.2</td>
<td>31.7±7.4</td>
<td>39.5±10.7</td>
</tr>
<tr>
<td>2V0b</td>
<td>8.5±1</td>
<td>20±0.52</td>
<td>23.7±1.9</td>
<td>26.1±1.1</td>
<td>27±1.1</td>
<td>27.5±1.4</td>
<td>28.3±1.5</td>
<td>28.7±1.6</td>
</tr>
</tbody>
</table>

n=6

Final colony diameters of the samples of 2V0a and 2V0b were 75.5±3.6 and 28.7±1.6 mm, V5a and V5b were 66.3±1.2 and 39.7±10.7 mm.

Figure 4.16.9. Comparison of colony diameter of fungi in Maha Varthikava Watee in Czapeck dox agar medium.
Commercial sample $2V_{0a}$ had the highest growth rate while the $2V_{0b}$ had the lowest in Czapeck dox agar medium.

**Table-4.16.15. Colony colours of Maha Varthikava Water samples in Czapeck dox agar medium.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Upside colour</th>
<th>Under side colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Middle</td>
</tr>
<tr>
<td>$V_{5a}$</td>
<td>green</td>
<td>light green</td>
</tr>
<tr>
<td>$2V_{0a}$</td>
<td>black/white</td>
<td>silky white</td>
</tr>
<tr>
<td>$V_{5b}$</td>
<td>black/white</td>
<td>white</td>
</tr>
<tr>
<td>$2V_{0b}$</td>
<td>green</td>
<td>greenish yellow</td>
</tr>
</tbody>
</table>

Fungus isolated from the sample $2V_{0a}$, colony diameter was $75.5 \pm 3.6$ mm upper surface colour was black to silky white lower surface was crystal white in colour when used Czapeck dox agar medium plate.

Fungus isolated from the sample $2V_{0b}$, colony diameter was $28.7 \pm 1.6$ mm upper surface colour was green to dark green lower surface was pinkish yellow in colour when used Czapeck dox agar medium plate.

Fungus isolated from the sample $V_{5a}$, colony diameter was $66.3 \pm 1.2$ mm upper surface colour was green to white, while the lower surface was yellow in colour when used Czapeck dox agar medium plate.

Fungus isolated from the sample $V_{5b}$, colony diameter was $39.5 \pm 10.7$ mm upper surface was black to white, while the lower surface was black in colour when used Czapeck dox agar medium plate.
Table 4.16.16. Colony diameters of selected fungi found in Seetharama Watee in malt extract agar medium.

<table>
<thead>
<tr>
<th></th>
<th>Day 1 /mm</th>
<th>Day 2 /mm</th>
<th>Day 3 /mm</th>
<th>Day 4 /mm</th>
<th>Day 5 /mm</th>
<th>Day 6 /mm</th>
<th>Day 7 /mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁</td>
<td>9.3±0.42</td>
<td>14.5±.43</td>
<td>20.8±.83</td>
<td>25.8±0.7</td>
<td>31.8±0.8</td>
<td>36.5±2</td>
<td>43.3±2.5</td>
</tr>
<tr>
<td>1S₀ₐ</td>
<td>17.5±7.5</td>
<td>39±1</td>
<td>45±5</td>
<td>45±5</td>
<td>45±5</td>
<td>45±5</td>
<td>45±5</td>
</tr>
<tr>
<td>S₅</td>
<td>17±0.82</td>
<td>32.3±1</td>
<td>50.3±.84</td>
<td>61.6±1.3</td>
<td>70.6±1.5</td>
<td>83.3±2.8</td>
<td>85±3</td>
</tr>
<tr>
<td>1S₀ₐ</td>
<td>16.7±.75</td>
<td>29.1±1.1</td>
<td>31.2±1.5</td>
<td>35±2</td>
<td>38.2±4</td>
<td>42.5±6</td>
<td>41.7±6.4</td>
</tr>
</tbody>
</table>

Final colony diameters of sample 1S₀ₐ and 1S₀ₐ were 45.5±5 and 41.7±6.4 mm, S₁ was 43.3±2.5 and the S₅ was 85±3 mm.

Figure 4.16.10. Comparison of colony diameter comparison of fungi in Seetharama Watee in malt extract agar medium.

Commercial sample S₅ had the highest growth rate while the S₁ had the lowest in malt extract agar medium.
Table-4.16.17. Colony colours of Seetharama Watee samples in malt extract agar medium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Upside colour</th>
<th>Under side colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Middle</td>
</tr>
<tr>
<td>S₁</td>
<td>dark green</td>
<td>white</td>
</tr>
<tr>
<td>1S₀a</td>
<td>dark green</td>
<td>dark yellow</td>
</tr>
<tr>
<td>S₅</td>
<td>black</td>
<td>black</td>
</tr>
<tr>
<td>1S₀b</td>
<td>greenish yellow</td>
<td>greenish yellow</td>
</tr>
</tbody>
</table>

Fungus isolated from the sample 1S₀a, colony diameter was 45±5 mm upper surface colour is dark green to dark yellow from centre, while the lower surface was greenish yellow in colour when used malt extract agar medium.

Fungus isolated from the sample 1S₀b, colony diameter was 4.7±6.4 mm upper surface colour was greenish yellow to white from centre, while the lower surface was light orange in colour when used malt extract agar medium.

Fungus isolated from sample S₁ colony diameter was 43.3±2.5 mm upper surface colour is dark green to white from center, while the lower surface was dark brown in colour when used malt extract agar medium.

Fungus isolated from sample S₅ colony diameter was 8.5±3 mm upper surface colour was black, while the lower surface was dark yellow in colour when used malt extract agar medium.
Table 4.16. Colony diameters of selected fungi found in Maha Varthikava Watee in malt extract agar medium.

<table>
<thead>
<tr>
<th>Day</th>
<th>V_{5a}</th>
<th>2V_{0a}</th>
<th>2V_{0b}</th>
<th>V_{5b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.83±1.3</td>
<td>15.33±.42</td>
<td>11.3±.5</td>
<td>15.8±.7</td>
</tr>
<tr>
<td>2</td>
<td>32.5±1.1</td>
<td>28.33±1</td>
<td>18.3±1.2</td>
<td>28.33±1.2</td>
</tr>
<tr>
<td>3</td>
<td>50±2.8</td>
<td>51.8±3.5</td>
<td>24±1.3</td>
<td>49.1±3.7</td>
</tr>
<tr>
<td>4</td>
<td>56.8±2.7</td>
<td>65.7±2.7</td>
<td>26.7±.8</td>
<td>60.83±4.6</td>
</tr>
<tr>
<td>5</td>
<td>65.3±3</td>
<td>77.5±1.4</td>
<td>27.7±1.2</td>
<td>71.8±3.3</td>
</tr>
<tr>
<td>6</td>
<td>74.5±3.5</td>
<td>90</td>
<td>29±1.8</td>
<td>84.8±1.2</td>
</tr>
<tr>
<td>7</td>
<td>86.7±2.1</td>
<td>90</td>
<td>32.3±2.4</td>
<td>75±8</td>
</tr>
</tbody>
</table>

Final colony diameters of 2V_{0a} and 2V_{0b} were 90 and 32.3±2.4 mm; V_{5a} and V_{5b} were 86.7±2.1 and 75±8 mm.

Figure 4.16.11. Comparison of colony diameter of fungi in Maha Varthikava Watee in malt extract agar medium.

Commercial sample V_{5a} had the highest growth rate while the V_{5b} had the lowest in malt extract agar medium.
Table-4.16.19. Colony colours of Maha Varthikava Watee samples in malt extract agar medium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Upside colour</th>
<th>Under side colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Middle</td>
</tr>
<tr>
<td>V₅a</td>
<td>green</td>
<td>dark yellow</td>
</tr>
<tr>
<td>2V₀a</td>
<td>silky white</td>
<td>silky white</td>
</tr>
<tr>
<td>V₅b</td>
<td>pink</td>
<td>white</td>
</tr>
<tr>
<td>2V₀b</td>
<td>green</td>
<td>yellow green</td>
</tr>
</tbody>
</table>

Fungus isolated from the sample, 2V₀a colony diameter was 75.5±3.6 mm upper surface colour was silky white from the centre, lower surface was white in colour when used malt extract agar medium.

Fungus isolated from the sample 2V₀b colony diameter is 28.7±1.6 mm upper surface colour was green to white from the centre, lower surface was pinkish yellow in colour when used malt extract agar medium.

Fungus isolated from sample V₅a colony diameter is 66.3±1.2 mm upper surface colour was green to light yellow from the centre, while the lower surface was brown in colour when used malt extract agar medium.

Fungus isolated from sample V₅b colony diameter is 39.5±10.7 mm upper surface colour was pink to white from the centre, while the lower surface was orange in colour when used malt extract agar medium.
4.17. Aflatoxins:

Aflatoxin is a liver toxic and potent carcinogen to many animals. There are at least 12 closely related difluranocoumarin compounds in the group of aflatoxins and the four most familiar are designated B₁, B₂, G₁ and G₂. Methods based on TLC and HPLC with various detection systems are used for aflatoxins determination. Aflatoxin have been detected in some raw herbal materials and spices, (Takahashi, 1993) so it is important to detect the aflatoxins in finished products. Aflatoxin analyses on spices were not simple because of the highly coloured contaminating materials that are co-extracted with aflatoxins (Romagnoli et.al, 2007).

4.17.1. TLC method based on fluorance:

After spotting, the samples in between the standard samples aflatoxin spots were observed under 364 nm wavelengths.

Aflatoxins B₁, B₂, G₁, and G₂ were not detected in the TLC of all prepared and commercial samples except S₂, which contained aflatoxin B₁. (Figure4.17.1)

Figure 4.17.1. TLC for the detection of aflatoxin in Seetharama Watee samples and standard aflatoxin samples - chloroform acetone (9: 1 v/v) solvent system and detected under long wave UV light 364 nm.
Table 4.17.1. TLC results of the authentically prepared and the commercial samples of Seetharama Watee for aflatoxins, sprayed with 25% H$_2$SO$_4$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B$_1$</th>
<th>B$_2$</th>
<th>G$_1$</th>
<th>G$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>2S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>3S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_1$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_2$</td>
<td>d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_3$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_4$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_5$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

n/d$^{23}$  d$^{24}$

$^{23}$ not detected
$^{24}$ detected
Figure 4.17.2. TLC for detection of aflatoxin in Maha Varthikava Watee samples and the standard aflatoxin samples - chloroform: acetone (9:1 v/v) solvent system and detected under long wave UV light 364 nm.

4.17.2. TLC analysis with 25% H₂SO₄:

Spraying with 25% H₂SO₄ is one of the confirmatory methods for the aflatoxin; otherwise, it is difficult to conform as aflatoxin. Occasionally blue fluorescent spots appear which can be easily mistaken as aflatoxin B₁. Therefore, chemical confirmation of the identity of the toxin in all positive test sample is essential (Anon, 2000).

Figure 4.17.3. TLC for detection of aflatoxin in Seetharama Watee samples and the standard aflatoxin samples - chloroform: acetone (9:1 v/v) solvent system, sprayed with 25% H₂SO₄ and examined under long wave UV light 364 nm.

Presence of aflatoxin B₁ the sample S₂ of the Seetharama Watee was confirmed by spraying with H₂SO₄ 25%.
Table 4.17.2. TLC analysis of the authentically prepared and the commercial samples of Maha Varthikava Watee for aflatoxins, sprayed with 25% H$_2$SO$_4$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$G_1$</th>
<th>$G_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>$V_1$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>$V_2$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>$V_3$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>$V_4$</td>
<td>d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>$V_5$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

Aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ were not detected in any of the authentically prepared or commercial samples except in commercial sample $V_4$. Aflatoxin $B_1$ was present in $V_4$ (Figure 4.18.5-Figure 4.18.6).

Figure 4.17.4. TLC for detection of aflatoxin in Maha Varthikava Watee samples and the standard aflatoxin samples - chloroform: acetone (9:1 v/v) solvent system, sprayed with 25% H$_2$SO$_4$, and detected under long wave UV light 364 nm.
4.17.3. Two-dimensional TLC method:

Two solvent systems were used after spotting the extracted drug samples and the aflatoxin standards. After developing the TLC plate using the 1st solvent system, 2nd solvent was used right angled to the 1st one. It produced better separation than the single solvent system.

Table 4.17.3. Two-dimensional TLC results of the authentically prepared and the commercial samples of Seetharama Watee for the detection of aflatoxin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>2S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>3S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₁</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₂</td>
<td>d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₃</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₄</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₅</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

Aflatoxins B₁, B₂, G₁, and G₂ were not detected in the TLC of any of the prepared samples and commercial samples except sample S₂, which contained B₁ (Figure 4.17.3).
Figure 4.17.5. Two-dimensional TLC fingerprint of the Seetharama Watee samples - chloroform: acetone (9:1 v/v) & toluene: ethyl acetate: 90% formic acid (5:4:1 v/v) solvent systems (1S₀, S₂ and S₅).

Note- No aflatoxins were present in sample 1S₀ and S₂ (Figure Sa1, Figure Sb1) while the aflatoxin B₁ present in the sample S₅ (Figure Sc1).
4.17.4. Two-dimensional TLC after spraying with 25% H$_2$SO$_4$:

After developing with the 2$^{nd}$ solvent system, the TLC plates were sprayed with 25% H$_2$SO$_4$.

Table 4.17.4. Two-dimensional TLC results of the authentically prepared and commercial samples of Seetharama Watee sprayed with 25% H$_2$SO$_4$ for the detection of aflatoxin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B$_1$</th>
<th>B$_2$</th>
<th>G$_1$</th>
<th>G$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>2S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>3S$_0$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_1$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_2$</td>
<td>d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_3$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_4$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S$_5$</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

While the aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ were not detected in any of the prepared samples and commercial samples except in commercial sample S$_2$, where aflatoxin B$_1$ was detected (Figure 4.17.4).
Figure 4.17.6. Two-dimensional TLC fingerprint of Seetharama Watee samples - chloroform: acetone (9:1 v/v) & toluene: ethyl acetate: 90% formic acid (5:4:1 v/v) solvent systems (1S0, S2 and S5) after spraying with 25% H2SO4.

Note- No aflatoxins were present in sample 1S0 and S2 (Figure Sa2, Figure Sb2) while the aflatoxin B1 present in the sample S5 (Figure Sc2) after spraying with 25% H2SO4.
Table 4.17.5. Two-dimensional TLC analysis of the authentically prepared and the commercial samples of Maha Varthikava Watee for the detection of aflatoxin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1V₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>2V₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>3V₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V₁</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V₂</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V₃</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V₄</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V₅</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

Aflatoxins B₁, B₂, G₁, and G₂ were not detected in any of the authentically prepared or commercial samples. (Table 4.17.6)
Figure 4.17.7. Two-dimensional TLC fingerprint of the Maha Varthikava Watee samples - chloroform: acetone (9:1 v/v) & toluene: ethyl acetate: 90% formic acid (5:4:1 v/v) solvent systems (V_4 and V_5).

Note-Any type of aflatoxin was not detected in sample V_4 and V_5.

Table 4.17.6. Two-dimensional TLC analysis of the authentically prepared and the commercial samples of Maha Varthikava Watee sprayed with 25 % H_2SO_4 for the detection of aflatoxin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B_1</th>
<th>B_2</th>
<th>G_1</th>
<th>G_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_0</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V_2</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V_3</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V_4</td>
<td>D</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V_5</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>
Aflatoxins B₁, B₂, G₁ and G₂ were not detected in any of the authentically prepared samples; but only B₁ was detected in the commercial sample V₄.

Sample V₄ - Va2

Sample V₅ - Vb2

Figure 4.17.8. Two-dimensional TLC fingerprint of the Maha Varthikava Watee samples - chloroform: acetone (9:1 v/v) and toluene: ethyl acetate: 90% formic acid (5:4:1 v/v) solvent systems (V₄ and V₅) after spraying with 25% H₂SO₄.

Note-Aflatoxin B₁ was present in sample V₄ after the 25% H₂SO₄ treatment (Figure Va2) and none of aflatoxin present in sample V₅ (Figure Vb2).

4.17.5. HPLC method:

The higher separation power and shorter analysis time of HPLC has resulted in the increased use of this method. The required detection in the low parts per billion (ppb) levels can be performed using simple enrichment and sensitive detection.
Table 4.17.7. HPLC analysis results of the authentically prepared and the commercial samples of Seetharama Watee for the detection of Aflatoxin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B₁,ppb</th>
<th>B₂,ppb</th>
<th>G₁,ppb</th>
<th>G₂,ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>315.7</td>
<td>0.6</td>
</tr>
<tr>
<td>2S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>227.3</td>
<td>13.4</td>
</tr>
<tr>
<td>3S₀</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>S₁</td>
<td>n/d</td>
<td>1.19</td>
<td>78.2</td>
<td>1.2</td>
</tr>
<tr>
<td>S₂</td>
<td>28.2</td>
<td>0.1</td>
<td>n/d</td>
<td>1.0</td>
</tr>
<tr>
<td>S₃</td>
<td>n/d</td>
<td>0.1</td>
<td>80.6</td>
<td>17.3</td>
</tr>
<tr>
<td>S₄</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>0.4</td>
</tr>
<tr>
<td>S₅</td>
<td>0.57</td>
<td>n/d</td>
<td>31.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

While the sample 1S₀ contained aflatoxin G₁ at 315.7 ppb level and G₂ at 0.6 ppb level, sample 2S₀ contained aflatoxin G₁ and G₂ at 227.3 ppb and 13.4 ppb levels. Sample S₁ contained B₂ at 1.19, G₁ at 78.2 and G₂ at 1.2 ppb levels respectively. Furthermore, the sample S₂ contained aflatoxin B₁ at 28.2 ppb level and S₁ contains B₂ at 0.1 ppb level G₁ at 80.6 levels and G₂ at 17.3 levels. Sample S₄ contains G₂ at 0.4 ppb level and sample S₅ contains B₁ at 0.57 ppb, G₁ at 31.3 ppb G₂ at 1.1 ppb levels respectively.

Although there were high levels of G₁ aflatoxin present in the HPLC analysis of the 1S₀ and the 2S₀ samples of the herbo-mineral formulations, it is not detected in two-dimensional TLC. HPLC analysis of sample S₅ also indicated that the aflatoxins B₁, G₁ and G₂ were present (Table-4.17.7); however there was no evidence to prove that the presence of aflatoxin G₁ and G₂ in the sample S₅, because in the 2 dimensional TLC they were in different Rₜ values and the level of florescence. If the aflatoxin B₁ in such
high (28.2 ppb) quantity, in sample S2 that should be indicated in 2 dimensional TLC within same Rf value and the fluorescence level. HPLC analysis of the sample S2 indicated that the presence of aflatoxin B1 and it was confirmed in the 2 dimensional TLC. That confirmed the sample S2 was contaminated with aflatoxin B1 and no evidence of aflatoxin B1 present in sample S5 in two dimensional TLC.

**Table 4.17.8. HPLC analysis of the authentically prepared and the commercial samples of Maha Varthikava Watee for the detection of aflatoxin.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>B1, ppb</th>
<th>B2, ppb</th>
<th>G1, ppb</th>
<th>G2, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1V0</td>
<td>n/d</td>
<td>n/d</td>
<td>44.15</td>
<td>n/d</td>
</tr>
<tr>
<td>2V0</td>
<td>n/d</td>
<td>n/d</td>
<td>444.40</td>
<td>0.75</td>
</tr>
<tr>
<td>3V0</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V1</td>
<td>n/d</td>
<td>n/d</td>
<td>13.6</td>
<td>n/d</td>
</tr>
<tr>
<td>V2</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V3</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V4</td>
<td>n/d</td>
<td>1.6</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>V5</td>
<td>1.21</td>
<td>58.7</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

Aflatoxin G1 was detected in 44.15 ppb level in the sample of 1V0, G1 and G2 were detected in 444.4 ppb and 0.75 ppb levels in 2V0, G1 was detected in V1 in 13.6 ppb level. Aflatoxins B1 and B2 were detected in sample V5 at 1.21 ppb and 58.7 ppb levels respectively. HPLC analysis of the 1V0, 2V0 and V5 samples of Maha Varthikava indicated that aflatoxins B1, B2, G1, and G2 present in indicated levels (Table-4.17.8). However, there was no evidence for the presence of aflatoxin in those samples, because in the 2 dimensional TLC they were in different Rf values and fluorescence levels.
Chapter 5

5. Conclusion:

Quality parameters identity and purity of the Seetharama Watee and Maha Varthikava Watee were taken for their standardization and quality assessment. Quality parameters of these preparations were weight variation, pH, weight loss on drying, specific gravity, extractable percentage into hexane, dichloro methane, ethyl acetate and methanol, mycological content, heavy metal content and aflatoxin activity.

TLC fingerprints, UV−vis spectrophotometric measurements and HPL chromatograms were used to establish the identity of these preparations. The purity was measured by means of total ash, acid insoluble ash and fiber content. The standard samples were prepared using the raw materials and following the standard methods described in Watika Prakaranaya. Proposed specifications for the Seetharama Watee and Maha Varthikava Watee are given below, which are prepared following the method described in Watika Prakaranaya.

5.1. Proposed specifications for Seetharama Watee:

Table-5.1.1. Test parameters for quality:

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weight of a pill</td>
<td>122-150 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>pH value</td>
<td>4.75- 5.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Specific gravity</td>
<td>1.14-1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Weight loss on drying</td>
<td>7.2-11.5% w/w</td>
<td>7-7.5% w/w</td>
<td>supported by Jayanetti,1987</td>
</tr>
<tr>
<td></td>
<td>Disintegration time</td>
<td>Friability</td>
<td>Hardness</td>
<td>Hexane extractable matter</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>------------</td>
<td>----------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>5</td>
<td>6-39 min</td>
<td>0.27-1% w/w</td>
<td>1.1-1.8 kg/cm²</td>
<td>23.3-29.8% w/w</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Heavy metal content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>7 x 10⁻²%</td>
<td></td>
<td>(≤ 3 x 10⁻⁴%)</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>6 x 10⁻⁴%</td>
<td></td>
<td>(≤ 1 x 10⁻³%)</td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>1.937 x 10⁻¹ - 5.287 x 10⁻¹%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>8.946 x 10⁻¹ - 1.7907%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*WHO Recommended value
### Table-5.1.2. Test parameters for purity:

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>Value range</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Total ash value</td>
<td>8.7-11.4% w/w</td>
<td>10.8-13% w/w</td>
<td>closely supported by Jayanetti, 1987</td>
</tr>
<tr>
<td>14</td>
<td>Acid insoluble value</td>
<td>0.03-0.07% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Crude fiber content</td>
<td>5.3-10% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>IMViC test</td>
<td>No E-coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Mycological content</td>
<td>2.4x10^2 CFU/g</td>
<td>&lt;10^3</td>
<td>below the WHO reference</td>
</tr>
<tr>
<td>18</td>
<td>Aflatoxin content</td>
<td>No aflatoxin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* WHO recommended value

### Table-5.1.3. Test parameters for identity:

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>Value range</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>TLC finger prints</td>
<td>(a) Fig.4.12.1.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Fig. 4.12.1.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Fig.4.12.1.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Fig.4.12.1.38</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>UV/vis λ max</td>
<td>287 and 290 nm.</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a) Figure 8.v.xxxi</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Figure 8.v.xxxii</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Figure 8.v.xxxiii</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>21</th>
<th>HPLC retention time</th>
<th>Five peaks can be identified at the level of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(a) 7-8 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) 14-17 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) 26-29 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) 37-40 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) 41-43 min</td>
</tr>
<tr>
<td></td>
<td>Solvent system-</td>
<td>acetonitrile: water</td>
</tr>
<tr>
<td></td>
<td>retention time</td>
<td>(20:30 v/v) with 254 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV detection.</td>
</tr>
</tbody>
</table>

As far as the quality assessment of *Seetharama Watee* is concerned pH value, specific gravity, friability, hardness, total ash, acid insoluble ash, Hg, As contents, TLC fingerprints, HPLC fingerprints and UV-vis absorption maximum can be considered as the quality parameters.
5.2. Proposed specification for Maha Varthikava Watee:

Table-5.2.1. Tests parameter for quality:

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Value</th>
<th>References</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weight of a pill</td>
<td>104-133 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>pH value</td>
<td>4.14-4.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Specific gravity</td>
<td>1.15-1.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Weight loss on drying</td>
<td>10-13.4% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Disintegration time</td>
<td>21-37 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Friability</td>
<td>0.8-1.3% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Hardness</td>
<td>0.5-1.2 kg/cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Hexane extractable matter</td>
<td>4.2-10.1% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Dichloro methane extractable matter</td>
<td>1.3-2.6% w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Ethyl acetate extractable matter</td>
<td>1.89-6.92 % w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Methanol extractable matter</td>
<td>5.3-25.3% w/w</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table-5.2.2. Test parameters for purity:

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Value</th>
<th>References</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Total ash value</td>
<td>5.43-7.50%</td>
<td>w/W</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Acid insoluble ash content</td>
<td>0.01-0.09%</td>
<td>w/w</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Crude fiber content</td>
<td>5.20-9.01%</td>
<td>w/w</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>IMViC test</td>
<td>No E-coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Mycological content</td>
<td>1.06x10² CFU/g</td>
<td>≤10¹⁸</td>
<td>below the WHO reference</td>
</tr>
<tr>
<td>17</td>
<td>Aflatoxin content</td>
<td>No aflatoxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Heavy metal content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0%</td>
<td>&lt;*3x10⁻⁴%</td>
<td>below the WHO reference</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>1.5 x 10⁻⁴ %</td>
<td>&lt;*1x10⁻³%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>0.7x10⁻⁴ %</td>
<td>&lt;*1x10⁻⁴%</td>
<td>below the WHO reference</td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>0%</td>
<td>&lt;*1x10⁻⁴%</td>
<td>below the WHO reference</td>
</tr>
</tbody>
</table>

* WHO recommended value
Table 5.2.3. Test parameters for identity:

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>Value range</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>TLC fingerprints</td>
<td>(a) Fig. 4.12.2.15</td>
<td><img src="image1.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Fig. 4.12.2.16</td>
<td><img src="image2.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Fig. 1.12.2.17</td>
<td><img src="image3.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Fig. 1.12.2.18</td>
<td><img src="image4.jpg" alt="Image" /></td>
</tr>
<tr>
<td>20</td>
<td>UV/Vis Spectrophotometric measurements</td>
<td>$\lambda_{\text{max}}$ 287 and 290 nm.</td>
<td><img src="image5.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a) Fig. 8.v.xxxvii</td>
<td><img src="image6.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Fig. 8.v.xxxviii</td>
<td><img src="image7.jpg" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Fig. 8.v.xxxix</td>
<td><img src="image8.jpg" alt="Image" /></td>
</tr>
<tr>
<td>21</td>
<td>HPLC retention time</td>
<td>Seven peaks could be identified at the level of</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a) 5-6 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) 6-7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) 15-17 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) 19-20 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) 21-23 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) 25-28 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g) 28-30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solvent system-</td>
<td>acetonitrile; water</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20:30 v/v) with</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>254 nm UV detection.</td>
<td></td>
</tr>
</tbody>
</table>

As far as the quality assessment of **Maha Varthikava Watee** is concerned pH, specific gravity, friability, hardness, total ash, acid insoluble ash content, TLC fingerprints, HPL chromatograms and UV-vis absorption maximum (λ max) can be considered as quality parameters.

Comparison of the results obtained from the commercial products of **Seetharama Watee** and **Maha Varthikava Watee** preparations with those obtained from their standard samples indicated a considerable deviation as shown by some of the commercial products. These deviations may be due to inconsistencies of recipes, different methods of preparations, and the variations among raw materials used by commercial manufacturers. The standardization of raw materials, use of standard recipes, and the standard methods of preparation are major factors that can be used to achieve the standard drugs. The drug manufacturers and the physicians who used the **Watika Prakaranaya as text**, for the manufacturing of **Seetharama Watee** and **Maha**
Varthikava Watee, the results published from this study, can be used to standardize and improve the quality of their products.

Except these physico chemical, biological and chromatographic parameters, the use of modern sophisticated equipment like LC/MS, ELISA can also be suggested for further studies. Although the clinical trials are not necessary for the traditional drugs which used for decades (Anon, 2004), clinical trials on standardized drugs will help in proving the safety of these drugs.
6. References:


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Kumarasinghe Aryadasa. (1962) Sharanghadhara Samhitha (Sinhalese) Department of official languages Colombo.


arsenic tetra-sulfide for the treatment of acute promyelocytic leukemia: a pilot

containing An Gong Niu Huang Wan (AGNH) is much less acutely toxic than
sodium arsenite and mercuric chloride. Chemico Biological Interactions, 189: 134-
140.

medicines: Is cinnabar toxilogically similar to common mercurials? Experimental
Biology and Medicine (Maywood), 233 (7): 810-17.

Mabrouk S S, Nefisa M A E S. (1980) Inhibition of Aflatoxin formation by some

concentration of Mercury, Lead, and Arsenic in selected Ayurveda Medicine.
SLAAS proceedings 66 th Annual Sessions. Abstract 803/ A.

aflatoxin production on black and white pepper and the inhibitory action of their


Nageeb B M. (2009) “Microbiology study of selected herbal preparation used in indigenous system of medicine in Sri Lanka with a view to defining microbial quality standards relevant to good manufacturing practice”. PhD Thesis Department of Microbiology, Faculty of Science, University of Kelaniya.


Ubayasekara D D. (1952) Rasa Shastra Colombo.


Chapter 7

Appendices:

Appendix I:

List of Publications and Communications from the thesis:


Appendix II:

Purification methods of mineral raw materials:

Purification methods of minerals:

Purification of Hingula- Put limejuice to cover and keep under sun rays until become hot, then stain the limejuice and then wash 7 times with limejuice.

Purification of Harithala- Ground and put into a Dola yantra containing buffalo milk: twice for eight hours then put into gingerly oil and kept for ten hours.

Purification of Manahshila- Ground and put into Dola yantra containing cows urine and then wash four times using hot water.

Pushkara- Put into bee honey and put under sunshade.

Redee thuthihan- ground and mixed with cows' ghee and put into dola yantra containing cows' urine for seven times.

Sasyaka- put into human urine and boil (Alvis, 1879).

Sinakara- Put into a metal vessel and fry until become white light powder.

Gal mada- Ground with Aloe vera gel and prepare chakrika and then one puta should be given (Ubayasekara, 1952; Ponnamperuma, 2004).
Appendix III:

III. A. Chemical compositions of reagents and stains used:

1. Kovac’s Reagent:

   P-dimethylaminobenzaldehyde 15 g  
   Isoamyl alcohol 150 mL  
   HCl 75 mL

2. Methyl red reagent:

   Methyl red 0.1 g  
   Distilled water 300 mL

3. Barritt’s reagent:

   Barritt’s A (VP I reagent) - 6 g alpha napthol in 100 mL 95% ethanol  
   Barritt’s B (VP II reagent) - 16 g of KOH in 100 mL water.

4. Lacto phenol Cotton blue Stain:

   Phenol crystals 20 g  
   Lactic acid 20 mL  
   Glycerol 40 mL  
   Distilled water 20 mL  
   Aniline blue 0.05 g

   Heat gently in hot water to dissolve and then add aniline blue dye.

III.B. Media used:

1. Triptopan Broth medium:

   Trypton 10 g  
   Distilled water 1 L
2. MRVP broth medium:

- Peptone: 7 g
- Glucose: 5 g
- Potassium Phosphate: 5 g
- Distilled water: 1 L
- pH: 6.9

3. Simmond's citrate agar medium:

- Sodium citrate: 2 g
- $\text{M}_3\text{SO}_4$: 0.2 g
- $(\text{NH}_4)\text{H}_2\text{PO}_4$: 1 g
- NaCl: 5 g
- Bromo-thymol Blue: 0.08 g
- Agar: 15 g
- Distilled water: 1 L
- pH: 7

4. Sabouraud's Dextrose Agar (SDA) medium:

- Peptone: 5 g
- Dextrose: 40 g
- Distilled water: 1 L
- Agar: 15 g

Sterilized by autoclaving. (0.05 g Chromphenicol may be added to arrest bacterial contaminants in heavily contaminated samples.)
5. Czapek- Dox Agar medium:

Sucrose 30 g
Sodium nitrate 2 g
Magnesium glycerol phosphate 0.5 g
Potassium chloride 0.5 g
Di potassium sulphate 0.35 g
Ferrous sulphate 0.01 g
Distilled water 1 L
pH 5.4-5.6
Sterilized by autoclaving.

6. Malt Extract agar medium:

Malt extracts 30 g
K₂HPO₄ 1 g
NH₄Cl 1 g
Citric Acid 15 g
Agar 30 g
Distilled water 1L (Karawa et. al, 2008)

III.C. Spray reagents:

1. Anisaldehyde Sulfuric reagent:

anisaldehyde 0.5 mL
Glacial acetic acid 10 mL
Methanol 85 mL
Conc. H₂SO₄ 85 mL added in order
2. Ninhydrin reagent:

Ninhydrine 30 g
n butanol 10 mL
Glacial acetic acid 0.3 mL

3. Vanillin sulphate reagent:

vanillin sulphate 3 g
Conc. H₂SO₄ 1.5 n·L
Ethanol up to 100 mL (Guptha at. el, 2006)

III. D. Solutions:

1. Stanus chloride solution:

SnCl₂·2H₂O 10 g
Conc. Hot HCl 20 mL
Deionised water top up to 100 mL (Ang, 2005)

2. Sodium boro hydrate solution:

0.5% Sodium borohydrate in 0.6% NaOH solution.

10 m HCl
Appendix IV:

Colony diameter in various media:

Table 7.iii.i. Colony diameters of selected fungi found in Seetharama Watee and Maha Varthikava Watee in Czapeck dox agar medium.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Diameters of colony means (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>A-V&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>B-S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>C-2V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>D-V&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>E-1S&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>F-S&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>G-1S&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>H-2V&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 7.iii.ii. Colony diameters of selected fungi found in Seetharama Watee and Maha Varthikava Watee in malt extract agar medium.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Diameters of colony means (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>A- V₅</td>
<td>0.3</td>
</tr>
<tr>
<td>B- S₁</td>
<td>0.3</td>
</tr>
<tr>
<td>C- 2V₀</td>
<td>0.3</td>
</tr>
<tr>
<td>D- V₅</td>
<td>0.3</td>
</tr>
<tr>
<td>E- 1S₀</td>
<td>0.3</td>
</tr>
<tr>
<td>F- S₅</td>
<td>0.3</td>
</tr>
<tr>
<td>G- 1S₀</td>
<td>0.3</td>
</tr>
<tr>
<td>H- 2V₀</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Appendix V:

TLC profiles/patterns and absorption spectra:

Figure 7.v.i. Seetharama 254 nm

Figure 7.v.ii. iodine vapour

Figure 7.v.iii. vanillin sulphate

Figure 7.v.iv. TLC profiles/patterns of Seetharama and Holy basil samples detected under visual light and 254 nm UV light.
Figure 7.v.v. TLC profiles/patterns of Seetharama and holy basil in iodine vapour and with vanillin sulphate spray.

Figure 7.v.vi. TLC profiles/patterns of Seetharama and Red Sandalwood detected under 254 and 366 nm UV lights.
Figure 7.vii. TLC profiles/patterns of Seetharama and Red Sandalwood in iodine vapour and with vanillin sulphate spray.

Figure 7.viii. TLC profiles/patterns of Seetharama and Sour Orange detected under 254 nm UV light, in iodine vapour and with vanillin sulphate spray.
Figure 7.v.ix. TLC profile/pattern of Seetharama, Indian privet, Ginger and Cinnomon sedge detected under visual light and 254 nm UV light.

Figure 7.v.x. TLC profiles/patterns of Seetharama Indian privet, Ginger and Cinnomon sedge in iodine vapour and with vanillin sulphate spray.
Figure 7.v.xi. TLC profile/patterns of Maha Varthikava and Betel detected under visual light and 254 nm UV light.

Figure 7.v.xii. TLC profile/patterns of Maha Varthikava and Betel in iodine vapour with vanillin sulfuric acid spray.
Figure 7.v.xiii. TLC profile/patterns of Maha Varthikava and Indian privet detected under 254 nm UV light, in iodine vapour and with vanillin sulphate spray.

Figure 7.v.xiv. TLC profile/patterns of Seetharama, Garlic, Red Sandalwood, Asafoetida and Cinnamon sedge samples detected under visual light.
Figure 7.v.xv. TLC profile/patterns of Seetharama, Holy basil, Neem, Indian privet, Ginger and Sour Orange samples detected under visual light.

Figure 7.v.xvi. TLC profile/patterns of Maha Vartikava, Betel, Indian privet, fresh Ginger, Iron wood and dry Ginger samples detected under visual light.
Figure 7.v.xvii. TLC profile/patterns of Maha Vartikava, Asafoetida, dry Ginger, Indian privet, Iron wood and fresh Ginger samples detected under visual light.

Figure 7.v.xviii. TLC profile/patterns of Seetharama, Garlic, Red Sandalwood, Asafoetida and Cinnamon sedge samples detected under 254 nm UV light.
Figure 7.v.xix. TLC profile/patterns of Seetharama, Holy basil, Neem, Indian privet, Ginger and Sour Orange samples detected under 254 nm UV light.

Figure 7.v.xx. TLC profile/patterns of Maha Vartikava, Betel, Indian privet, fresh Ginger, Bees’s honey and dry Ginger samples detected under 254 nm UV light.
Figure 7.v.xxi. TLC profile/patterns of Maha Vartikava, Asafoetida, dry Ginger, Indian privet, Iron wood and fresh Ginger samples detected under 254 nm UV light.

Figure 7.v.xxii. TLC profile/patterns of Seetharama, Garlic, Red Sandalwood, Asafoetida and Cinnamon sedge samples detected under 366 nm UV light.
Figure 7.v.xxiii. TLC profile/ patterns of Seetharama, Holy basil, Neem, Indian privet, Ginger and Sour Orange samples detected under 366 nm UV light.

Figure 7.v.xxiv. TLC profile/ patterns of Maha Vartikava, Betel, Indian privet, fresh Ginger, Bee's honey and dry Ginger samples detected under 366 nm UV light.
Figure 7.v.xxv. TLC profile/patterns of Maha Vartikava, Asafoetada, dry Ginger, 
Indian privet, Iron wood and fresh Ginger samples detected 
under 366 nm UV light.

Figure 7.v.xxvi. TLC profile/patterns of Seetharama, Garlic, Red Sandalwood, 
Asafoetada and Cinnamon sedge samples detected after spraying 
anisaldehyde in sulfuric acid.
Figure 7.v.xxvii. TLC profile/patterns of Seetharama, Holy basil, Neem, Indian privet, Ginger and Sour Orange samples detected after spraying anisaldehyde in sulfuric acid.

Figure 7.v.xxviii. TLC profile/patterns of Maha Vartikava, Betel, Indian privet, fresh Ginger, Bees’s honey and dry Ginger samples detected after spraying anisaldehyde in sulfuric acid.
Figure 7.v.xxix. TLC profile /patterns of Maha Vartikava, Asafoetida, dry Ginger, Indian privet, Iron wood and fresh Ginger samples detected after spraying anisaldehyde in sulfuric acid.

Figure 7.v.xxx. UV Spectrophotometer (UV mini 1240).
Figure 7.v.xxxi. UV absorption spectrum of $1S_0$.

Figure 7.v.xxxii. UV absorption spectrum of $2S_0$.

Figure 7.v.xxxiii. UV absorption spectrum of $3S_0$. 
Figure 7.v.xxxiv. UV absorption spectrum of $S_1$.

Figure 7.v. xxxv. UV absorption spectrum of $S_2$.

Figure 7.v.xxxvi. UV absorption spectrum of $S_3$.
Figure 7.v.xxxvii. UV absorption spectrum of $S_6$.

Figure 7.v.xxxviii. UV absorption spectrum of $S_5$. 
Figure 7.v.xxxix. UV absorption spectrum of $1V_0$.

Figure 7.v.xli. UV absorption spectrum of $2V_0$.

Figure 7.v.xlii. UV absorption spectrum of $3V_0$. 
Figure 7.v.xlii. UV absorption spectrum of V₁.

Figure 7.v.xliii. UV absorption spectrum of V₂.

Figure 7.v.xliv. UV absorption spectrum of V₃.
Figure 7.v.xlv. UV absorption spectrum of V$_4$.

Figure 7.v.xlvi. UV absorption spectrum of V$_5$. 