ETHNO-MEDICAL USE OF *Drymoglossum pioselloides* AND SCREENING FOR CHEMICAL CONSTITUENTS

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UNIVERSITY OF SRI JAYEWARDENEPURA

SRI LANKA

2010
DECLARATION

I do hereby declare that the work reported in this project report/thesis was exclusively carried out by me under the supervision of Prof A.M. Abeysekera. It describes the result of my own independent research except where due reference has been made in the text. No part of this project report/thesis has been submitted earlier or concurrently for the same or any other degree.

Date: 20/07/2010

Signature of the Candidate

Certified by:

1. Supervisor (Name):.......................... Date:..........................
   (Signature):............................

2. Co-Supervisor (Name):...................... Date:......................
   (Signature):............................
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ABSTRACT

Drymoglossum piloselloides or “Panam pethi” of the Polypodiaceae family is a small epiphytic fern having thick, simple and numerous leaves commonly seen on the trunk of older trees and is found throughout tropical Asia below an elevation of 2,000 feet. In Ceylon Drymoglossum piloselloides is used for the treatment of bones fraction, arresting capillary hemorrhages and skin diseases.

Drymoglossum piloselloides were collected in the Kurunegala district in Sri Lanka and identified. A voucher specimen has been deposited at the Herbarium of University of Sri Jayawardenepura. Leaves of Drymoglossum piloselloides were washed & dried for about one month under normal environmental conditions. Dried leaves were grounded into a paste form and extracted with methanol using soxhlet apparatus around 50-70°C temperature for 1½ hrs. The solvent was concentrated under vacuum using a rotary evaporator. The yield was 4.706%. Then 50ml of the above basic solution was extracted with hexane. The yield was 0.012 %.

Phytochemical screening test for alkaloids, unsaturated sterols, flavanoids, polyphenolic compounds was done. Preliminary phytochemical investigation by thin layer chromatography (TLC) using authentic standards and UV Spectrometry led to the detection of unsaturated sterols, polyphenolic compounds, flavonoids.

To gather scattered & hidden ethno-medical knowledge about Drymoglossum piloselloides, field survey was carried out in Kurunegala, Gampaha and Colombo districts of Sri Lanka with randomly selected 50 indigenous practitioners using semi-structured questionnaire.
The results obtained from the phytochemical analysis of the *Drymoglossum piloselloides* L showed the presence of flavonoids, triterpenoids, steroids, and the absence of alkaloids. The survey revealed that *Drymoglossum piloselloides* has maximum effect for fraction healing, reduce pungent feeling activities, rectal prolepses, Hemorrhoids, Anemia, Hemorrhagic condition, wound healing and reduce swelling. And it considers that *Drymoglossum piloselloides* may have to a certain extant act for curing Dengue fever. So it implies the importance of carrying out further studies on the chemical constituents in *Drymoglossum piloselloides* and bio assay.
CHAPTER 1

1. INTRODUCTION

Drymoglossum piloselloides L or “Panam pethi” is a species of family Polypodiaceae found throughout tropical Asia. It is distributed in India, Burma, Ceylon, Malaya, Indo-china and Philippine islands below an elevation of 2,000 feet\(^1\). Normally it is called “Dragon Scales”, “Sita's Necklace”. But in Sri Lanka it is called “Kimbul Venna” which means “Crocodile Scales” because sometimes the trees are seen with a coating which appears like crocodile scales.

According to medical journals, Oka leaves-traditional published form and published studies Drymoglossum piloselloides may provide a wide range of health benefits. Apart from that Drymoglossum piloselloides is used for some kind of medicine in folk systems of medicine all over the world. Since Drymoglossum piloselloides is believed to have medicinal properties, they are used for various medicinal preparations. But there has not been much scientific work carried out of Drymoglossum piloselloides.

The traditional remedies of Drymoglossum piloselloides obtained from the literature give promising evidence for its phytochemical properties. Although Drymoglossum piloselloides which was familiar beyond two or three decades in Sri Lanka it is unusual plant now. But due to various reasons this valuable knowledge is in danger at present. Urbanization destroy much of the habitats, and lack of interest for traditional medicine among communities with the modern lifestyle and system of western medicine and elder traditional healers pass away without handing down their knowledge are some of the
main courses for losing the traditional knowledge from the society. Even the existing knowledge also is scattered in all parts of the country. So it is essential to gather, conserve and develop scattered and hidden knowledge about Drymoglossum piloselloides L and its medicinal values for the future generation.

The objectives of this research project were to gather scattered and hidden ethnomedical knowledge about Drymoglossum piloselloides and identify the chemical constituents of the Drymoglossum piloselloides.

The research project consists of two parts.

Part 1: Field survey of therapeutic utilization of Drymoglossum piloselloides in indigenous medicine in Sri Lanka

Part 2: Identification of chemical constituents in Drymoglossum piloselloides

1.1. Morphology

Fig: 1.1 Drymoglossum piloselloides
*Drymoglossum piloselloides* is an epiphytic fern climbing on tree trunks and shrubs of exposed areas and on humus deposit of rocks (Fig: 1.1) but not on the ground.

Rhizomes are slender; sometimes hair-pointed. Its slender roots are covered with green to brownish leaves, creeping strongly on trunks and branches. It is given as Fig: 1.2.

![Fig: 1.2 Rhizomes of *Drymoglossum piloselloides*](image)

Fronds are two kinds. They are sterile and fertile. Sterile fronds are thick and fleshy; vary from elliptic (Fig: 1.3 A) to rounded shape (Fig: 1.3 B), 1 to 5 cm long and 1 to 2 cm wide. Sterile fronds have rounded apex and cuneated base covered with stellate hairs when young.
Fig: 1.3 Variations of sterile fronds of *Drymoglossum piloselloides*

The fertile fronds (Fig: 1.4) variety is narrower, 3 to 5 cm long and 3 to 10 mm wide, on stipes up to 2 cm long veins immersed, areoles with copious free veinlets stipes about 2.5 cm long.

Fig: 1.4 the fertile fronds of *Drymoglossum piloselloides*

The sori (clusters of sporangia) arranged in a broad and sub marginal line not filling the whole surface of the frond (Fig: 1.5 a), capsules mixed with a few stellate paraphyse. *Drymoglossum piloselloides* grows through spore and separation of roots. The spore is brown colour. (Fig: 1.5 b)

Fig: 1.5 a. A Cluster of sporangia (Sori) of *Drymoglossum piloselloides*, b. Brown spores on the fertile frond.
1.2. Taxonomy

The taxonomy of *Drymoglossum piloselloides* is given as below.

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Pteridophyta

Class: Pteridopsida

Sub-class: Polypoditae

Order: Polypodiales

Family: Polypodiaceae

Genus: *Drymoglossum*

Species: *Drymoglossum piloselloides* (L.)Presl.

1.2.1. Synonyms

*Drymoglossum piloselloides* was earlier named as *Drymoglossum heterophyllum* (Linn.) Trimen.

1.2.2. Vernacular name

*Drymoglossum piloselloides* is distributed throughout tropical Asia. There are several vernacular names among countries and nationalities. There are given as below.
Indonesia: Paku sisik naga, picisan, duwitan (Jawa), duduwitan (Sunda)  
Sri Lanka: Panampethi 1,2,9,13,14, Kimbulvenna 7,8,9,13,14  
English: Sita's Necklace18  
Malaya: Dragon Scale, Sisek naga  
Malaysia: Paku Sisek Naga, Paku Sakat Ribiribu 4,5  
India: Sikitang 6  
Philippine: Pagong-pagongan 21  
Java: Duwitan  
Tamil: Shallikkodi 14  
Sunda: Duduwitan  
Sanskrit: Swasthika 8,14  

1.3. Literature Review

There has not been much scientific work carried out of Drymoglossum piloselloides. Its anti-bacterial activity (www.sukan.upm.edu.my) and anti-fungal activity (www.sukan.upm.edu.my) had been studied.

A study had been to evaluate the anti-bacterial property of Drymoglossum piloselloides against several common bacteria. Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia, Bacillus subtilis and Salmonella enteritidis had been utilized.
According to results the anti-bacterial activity of the water extract of the plant had been statistically less potent when compared to ampicillin and chloramphynicol which are anti-bacterial drugs. This study had been revealed that *Drymoglossum piloselloides* has minimum anti-bacterial activity. (University Putra Malaysia 2008)

Other study also had been to evaluate the anti-fungal property of *Drymoglossum piloselloides* against several common bacteria and fungi. *Trichophyton rubrum*, *Trichophyton mentagrophytes* which are two of the most common causes of Athlete's foot, *Candida albicans*, *Candida tropicalis*, *Microsporum canis* and *Aspergillus fumigates* had been utilized. The results had been revealed that the chloroform and ethanol extracts only has mild activity against the *Trichophyton spp* and the water has devoid of any activity. The anti-fungal activity was statistically less potent than griseofulvin and fluconazole or itaconazole. (University Putra Malaysia 2008)

Based on Ethno-medicinal importance several usages of *Drymoglossum piloselloides* were reported.

Exhaustive field survey had been undertaken from 2003 to 2004 for gathering information on each and every species useful in herbal medicine including *Drymoglossum piloselloides* in different villages of North Tripura, India. According to the survey *Drymoglossum piloselloides* is used as a paste obtained by crushing and applied externally in the form of poultice on fractured bones. (Department of Life Science, Assam University Silchar, India)
The ground leaves are being used as styptic for coagulating blood and arresting capillary hemorrhages and also used in small subcutaneous lumps and nodes.\textsuperscript{21} 

www.stuartxchange.org/ChineseList.html

The medicinal value of leaves of \textit{Drymoglossum piloselloides} have been summarized as reduce swelling, sprains and for relieving pain.\textsuperscript{23} (M.Maridass, 2008)

There has been reported that \textit{Drymoglossum piloselloides} was value in urinary disorders and diseases of genital organ.\textsuperscript{18} www.herbalking.in/healthwar.htm

An article was found on conducing the research (written by A.Putradjaja\textsuperscript{25-2009}) \textit{Drymoglossum piloselloides} was used as an herbal medicine because it has anti-inflammation, anti-toxin and anti-cancer specially breast, and prevent bleeding (anti-hemorrhages). Apart from that plant could be cured some diseases like gonorrhea, jaundice, stomach pain, constipation, cough, blood coughing, rheumatism, leucorrhoea, gingivitis, stomachitis, external the skin disease, such as scabies and ringworm. The chemical composition also has been included in that article. According to that \textit{Drymoglossum piloselloides} has aetheric oil, sterol, triterpen, phenol, flavonoid, tannin and glucose. And some of them could be potentially inhibit cancer cells in the body and particularly in the breast.
1.4. Properties of *Drymoglossum piloselloides* according to Ayurveda.

Based on Ayurvedic philosophy there is a way to explain a drug. According to that a broad knowledge of a drug could be obtained. Pharmacodynamics or Identification of Dravya, pharmacy (Preparation of drugs), clinical pharmacy (How to use this drug), phamocodynamics, phamocotherapeutics and action of drugs, properties of drugs are among them.

Considering the Ayurvedic term it is difficult to give the perfect meaning. Those illustrate several meanings. There are six categories to should know about the drugs. They are based on physical and chemical properties.

1. “Rasa” (taste)

There are six kind of “Rasa” or taste; Madhura (sweet), Amla (sour), Katu (pungent), Thikta (bitter), Lavana (salty) and Kashaya (astringent).

2. “Guna” (qualities)

As per Ayurveda, twenty physical (20) qualities known as “gunas” are found in living beings.

3. “Vipaka” (Post digestive effects on taste)

“Vipaka” is also based on taste. There are three types: Madhura (sweet), Amla (sour), Katu (pungent).

4. “Virya” (energy)

There are two type of “Virya”; “Shita” (cool) and “Ushna” (hot) based on chemical reactions.
5. "Prabhava" (special action of a drug)

"Prabhava" is defined as a special action of a drug. It is depended on a plant (drug).

6. "Karma" (pharmacological action).

If any action happens in the body from "Rasa", "Gunas", "Vipaka", "Virya" and "Prabhava" after administrating the drug, it is called "Karma" (pharmacological actions), it is depended on the plant having its qualities.

A drug acts partially through "Rasa" (taste), partially through "Virya" (energy) and some act through their "Guna" (qualities), "Vipaka" (Post digestive effects on taste) and "Prabhava" (special action of a drug) and "karma" (pharmacological action).

According to Ayurvedic literature *Drymoglossum piloselloides* provide a wide range of health benefits as the following table.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Properties of <em>Drymoglossum piloselloides</em> according to Ayurveda</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Rasa&quot; (taste)</td>
<td>&quot;Kashaya Rasa&quot;9, 9, 10, 14, 13, 18 (astringent)</td>
</tr>
<tr>
<td></td>
<td>&quot;Madhura Rasa&quot;14, 10 (slightly sweet)</td>
</tr>
<tr>
<td>&quot;Guna&quot; (qualities)</td>
<td>&quot;Jaghu&quot;14, 10 (light), &quot;Rooksha&quot;10 (dry), &quot;Vishada&quot;10 (clear);</td>
</tr>
<tr>
<td>&quot;Vipaka&quot; (Post digestive effects on taste)</td>
<td>&quot;Madura vipaka&quot;9, 10</td>
</tr>
<tr>
<td>&quot;Virya&quot; (energy)</td>
<td>&quot;Sheeta&quot;9, 10 (cooling effect.)</td>
</tr>
<tr>
<td>“Prabhava” (special action of a drug)</td>
<td>“Karma” (pharmacological action).</td>
</tr>
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<td>--------------------------------------</td>
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<tr>
<td></td>
<td>“Vishagnam”&lt;sup&gt;9&lt;/sup&gt; (Antidotes)</td>
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<tr>
<td></td>
<td>“Arshogna”&lt;sup&gt;21&lt;/sup&gt; (cure for piles)</td>
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<td></td>
<td>“Mehagna”&lt;sup&gt;10&lt;/sup&gt; (Anti-diabetic)</td>
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<tr>
<td></td>
<td>“Rasayana”&lt;sup&gt;14, 10&lt;/sup&gt; (Anti- oxidant)</td>
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<tr>
<td></td>
<td>“Raktawardaka”&lt;sup&gt;14&lt;/sup&gt; (Blood formation)</td>
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<tr>
<td></td>
<td>“Medasnashaka”&lt;sup&gt;10&lt;/sup&gt; (Reducing body fat)</td>
</tr>
<tr>
<td></td>
<td>“Kotaprasamana”&lt;sup&gt;23&lt;/sup&gt; (Reduce swelling)</td>
</tr>
<tr>
<td></td>
<td>“Rakthastambana”&lt;sup&gt;21&lt;/sup&gt; (Arresting capillary hemorrhages)</td>
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<tr>
<td></td>
<td>“Purisha sangrahaniya”&lt;sup&gt;14, 10&lt;/sup&gt; (Stool formations)</td>
</tr>
<tr>
<td></td>
<td>“Srotovishodana”&lt;sup&gt;23&lt;/sup&gt; (Effect on body tissues)</td>
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<tr>
<td></td>
<td>“Mootra virechaniya”&lt;sup&gt;18&lt;/sup&gt; (Diuretics)</td>
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<tr>
<td></td>
<td>“Vran ropana”&lt;sup&gt;14&lt;/sup&gt; (Wound healing)</td>
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<tr>
<td></td>
<td>“Vrana shodana”&lt;sup&gt;14&lt;/sup&gt; (wound cleaning)</td>
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<td></td>
<td>“Kushtagna”&lt;sup&gt;14, 10&lt;/sup&gt; (Skin diseases)</td>
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<td></td>
<td>“Sandhaniya”&lt;sup&gt;14, 6&lt;/sup&gt; (Fraction healing)</td>
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<tr>
<td></td>
<td>“Dahaprashamana”&lt;sup&gt;10&lt;/sup&gt; (Reduce pungent feeling)</td>
</tr>
<tr>
<td></td>
<td>“Agnidepaka”&lt;sup&gt;14, 10&lt;/sup&gt; (Increasing enzymes)</td>
</tr>
<tr>
<td></td>
<td>“Raktashodana”&lt;sup&gt;9&lt;/sup&gt; (Blood purification)</td>
</tr>
</tbody>
</table>

Tab: 1.1 Properties of *Drymoglossum piloselloides* according to Ayurvedic literature
1.5. Drug preparation using *Drymoglossum piloselloides* L

Freshly taken the leaves of *Drymoglossum piloselloides* L is consumed as a single material or with combination of other herbs.

There are many ways in which herbs can be prepared and administered to the patient. They are named as “Kalpana”. The several different methods of preparation including *Drymoglossum piloselloides* are as follow,

i. Oil or “Sneha Kalpana”

ii. Juice

iii. Decoction

iv. “Yavagu”

v. Poultice

1.5.1. Oil or “Sneha Kalpana”

Herbal oil is used for internal and external applications. According to Ayurvedic pharmacopoeia there is a standard method and three essential components with standard ratio.

1. A liquid (decoction, juice, milk)- 16 parts

2. A fine paste- 1 part

3. Ghee or oil- 4 parts

The paste which consists of several ingredients is prepared by finely grinding the drugs in a “kalva yanthra” (mortar and pestle) with water or prescribed juice till it becomes a fine paste. Here drugs should be taken in dry form. Liquid may be one or more decoction, juice, milk, meat soup and etc. The oil which is prescribed oil in the recipe
should be used. If the variety of oil is not mentioned Sesame oil should be used. Normally Copper, Iron or earthen pan with wide mouth and less depth is used.

For preparation, one part of herbs is cooked along with four parts of oil and sixteen parts of water, over a low flame for a period of four to eight hours, until all the water evaporates.

Alternately, one can first make the decoction of herbs by itself. Then equal parts of the decoction and the oil are used, and the mixture is similarly cooked until the decoction evaporates. Some herbs that are sensitive to heat can be added directly to the oil and prepared without water. One part of herbs of four parts oil and the mixture is allowed to stand for two days. Strain the mixture and use. Other not quite as sensitive can be added directly to the oil, but they should be cooked over a low flame for several hours, then they may be strained and used. Fresh juice of certain herbs may be added in equal amounts to the oil (as in decoction), and similarly cooked until all water evaporates. Special care must be taken not to over boil them. The oil thus prepared is ready to use.

1.5.2. Decoction ("Kashaya Kalpana")

Ayurvedic pharmacopeia describes several “kashaya kalpana”. It depends on author. Water is used as the media and the main aim of these is to extract the active principles into water by heating or without heating. They are as follow.

1. Swarasa kalpana – Juice
2. kalka kalpana – Decoction using Paste
3. srra kalpana – Decoction
4. sita kalpana – Cold infusion
5. Phanta kalpana – Hot infusion

7. Abhisava kalpana – Alcoholic extraction

8. Curna kalpana – Decoction with Powder

1.5.2.1. Swarasa kalpana – Juice

According to Ayurvedic pharmacopeia the juice is prepared as follows. The juice taken out from a fresh green herb, well pounded and squeezed through a piece of cloth is known as “Swarasa”. To make the “Swarasa” the green fresh drug should be collected on the same day. The juice thus collected should be kept in a glass, metal or clay container covered with a lid. The juice should be prepared only for 24 hours.

1.5.2.2. Srta kalpana– Decoction

Medicine prepared by boiling a drug and water on fire is called srta. One palam(60g) of coarsely powdered drug is boiled with sixteen (16) parts of water in an earthen pot over a mild to moderate fire till liquid is reduced to one eighth of the original quantity (240 ml). The pot should not be covered with a lid. Barks, roots and leaves etc of medicinal plants should be dried in sun light before they are converted into coarse powder. The decoctions are usually prepared fresh just before the application and are generally administered before meals or empty stomach. At one time, decoction required for 24 hours can be prepared.
1.5.3. Porridge or “Yavagu”\textsuperscript{16}

“Yavagu” is used as a meal as well as a remedy. According to “Bisajjaratna sangrahaya”\textsuperscript{16} to make “Yavagu”, thirty two (32) “Palam” of water (1920 ml) and six (6) “Kalan” of drugs (30 g) should be boiling to reduce to sixteen “Palam” of liquid (960 ml). Then after filtered drugs parts, one “Palam” (60 g) of rice should be added and boiled to reduce eight “Palam” (480 ml).

1.5.4. Poultice\textsuperscript{20}

There are several types of methods to prepare poultice. According to “Sharangadhara Sanhitha”\textsuperscript{20} there are three types of poultice based on its actions.

“Patthu” and “Mellum” are two methods which are treatment method for bone fracture especially in Indigenous system of medicine. Both are used for only external application.

Some plants materials with salt and curcuma which are two main ingredients are pounded then used mildly heated to prepare “mellum”. “Mellum” appears like a coarse mixture.

But when preparing the “patthu” heat is not used always. Plant materials should be crushed through a stone grinder (mortar and pestle). During grinding; some kind of liquids which are mentioned like water, gee, milk, and juice of leaves should be added. It should be applied on affected area and not allowed to let dry. The appearance of “patthu” is like a paste or should be uniformly fine. Generally, such poultice should be used within 24 hours. For the next day, fresh paste of drugs should be prepared.
1.6. Systems of Medicine in Sri Lanka

Sri Lanka is a country of rich heritage, one of which is its indigenous system of Medicine, which has been practiced by the people since time immemorial.

According to the present situation there are mainly two medical systems in Sri Lanka. They are divided as Western system and Indigenous system of Medicine. The Western system of medicine arrived with Western invasions.

The Western medical system is conducted under the Ministry of Healthcare and Nutrition. The Indigenous system of Medicine is conducted under the Ministry of Indigenous medicine.

The Ayurvedic system of medicine from North India, the Siddha system of medicine from South India and the Unani system of medicine of Arabs enriched with contributions from the traditional system of medicine called “Desheeya Chikitsa” is popularly known as the Indigenous system of medicine in Sri Lanka.

It is simply illustrated as below Fig: 1.6.

![Diagram of Systems of Medicine in Sri Lanka]

Fig: 1.6 Systems of Medicine in Sri Lanka
According to the above classification there are mainly two kinds of medical practitioners in Sri Lanka. They are Western medical practitioners and non Western medical practitioners. Western medical practitioners should be registered and regulated under Western medical council conducted by the Ministry of Healthcare and Nutrition. Among the non-western medical practitioners, physicians are two kinds. They are Ayurvedic physicians and other physicians who are registered under the Ayurvedic Department. There are five kinds of physician who should be registered and regulated under the Ayurvedic Medical Council, Department of Ayurvedic in Sri Lanka. They are Ayurveda, Traditional, Unani, Siddha and Homeopathy physicians. It is given as below (Fig:1.7).

![Fig: 1.7. Physician who should be registered from Ayurvedic Department](image)

To be an Ayurvedic physician, a Diploma of Ayurveda (DA) conducted by Department of Indigenous medicine or degree, Bachelor of Ayurvedic Medicine and Surgery (BAMS) in universities of Colombo or Kelaniya should be completed. Unani, Siddha and Homeopathy medicine system also are conducted by the Ayurvedic Department in
Sri Lanka. The Bachelor of Unani Medicine and Surgery (BUMS) at University of Colombo and Bachelor of Siddha Medicine and Surgery (BSMS) at University of Jaffna should be completed to be a Unani and a Siddha practitioner. To be a Homeopathic practitioner a course should be follow by Institutes which are registered under Ayurvedic Department in Sri Lanka. Traditional medicine is mainly inherited from ancestors or from elders to their new generation. But it was that, there is a rule (Ayurvedic Act No.31 of 1961) that traditional physicians should also be registered by the Ayurvedic Department.

1.7. Geographical study area of Field survey

Sri Lanka consists of nine (9) provinces and twenty four (24) districts. Out of them three districts from two provinces were chosen for conducting the survey. The reasons for selecting above mentioned districts are the homogeneous population distribution and climatologically it varies from wet zone climates in “Gampaha” District and Colombo District to dry zone in “Kurunegala” District.

Interviews of the field survey were carried out “Polpitigama” divisional secretary in “Kurunegala” District, “Wikkramarachchi” Ayurvedic hospital (teaching) at “Yakkala” in “Gampaha” District and Ayurvedic hospital (teaching) at “Boralla” in Colombo District.
1.7.1. The Western province of Sri Lanka

The Western province which is the most densely populated province of Sri Lanka consists of three (3) districts as Colombo, “Gampaha” and “Kaluthara”.

In Western province, ten (10) Ayurvedic hospitals and sixteen (16) central dispensaries are situated. There are 642 registered Ayurvedic practitioners and 360 specialized physicians were present in Colombo district.

Ayurvedic hospital (teaching) in Colombo and “Wikramarachchi” Ayurvedic hospital (teaching) in Gampaha are main hospitals situated in Western province.

There are General physicians, traditional physicians as well as specialized physicians.
1.7.2. The North-Western province of Sri Lanka

The North-Western province consists of two Districts “Kurunegala” and “Puttalam”. (See appendix II, page 72).

In North-Western province six (6) Ayurvedic hospitals and thirty two (32) central dispensaries are situated.

“Polpitigama” divisional secretary which is 380 km² in the “Kurunegala” district (see appendix II, page 72) was the main area of the survey. According to “Sampath patikada” in 2008 the population of “Polpitigama” was 87,000. That district secretary division consist 311 villages and 82 “Gramaseva vasams”. “Polpitigama” belongs to dry zone. There are well famous traditional physicians there. According to Ayurvedic protecting committees (Ayurvedic “Sagnrakshna saba”) of “Polpitigama” divisional secretary conducted by the Ayurvedic department hundred (100) of Ayurvedic physicians has been registered.
Figure 1.9 Polpitigama divisional secretary areas
CHAPTER 2

2. Methodology of field survey: Part I

The data presented here was collected during the field survey at the above mentioned area from 1 February to 31 August in 2009.

A Questionnaire which contained eighteen (18) questions (See appendix I, page 68), which were multiple choices, text open end and rating scales questions for gathering details about “panampethi”, were designed. The Questionnaire was presented to Ayurvedic practitioners to tick on correct answer or suitable data.

2.1. Sample of survey

The Target populations were fifty (50) of both traditional and Ayurvedic practitioners. Those populations were selected by randomly sampling. Some keen persons were involved. Among them Dr Danister Perera who is an Ayurvedic practitioner having broad and specialized knowledge at subject, Professor Sarath Ranasingh who is a traditional as well as an Ayurvedic practitioner and Mr Piyal Marasingha who is a Botanist at Ayurvedic Department in Sri Lanka.

2.2 Geographical studies area

There were three geographical studies area chosen for the survey. As mentioned in chapter 1.8, the areas were “Polpitigama” divisional secretary in “Kurunegala” District
of the North-Western province and "Wikkramarachchi" Ayurvedic hospital (teaching) at Yakkala in Gampaha District and Ayurvedic hospital (teaching) at "Boralla" in Colombo District of the Western province.

The main area out of them was "Polpitigama" divisional secretary in "Kurunegala" District.

2.3 Calculation of Percentages

The answers taken by Ayurvedic and traditional practitioners were analyzed by calculation of percentages.

The percentages were calculated by the formula: \( \% = \frac{NT \times 100}{n} \)

- \( NT \) - Number of respondents agree with the selected answer
- \( n \) - Total number of respondents
CHAPTER 3

3. MATERIALS AND METHODS : PART 2

3.1. Materials for Chemical

There were several chemicals used in chemical analysis of *Drymoglossum piloselloides*.

3.1.1 Preparation of spray reagents

For TLC detection, several spray reagents were used to identify active compounds of *Drymoglossum piloselloides*. There were given below.

3.1.1.1 Natural products reagent (NPG) \(^8\)

The plate is spread with 1% methanolic diphenylboric acid, β-ethylamino ester (= diphenylcarboxyethylamine, NP)

Detection of flavonoids, Aloin, Intense fluorescence is produce in UV 356 nm PEG increase sensitivity. (From 10 μg to 2.5 μg) The fluorescence behavior is structure depend.

3.1.1.2 Anisaldehyde Sulphuric acid reagent (ASG) \(^8\)

About 5 ml anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulfuric acid, in that order.
The TLC plate was sprayed with about 10 ml, heated at 100 °C for 5-10 min, and then evaluated in vis. or UV 356 nm.

The reagent has only limited stability and is no longer useable when the colour has turned or red-violet. Detection – terpenoids, propylpropanoids, pungent and bitter principles, saponins.

3.1.1.3 Alcoholic Potassium hydroxide reagent (KOH) 

Dissolved 16 g of KOH pellets in 800 cm³ of alcohol (Ethanol) after standing for 24 hrs the clear solution should be separated by decanting/filtering. (The bottle should be closed by using a cork.)

The plate is sprayed with 10 ml and evaluated in visual and in 356 nm with or without warming.

- Detection – anthraquinones (red), anthrone (yellow, UV 356 nm), Coumarins (blue).

3.1.1.4 Libermann-Burchard reagent (LB) 

About 5 ml acetic anhydride and 5ml concentrated sulphuric acid were added carefully to 50 ml of absolute ethanol, while cooling in ice. The reagent must be freshly prepared.

The sprayed plate is warmed at 100 °C for 5-10 min, and then inspected in UV 356 nm.

- Detection of triterpenes and steroids.
3.1.1.5 **Folin Denis reagent**

About 100 g sodium tungstate and 20 g phosphomolybdic acid were added with 750 ml water into 2 L flask. 50 ml orthophosphoric acid was added and refluxed for 2h. After that it was allowed to cool and make up to 1 L. Then it was stored in dark.

3.1.2 **Preparation of Thin layer chromatography plates**

Commercial TLC plates and TLC plates prepared in the laboratory were used for chromatographic analysis.

About 30 g of silica gel and 60 ml of distilled water were mixed well in a stoppered conical flask to prepare slurry. This slurry was carefully poured over clean dry glass plates (10 x 5 cm) so as to obtain a thin, uniform coating of silica on the plates. Finally the coated plates were first allowed to dry in air and then in an oven maintained the temperature at 100 °C.

Commercial TLC plates, Pre coated with silica gel \( \text{Gf 254, (E.Mark), 0.2mm thickness} \) termination the \( R_f \) value was calculated using fallowing formula,

\[
R_f = \frac{\text{Distant travel by the compound}}{\text{Distant travel by the solvent front}}
\]
3.2 Sample preparation for Chemical Analysis

*Drymoglossum piloselloides* was harvested at several areas in “Kurunegala” district. Only leaves of *Drymoglossum piloselloides* were picked and washed to remove waste materials. The leaves were dried in open air in the laboratory about one month under normal environmental condition. Temperate was in that periods at 28-29 °C. The leaves were placed under protection not to allow pest attack.

3.3 Chemical Extraction and Phytochemical Screening tests

Extraction of plant material was performed using methanol, distilled water and hexane.

3.3.1 Methanol Extraction

Dried plant materials were pounded to paste form in laboratory using molar. Hundred grams (100.003 g) of pounded leaves of *Drymoglossum piloselloides* were weighed and extracted at 60 °C for 1½ hrs in 100% methanol using Soxhlet apparatus. About 250 ml of 100% methanol was used for the extraction. (Weights of 35.00 g, 36.03 g and 29.00 g were put into the container for three times because the container of Soxhlet apparatus was not large enough to accommodate whole leaves). The methanol extracts were filtered using filter paper. The solvent was evaporated under vacuum using a rotary evaporator.

The phytochemical tests below were carried out on the sample of methanol extraction to determine the presence of alkaloids, sterols, terpenoids and flavanoids.
3.3.1.1 Test for Alkaloids

A quantity 1 g of methanol extract was stirred and heated with 10 ml of 2 M hydrochloric acid on steam bath for 5 minutes. After been cooled to room temperature, 0.5 g of sodium chloride (NaCl) was added and mixed with stirring. Then 2 M hydrochloric acid (HCl) was added to the filtrate to bring the final volume up to 10 ml. This final solution was divided into two equal 5 ml portions in two test tubes. A few drops of Mayer’s reagent and a few drops of Wagner’s reagent were added separately into the two tubes.

3.3.1.2 Test for Steroids and Terpenoids

A quantity 1 g of methanol extract repeatedly extracted with 10 ml portions of petroleum ether by triturating and decantation. Then 10 ml of chloroform was poured into the dish and stirred well to extract. Then it was poured through anhydrous sodium sulphate to dry. 3 ml portions of the chloroform extract were poured into clean dry test tubes (test tube A and test tube B) and the tests mentioned below were carried out.

- Test for Unsaturated sterols and Triterpenes (Liebermann-Burchard Test)

Three drops of acetic anhydride was added into the tube A and mixed by swirling. One drop of concentrated sulfuric acid was added and mixed. Then colour changes were observed immediately and over a period of an hour.
- **Test for Unsaturated sterols (Salkowski Test)**

The test tube B was held at angle of 45° angle and 1-2 ml of concentrated sulfuric acid was poured along the side of the test tube. Then the colour at the junction of the two liquids was observed immediately and over a period of an hour.

### 3.3.1.3 Test for Flavanoids

A quantity 1 g of the methanol extract was repeatedly extracted with 10 ml portions of petroleum ether to remove pigmented materials by triturating and decantation. Then the defatted residue was dissolved in 30 ml of 80% ethanol and filtered. The colour was light yellowish orange. The filtrate was poured into two test tubes (tube C and tube D). Tube C was used as a blank control. 0.5 ml of concentrated hydrochloric acid and Magnesium turnings were added to the tube D. Then the colour change was observed.

### 3.3.2 Water Extraction

Four- five of *Drymoglossum piloselloides* were taken into a beaker with 25 ml of distilled water and boil for few minutes. After cooling to the room temperature, the extract was filtered.
3.3.2.1 Test for Poly phenolic compounds

Portion of 10 ml aqueous extract was divided into two portions. Ferric chloride-potassium ferricyanide reagent and ferric chloride (2%) solution were added to each of the portions and observations were recorded.

3.4 Chemical Analysis

After the identification of chemical compounds in *Drymoglossum piloselloides*, thin layer chromatography and Ultraviolet visible spectroscopy were used for the analysis of compounds.

3.4.1 Extraction and TLC detection of Polyphenolic compounds

About 5 ml methanol extract was reduced to 1 ml using a hot water bath. Then the TLC plate was spotted with the extract using a thin capillary tube. The chromatogram was run in the solvent systems which were Methanol to Chloroform (6:4) and Ethyl acetate to Glacial acetic acid to Formic acid to water (100:11:11:26). After that about 10 ml of anisaldehyde -Sulphuric acid reagent was sprayed on the TLC plates and heated at 100 °C for 5-10 min. The developed chromatograms were then inspected under visible light and UV radiation at 356 nm.

3.4.2 Extraction and TLC detection of Unsaturated Sterols

About 5 ml methanol extract was reduced to 1 ml using a hot water bath. Then the TLC plate was spotted with the extract using a thin capillary tube. The chromatogram was
run in the solvent systems which were Methanol to Chloroform (6:4) and of Ethyl acetate to Glacial acetic acid to Formic acid to water (100:11:11:26). After that; about 10 ml of the Libermann-Burchard (LB) reagent was sprayed on the TLC plates and heated at 100 °C for 5-10 min, and then inspected in visible light or UV at 356 nm.

3.4.3 Extraction and TLC detection of Flavonoids

Powdered leaves of *Drymoglossum piloselloides* (1 g) was extracted with 10 ml methanol for 5 min on a water bath at about 60 °C and then filtered. The extract was concentrated to about 2 ml. Then 1ml water and 10 ml ethyl acetate was added and the mixture was shaken several times. After that, it was kept to allow the separation of the two phases. The ethyl acetate phase was separated and reduced to 1 ml by evaporation. About 10 μl of evaporation was used to put on the TLC plates (5x10 cm) for investigation.

Chromatography solvent mixture was composed of Ethyl acetate: Glacial acetic acid: Formic acid: water (100:11:11:26). Then NP reagent sprayed and the spots on the TLC were observed under UV lamp (356 nm).

3.5 Determination of Carotinoids

3.5.1 Sample preparation-Hexane extraction

About 50 ml of the methanol extract of *Drymoglossum piloselloides* was taken into the separation funnel and extracted repeatedly with 10 ml of hexane until hexane portion colorless. Then funnel was moved slowly and kept to separate two liquids. Lower
liquid layer was separated out into a conical flask. Then the solvent was evaporated under vacuum using a rotary evaporator.

3.5.2. Determination of UV-Visible spectrum of Hexane sample

The spectrum was determined using model-Heλios Alpha & beta UV-Visible spectrometer. Amount 1 ml of the hexane extraction was diluted with 3ml of hexane and placed in a transparent cell providing one centimeter diameter. Readings were taken after blanking the machine with hexane.

3.6. Determination of Chlorophyll

3.6.1. Sample preparation

A quantity 0.5 g of dark green colored filtrate which obtained by methanol extraction was dissolved in 20 ml of absolute Methanol.

3.6.2. Determination of UV-Visible spectrum of Methanol sample

Small amount of sample was placed in a transparent cell providing one centimeter diameter to analyze by using UV-visible Spectroscopy. Readings were taken after blanking the machine with absolute Methanol. Because the filtrate was dark green, it may be present chlorophyll.
CHAPTER 4

4 RESULTS AND DISCUSSION: PART 1

The data results of survey were analyzed as percentages.

4.1 Identification of “Panam-peti” or Drymoglossum piloselloides

All physicians were identified Drymoglossum piloselloides which is an epiphytic fern climbing on trees, thick and fleshy rounded shape leaves like the ancient the Ceylon coin called “Panama”. That is the main reason to call it as “Panam peti”.

There are several vernacular names for Drymoglossum piloselloides in Sri Lanka. They are “Panam-peti” and “Kimbul venna”.

“Nighantu”, a kind of books belong to Ayurvedic medicine, play as Herbal Materia Medica. The similarities of plants, the synonyms of plant, the action of plant, the classification of plant, the morphology and more details are indicated in the “Nighantu”. “Wanawasa Nighantu” and “Saraswathi Nighantu” are two books out of them belonged to Sri Lanka. There is a plant category called as “Vanna hata” meaning seven creepers in those books. “Kimbul venna” is mentioned as a creeper out of seven. According to above literatures7, 8 “Kimbul venna”, is climbing on tree or somewhere but never growing on ground.

But “Kimbul venna” is also used as synonym of “Petipala”.

A question was included in asking whether Drymoglossum piloselloides is belonged to “Vanna hata”. The result by percentage show as follow pie chart in Fig: 4.1.
According to above result 12% of physicians have mentioned, *Drymoglossum piloselloides* is not included in “Vanna hata”. Although “Kimbul venna” is included in, it is not considered as *Drymoglossum piloselloides*. They expressed “Kimbul venna” is also a creeper having four leaflets, grow on the ground, called as “Petipala”.

About 51% of the physicians have mentioned there have no idea about “Vanna hata” and they wanted to find out clear details from some books specially “Nighantu” to express that.

The rest of the physicians, 37%, have mentioned that *Drymoglossum piloselloides* is “Kimbul venna” and it belongs to “Vanna hata”.

In view of Piyal Marasingha who is a botanist in Ayurvedic Department in Sri Lanka, having a broad and specialized knowledge of medicinal plants, although few plants are used “Kimbul venna” as synonym it is not declared yet, but it depends on areas.
“Kimbul venna” is also like that. His opinion was that “Kimbul venna” is an unknown plant.

4.2 Methods of preparation

There are so many methods of drug preparation using *Drymoglossum piloselloides*. Those are as follow bar chart.

Fig: 4.2 Drugs preparation using *Drymoglossum piloselloides*

As a result, oil is used most of physicians. And poultice ("Pattu" and "Mellum") and juice also are used in high percentage. Decoction and "Kenda" are used in comparatively lower percentages.

Oil

Due to several administrates the percentage of oil was high.

The oil is prepared including this plant for Wound healing, Hair blacking, Alopecia, Hair growing, Ortho-pediatric, Paralysis and some brain and nerve disorder.
The “Henaraja thylaya”\textsuperscript{15} is specific oil for brain and nerve disorders. “Wata viduranga thylaya”\textsuperscript{15} too is used for the same remedy. \textit{Drymoglossum piloselloides} is one ingredient in both oils and used as external as well as an internal remedy. Oral, suppository (“Anuwasana or Sneha Vasti”) and nasal inhalation (“navana”) were routes of internal administrations of the above mentioned oils.

“Vasti” is applied through the rectum and may also be applied per uretra. According to Ayurvedic Medicine “Vasti” is the main treatment plan for balancing “vatha dosa”. The brain and nerve disorders consider as a progresses of unbalancing “vatha dosa”. Above two oils are used as also “vasti”.

“Navana” or “nasna” and drinking oil are other internal administrative methods of oils. One tea spoonful of oil normally is used for mixing with some liquids like decoctions or directly drinking per twice a day. One to three drops of oil is used for nasal administration. It depended on diseases, state of patient and stages of disease.

The oil is used for Wound healing, Hair blacking, Alopecia, Hair growing, Orthopediatric as external application. A piece of cloth soaking with oil is bandaged on affected area for Ortho-pediatric condition. That is the first treatment step of Ortho-pediatric condition after bones is located in the write position.

\textbf{Decoction and juice}

When preparing a juice \textit{Drymoglossum piloselloides} is used as single ingredient for treatment of cancer relieving especially in Breast cancer, piles, diarrhoea and thirst. The percentage preparing juice was fifty four (54%).
The boiled water of *Drymoglossum piloselloides* is used for piles, diarrhea and thirst, cancer relieving, Jaundice, Pediatric diseases, reduce swelling, urinary disorder, Anemia and blood purification.

*Drymoglossum piloselloides* is used with other ingredients in some decoctions which are treated for piles, diarrhea and thirst, Diuretics, urinary disorder, Anemia and blood purification, Paralysis and anti-Hemorrhagic condition. The percentage was thirty four (34%).

**Porridge or “yavagu”**

Porridge including *Drymoglossum piloselloides* is used for Jaundice, Blood formation, Blood purification and Anemia. The percentage was thirty four (34%).

**Poultice**

*Drymoglossum piloselloides* is used as single ingredient of preparing the poultice ("Pattu") to Reduce pungent feeling, Antidotes, Reduce swelling, Eye diseases, subcutaneous lumps, wound healing, Hemorrhagic condition, Alopecia. And it is used as “Mellum” for vaginal prolapses, rectal prolapses, Ortho-pediatric as dislocations and bone fractures.

Psychiatry named as “Unmada” treat for disturbance of memory and sleep, unconsciousness and all brain disorders. Special treatment of “Unmade” is “hisa kuduchchiya”, some kind of paste, applies on the head for expecting a cooling effect. *Drymoglossum piloselloides* also is used for that remedy because of its cooling effect.

The following chart indicates simply the method of preparation of *Drymoglossum piloselloides* based on Therapeutic value.
<table>
<thead>
<tr>
<th>Method of preparation</th>
<th>Therapeutic Usages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>Wound healing, Hair blacking, Alopecia, Hair growing, Ortho-pediatric, Paralysis, Nerve disorder</td>
</tr>
<tr>
<td>Porridge</td>
<td>Jaundice, Blood formation, Blood purification and Anemia</td>
</tr>
<tr>
<td>Juice</td>
<td>Breast cancer, Piles, Diarrhoea and Thirst.</td>
</tr>
<tr>
<td>Poultice (&quot;Pattu&quot;)</td>
<td>Reduce pungent feeling, Antidotes, Reduce swelling, Eye diseases, Subcutaneous lumps, Wound healing, Hemorrhagic condition, Alopecia, Headache</td>
</tr>
<tr>
<td>Poultice (&quot;Mellum&quot;)</td>
<td>Vaginal prolegs, Rectal prolegs, Ortho-pediatric</td>
</tr>
<tr>
<td>Decoction</td>
<td>Piles, Diarrhea, Thirst, Diuretics, Urinary disorder, Anemia, Blood purification, Paralysis, anti-Hemorrhagic condition</td>
</tr>
</tbody>
</table>

Table 4.1: Method of preparation based on Therapeutic value
4.3 Educational and Professional qualification

Traditional and Ayurvedic Physicians were selected randomly from the Western and North western provinces in Sri Lanka.

According to results traditional physicians were 58%, Diploma of Ayurvedha 8%, Diploma holder of Ayurvedic medicine and surgery 15%, and Bachelor of Ayurvedic medicine and surgery 19%. It is as follow.

![Chart showing percentages of Physicians participation for survey]

Physicians were categorized according to their generation and registration under Ayurvedic department in Sri Lanka. Normally DA, DAMS and BAMS physicians get the registrations as General physicians. But traditional physicians are different; they get the registrations due to their generation which come from ancestors or as General. Their generations illustrate which categories were specialized.

Traditional physicians treat in their specialized field. So they automatically become as specialist physicians for several diseases. There were well known several diseases treated by specialized traditional physicians in Indigenous system of medicine like
Paralysis, Ortho-paedics, Psychiatry (Unmada”), Venom logy, ENT, Dermatological condition and so on.

According to the results the physicians were specialized in Dermatologists 7%, Ortho-paedics 22%, Psychotherapist 2%, Eye surgeon 4%, Burnt injury 5%, Venologist 9% and General 51%.

![Diagram showing the distribution of physicians by specialty.](image)

Fig: 4.4 Position of physician

Considering the above data *Drymoglossum piloselloides* is used for whatever preparation in Ortho-paedicts field, Psychotherapist, Dermatologists, Eye surgeons, Burnt injury, Venologist.

### 4.4 Used formation

Ayurvedic pharmacopeia has mentioned that in general freshly collected drugs should be used and dried. All the time freshly collected and dried drugs are to be used. Wet
drugs are to be used in double prescribed quantity. But it depends on methods. Leaves of *Drymoglossum piloselloides* are thick and fleshy.

![Graph: How to taking Drymoglossum piloselloides for preparing drugs]

**How to taking Drymoglossum piloselloides for preparing drugs**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Dried Form</th>
<th>Fresh Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>80%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Taking forms**

Fig: 4.5 Usage form for drug preparation

This plant was used in fresh form by all physicians. *Drymoglossum piloselloides* should be taken in fresh form due to some reasons. As *Drymoglossum piloselloides* is very fleshy, a lot of qualities may disappear during drying process. On view of Ayurvedic opinion *Drymoglossum piloselloides* has cooling effect. That is called as “Shita Veerya” in metabolic activity. To cure some diseases which cause aggravation of “Pitta Dosha” that quality of the drugs should be needed.

In other hand *Drymoglossum piloselloides* may have some vitamins. So during drying process those might be destroyed. So according to them fresh form is the best form for drug preparation. That was the reason to get the 100% answer.

But according to the above result there is 30% of using dried form. There were the reasons. Some physicians mentioned that *Drymoglossum piloselloides* grows in humid
environment so in some time periods this plant could not be found in their areas which belong to the dry zone. According to necessity of the medicine preparation, dried form is also to be used.

The *Drymoglossum piloselloides* in fresh form is used for preparing the juice, the paste, the oil and the “kenda”.

The dried form is used for the preparation of decoctions and some oils especially when fresh leaves could not be found.

4.5 **Ethno medical Usages of Drymoglossum piloselloides**

According to the Ayurvedic system of medicine, it is believed that the body becomes susceptible to a host of disease mainly due to accumulation of various metabolic wastes and toxins in the body. Hence, treatment is directed towards proper elimination of these wastes and toxins from the body. According to ancient Indian philosophy, the universe is composed of five basic elements or “Pancha Bhutas”: “Prithvi” (earth), “Jala” (water), “Agni” (fire), “Vayu” (air), and “Akash” (sky). The Pancha Bhutas are represented in the human body as “Doshas”, “Dhatus” and “Malas”. To be healthy, equilibrium of three “Doshas”, sevens “Datus” and three “Malas” is essential. When at their natural state of balance, our “Doshas” provide the strength to our bodies and need to prevent conditions that may allow disease. When out of balance, the body becomes susceptible to disease. Our “Dosha” can go out of balance due to toxin accumulation, improper diet, climatic condition, unhealthy habits and stress. In order to derive any benefit from Ayurvedic medicines, it is of utmost importance that we evaluate our constitution on the basis of these “Doshas”.
There were a lot of diseases which be cured from *Drymoglossum piloselloides*. The diseases and percentage given by Fig: 4.6

![Ethno-medical usages of Drymoglossum piloselloides](image)

**Fig: 4.6.Ethno- medical Usage of *Drymoglossum piloselloides* as the percentages.**
According to above result there are high rates of usage *Drymoglossum piloselloides* in several diseases. They are Orthopaedics, Rectal prolapsed and for reducing pungent feeling. The percentage was more than 50%. Some diseases were between 20%- 40%, Diarrhoea, Haemorrhoid, Anemia, Hemorrhagic condition, Reduce swelling, wound healing and Blood formation.

Any bone fracture and cracking, joint dislocation, sprains of any site in the body could be cured under Ortho-paedics. “Mellum” and “Patthu” are famous as special remedies for this field. If any plant is used for “Mellum” and “Patthu” it should have fraction healing properties, reduce swelling and relieving pain. Due to the above properties, *Drymoglossum piloselloides* is used in Ortho-paedics.

Diarrhoea\(^{22}\) is deviation from established bowel rhythm characterized by an increase in frequency and fluidity of the stools. To recover this problem should decrease frequency as well as fluidity. In Ayurvedic system, if some drug consists of stool formation action (“Purisha sanghahaniya\(^{10, 14}\)”) it is able to cure that problem. Considering *Drymoglossum piloselloides* it has the stool formation action. Some decoctions, boiled water were used to cure that problem.

A haemorrhoid\(^{22}\) or pile is mentioned as varicosity of the veins around the anus. There are five type based on aggravation of “Dosha” in Ayurveda. Cause of “Raktaga” type (oozing of the blood) is due to the rupture of capillaries. It is the type of haemorrhagic condition.
Haemorrhagic condition means capillary bleeding. If bleeding consist wherever the body, *Drymoglossum piloselloides* can arrest that condition. Some paste, Decoctions, “kenda”, fluids, boiled water and pastes are used for anti- Hemorrhagic action. The percentage of anti- Hemorrhagic action was 32%.

Anemia[^22] is defined as a deficiency of haemoglobin in the blood due to lack of red blood cells and or their haemoglobin content. “Raktawardaka[^14]” (blood formation) action of *Drymoglossum piloselloides* declared there may be action to settle the lack of red blood cells and or their haemoglobin content at corresponding level. The percentage for treatment for Anemia was 34%. Decoctions, “kenda”, fluids and boiled water are utilized to cure Anemia.

Rectal prolapsed[^22] or rectocele define as prolapsed of the rectum, usually herniation of anterior rectal wall. Vaginal prolapsed also like that. The main reason for both is due to decrease of muscular tone of those walls. Therefore *Drymoglossum piloselloides* consist of “Rasayana[^14]”, it helps to cure that problem. The “mellum” prepared from *Drymoglossum piloselloides* normally with Gee is used for rectal prolepses. According to Ayurvedic physicians it is answered well for rectal prolapsed.

Reduce pungent feeling was other main action, percentage 61%. “Shitha Virya[^9, 10]”, (cooling effect) of *Drymoglossum piloselloides* act to reduce pungent feeling .The pungent feeling is one kind of disease according to Ayurveda, called as “Dahaya” (burning sensation). The aggregation of “Pita dosha” cause pungent feeling. Apart from that burn injury also induces pungent. “Vran ropana[^14]” (Wound healing)
and “Kushtagna” action need to cure burn injury. The paste including only *Drymoglossum piloselloides* is applied on affected area as remedy for pungent feeling.

Antidotes are a remedy which counteracts or neutralizes the action of a poison. Some pastes are used for neutralizes poison.

One focused area in the survey was assessing the ability of *Drymoglossum piloselloides* to cure Dengue fever.

Dengue is defined as one of the mosquito transmitted haemorrhagic fever, characterized by rheumatic pains, fever and a skin eruption. The haemorrhagic form has a high mortality. According to Western medicine there is no specific treatment. They indicate pain killer, volume replacement, blood transfusions and management of stock in the capillary leak syndrome. *Drymoglossum piloselloides* have “Rakthasthambana” (Arresting capillary hemorrhages), “Raktashodana” (Blood purification), “Raktawardaka” (blood formation) “Shitha Virya” (cooling effect), “Vran ropana” (Wound healing) and ability to cure Anemia. Considering the results and above mentioned properties of *Drymoglossum piloselloides*, it may have to a certain extent act for curing dengue fever.

Reduce swelling is abnormal infiltration of tissues with fluid. It is a symptom of several diseases. The action of “Kotaprasama” (Reduce swelling) help to reduce or remove abnormal infiltration of tissues with fluid. They are using some paste for external application and some decoction consisted other plants also used as a diuretic (“Mootra virechaniya”) which also help to balance body fluids. The percentage for treatment for that was 23%.
Cancer\textsuperscript{22} is a general term which covers any malignant growth in any part of the body. The growth is purposeless, parasitic, and flourishes at the expense of the human host. There is a new opinion, not only cancer but also all types of diseases arise due to accumulation of oxidant in the body. So \textit{Drymoglossum piloselloides} acts as an antioxidant. There is some evidence\textsuperscript{25,12} to prove anti cancer action.
CHAPTER 5

5 RESULTS AND DISCUSSION: PART II

5.1 Results of extraction

During extraction of the plant, the colour of solvent was dark green. After becoming to room temperature the colour was greenish brown with turbidity (Fig: 5.1). There was some precipitate in dark greenish. After filtered about 230 ml amount of extraction was taken in brownish yellow colour with little bit turbidity.

Fig: 5.1 Methanol extraction of Drymoglossum piloselloides

There was some precipitate on the filter paper. That was dark green powder form and weight was 6.701 g. (Fig: 5.2)

Fig: 5.2. The precipitate on filter paper
After evaporated solvent under vacuum using a Rotary Evaporator the methanol extracts was afforded 15.065 g.

- **Hexane extraction**

About 100 ml of hexane portion was taken. It was bright light yellow, clear and transparency (Fig: 5.3). Ongoing procedure gradually hexane colour was reducing from bright light yellow to colourless. After evaporated solvent under vacuum using a rotary evaporator the hexane extract was afforded 0.0528 g. The yield was 0.012 %.

The Methanol extraction after taking hexene partition was tea water colour (Fig: 5.3).

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**Fig: 5.3 Hexane extraction is yellow colour and Methanol extraction after hexene partition is tea water colour**

### 5.2 Result of screening tests

The results of the phytochemical analysis carried out using different extraction methods revealed the presence and the absence of some secondary plant metabolites. The results of the phytochemical analysis are shown in tables 5.1.
Tab: 5.1 Observation and Results of the phytochemical screening tests on using methanolic extraction.

<table>
<thead>
<tr>
<th>Screening test for</th>
<th>Testing methods</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>using Mayer’s and Wagner’s reagent</td>
<td>no any colour changes or precipitate with Mayer’s and Wagner’s reagent</td>
<td>Absence of Alkaloids</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>with Cyaniding Test</td>
<td>Reddish pink colour was observed (See Fig: 5.4)</td>
<td>Presence of Flavonoids</td>
</tr>
<tr>
<td>Unsaturated Sterols and Triterpins</td>
<td>using .Libermann-Burchard Test and Salkowski Test</td>
<td>Immediately no colour change After an hour, A small brown coloured oil drop in the bottom Reddish brown colour ring at the junction After an hour two liquid portions were observed brownish colour below and above colourless.</td>
<td>Presence of steroids</td>
</tr>
</tbody>
</table>
According to the table 5.1, it was evident that methanolic extract did not contain alkaloids.

The Liebermann-Burchard test was used to detect the presence of polycyclic substances like sterols and triterpenes in the extract. Triterpenoids give purple and pink colours than the blue shades suggestive of steroids\textsuperscript{30} (L.J Webb, 1955). A reddish-brown interface for Salkowski Test shows the presence of steroids (Parker Elijah and EZE, Nkechi J, 2009).

The observations of some screening tests are shown by following figures. According to the figure 5.4,

![Fig: 5.4 Observation of Cyaniding Test](image1)

![Fig: 5.5 Observation of test with Ferric chloride](image2)
According to the above results methanolic extraction of *Drymoglossum piloselloides* contain some chemical compounds such as Flavonoids, Sterols and Terpenoids.

Tab: 5.2 Observation and Results of the phytochemical screening tests on using water extract

<table>
<thead>
<tr>
<th>Screening test for</th>
<th>Testing methods</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenolic compound</td>
<td>with Ferric chloride</td>
<td>Blackish blue precipitate and brown colour solution (see Fig: 5.5)</td>
<td>Presence of Polyphenolic compound</td>
</tr>
</tbody>
</table>

According to table 5.2, water extract of *Drymoglossum piloselloides* contain poly phenolic compounds.

That was confirmed the article written by A. Putradjaja\textsuperscript{25}, August 2009. According to that chemical compound of *Drymoglossum piloselloides* were aetheric oil, sterol, triterpen, phenol, flavonoid, tannin and glucose.

5.3 Result of TLC

5.3.1 Treatment with NP reagent (Flavonoids)

Two solvent systems were used. They were Ethyl acetate: Glacial acetic acid: Formic acid: water-100:11:11:26 (v/v/v/v) and Toluene: Ethyl acetate: formic acid-5:4:1(v/v/v). A sample, concentrated extractions ethyl acetate, was used on each TLC plate.
According to results there were four spots; colored yellow, orange, yellowish green and blue both solvent systems. The colour of spots were same but the arrangement of fraction considering colour were counter current in both solvent systems. They were given bellow figure 5.6.

Fig: 5.6 TLC treatments with NP reagent in solvent systems, a: - Ethyl acetate: Glacial acetic acid: Formic acid: water, 100:11:11:26 (v/v/v/v), b: - Toluene: Ethyl acetate: formic acid, 5:4:1 (v/v/v) (EA: extraction of Ethyl acetate)

The solvent front as figure 5.6 a, was 7.2 cm and compound fronts were from top to down 7.1 cm, 6.9 cm, 6.3 cm in ethyl acetate sample that three colour were seen as inflorescent. And at 2.4 cm there was a blue spot.

Considering figure 5.6 b, solvent front was 7.3 cm. There were four spots at 2 cm, 1.2 cm, 0.7 cm, 0.4 cm in inflorescent in ethyl acetate sample.

According to the above results responsible compounds may be as table 5.3.
<table>
<thead>
<tr>
<th>Colour of spots</th>
<th>$R_f$ value</th>
<th>Responsible compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>orange</td>
<td>0.05 0.95</td>
<td>Flavones</td>
</tr>
<tr>
<td>Yellowish orange</td>
<td>0.09 0.98</td>
<td>Flavonols</td>
</tr>
<tr>
<td>Yellow green</td>
<td>0.14 0.87</td>
<td>Flavonols</td>
</tr>
<tr>
<td>blue</td>
<td>0.27 0.33</td>
<td>phenol carboxylic acids</td>
</tr>
</tbody>
</table>

Tab: 5.3 the retention factors ($R_f$) and responsible compound for Flavonoids.

Flavonoids are Polyphenolic compounds that are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, anthocyanidins and chalcones. They have potential beneficial effects on human health having antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities.

Treatment with NP reagent generates in UV 356 nm predominantly orange and yellow-green fluorescence for the, Flavone and Flavanol type and phenol carboxylic acids appear as intense, light-blue zones.$^{13}$

Fig: 5.7 the flavanoid skeleton
According to results there are four flavonoids. They may be Flavone type, Flavanol type. Apart from that there are phenol carboxylic acids. To express perfectly that, further investigation should be carrier and identify which were consisted.

5.3.2 Treatment with Folin-Denis reagent (Poly phenol)

Three solvent systems were used. They were Methanol: Chloroform in ratios 4:6 and 6:4 and Ethyl acetate: Glacial acetic acid: water in ratio 7:1:2.

After sprayed Folin Denis reagent, a black streak was observed up to 2.4 cm in Fig: 5.8a. The solvent front of that was 8.4 cm.

The solvent front as Fig: 5.8 b was 9.1 cm and in which black spot at 7.2 cm and black streak were observed up to 1.1 cm. And Fig: 5.6 c there was blackish streak up to 6.8 cm and solvent front was 7.8 cm in same solvent complex but only on laboratory prepared TLC plate.

Fig: 5.8 d, Ethyl acetate: Glacial acetic acid: water in ratio 7:1:2 (v/v/v), the solvent front was 8.5 cm and there was a black spot at 2.6 cm.

The TLC plates after treatment with Folin Denis reagent illustrate by following Figure 5.8.
Fig: 5.8 TLC analyses for poly phenol using Folin Denis reagent a: - Methanol: Chloroform, 4:6 (v/v), b and c: - Methanol: Chloroform in ratio 6:4 (v/v), d: - Ethyl acetate: Glacial acetic acid: water, 7:1:2 (v/v/v)

According to the above figure 5.8 blackish coloured spots were observed on all TLC. But component front were differ due to polarity of solvent systems.

The retention factors (Rf) and responsible compound are as follow.

<table>
<thead>
<tr>
<th>Component front</th>
<th>Rf value</th>
<th>responsible compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig:4.6 a- up to 2.4 cm blackish streak</td>
<td></td>
<td>Phenol$^{11}$</td>
</tr>
<tr>
<td>Fig:4.6 b- @7.2 cm black &amp; 1.2 cm black</td>
<td>0.96</td>
<td>Phenol$^{11}$</td>
</tr>
<tr>
<td>Fig:4.6 c- up to 6.8 cm blackish streak</td>
<td>0.16</td>
<td>Phenol$^{11}$</td>
</tr>
<tr>
<td>Fig:4.6 d -@2.6 cm black</td>
<td>0.30</td>
<td>Phenol$^{11}$</td>
</tr>
</tbody>
</table>

Tab: 5.4 the retention factors (Rf) and responsible compound for poly phenol.

Treatment with Folin Denis reagent $^{11}$ generates in visual blackish colour for phenols. According to results there are more phenol compounds.

5.3.3 Treatment with Anisaldehyde Sulphuric acid reagent (Triterpenes)

Three solvent systems were used they were Ethyl acetate: Glacial acetic acid: Formic acid: water, 100:11:11:26 (v/v/v/v) and Methanol: Chloroform in ratio 6:4 (v/v).
Anisaldehyde Sulphuric acid reagent a: - Methanol: Chloroform, 6:4 (v/v) b:-Ethyl acetate: Glacial acetic acid: Formic acid: water, 100:11:11:26 (v/v/v/v)

Fig: 5.9 a shows each one spot same sample in Ethyl acetate: Glacial acetic acid: Formic acid: water, 100:11:11:26 (v/v/v/v) solvent systems. The solvent front was 8.9 cm and at 4 cm was each red-violet spots.

Methanol to Chloroform 6:4 (v/v) was also used. It shows in Fig: 5.9 b. According to that there were three different colored streaks combined together. They were about 2.3 cm in bluish black, about 2.9 cm in red-violet and about 3.9 cm in lighter than violet. But in UV 356 nm there was inflorescent as considering above 3.9 cm in light yellow and bellow combination of two in dark colour.

According to Wagner, treatment with Anisaldehyde Sulphuric acid reagent red-violet colour defined isoflavone especially polar compound as terpenoids, propylpropanoids, pungent and bitter principles, saponins.
Therefor *Drymoglossum piloselloides* may consist of terpenoids, propylpropanoids, pungent and bitter principles, saponins. Further investigation should be apply to identify above compounds.

### 5.3.4 Treatment with Liebermann-Burchard reagent (Steroids)

After treat with Liebermann-Burchard (LB) the result as follow figure 5.10 the solvent system was Methanol and Chloroform in 6:4. The solvent front was 8 cm. In visually two different colour spots at 3.4 cm and 2.4 cm were seen in streak. One spot was yellowish colour which was by 3.4 cm distance and other was brownish colour by 2.4 cm distance. But above yellowish colour spot was only seen under UV 356 nm.

![TLC analyses for Unsaturated Sterols using Liebermann-Burchard reagent in Methanol: Chloroform, 6:4 (v/v)](image)

Fig: 5.10 TLC analyses for Unsaturated Sterols using Liebermann-Burchard reagent in Methanol: Chloroform, 6:4 (v/v)

The $R_f$ values for component front were given table 4.5.
<table>
<thead>
<tr>
<th>Component front</th>
<th>$R_f$ value</th>
<th>responsible compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>@3.4 cm yellowish spot</td>
<td>0.42</td>
<td>sterol</td>
</tr>
<tr>
<td>@ 2.4 cm brownish spot</td>
<td>0.3</td>
<td>sterol</td>
</tr>
<tr>
<td>@3.4 cm yellow spot UV 356 nm</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

Tab: 5.5 the retention factors ($R_f$) and responsible compound for Unsaturated Sterols

Treatment with Liebermann-Burchard (LB) generates colour defined, *Drymoglossum piloselloides* may have several sterols.

### 5.3.5 Treatment with Potassium hydroxide reagent

![TLC analyses using KOH reagent in Methanol: Chloroform, 6:4 (v/v)](image)

Fig: 5.11 TLC analyses using KOH reagent in Methanol: Chloroform, 6:4 (v/v)

The TLC plate (Fig: 4.11) was prepaid for spray Potassium hydroxide reagent. Before spraying Potassium hydroxide it was seen in visually yellow colour spot and blue streak.
from 1.3 cm to 4.5 cm under UV 356 nm. After spraying Potassium hydroxide a yellow colour spot was become bright yellow and there was inflorescence in visually above yellowish meddle brownish and below reddish brown. Under UV 356 nm at 2.4 cm was blue spot. The Solvent front of that TLC was 8.4 cm.

The Rf values and responsible compound are given table 5.6.

<table>
<thead>
<tr>
<th>Component front</th>
<th>Rf value</th>
<th>responsible compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>@0.9 cm reddish brown</td>
<td>0.1</td>
<td>Anthraquinones</td>
</tr>
<tr>
<td>@1.9 cm brown</td>
<td>0.22</td>
<td>Anthraquinones</td>
</tr>
<tr>
<td>@4.4 cm yellow</td>
<td>0.52</td>
<td>Anthrone</td>
</tr>
</tbody>
</table>

Tab: 5.6 the retention factors (Rf) and responsible compound after treatment of KOH

Treatment with KOH if spots show in red colour there may be anthraquinones (red), and yellow, UV 356 nm anthrone. Considering results *Drymoglossum piloselloides* may be consist of anthraquinones and anthrone. But further investigation should be done.

![Fig: 5.12 Structure of anthrone](image)

![Fig: 5.13 Structure of anthraquinones](image)
5.4 Determination of UV-Visible spectrum of precipitate.

Two samples were used to analysis by UV spectroscopy. The first was the precipitate which was in methanol extraction. The second was the hexane extraction.

![UV-Visible spectrum of precipitate in methanol of Drymoglossum piloselloides](image)

**Fig: 5.14 Ultra violet -visible spectrum of precipitate in methanol 515-713 nm**
UV-visible spectrum of precipitate in Methanol of *Drymoglossum piloselloides*

![Graph](image)

Fig: 5.15 Ultra violet -visible spectrum of precipitate in methanol 450-600 nm

According to results of above two figures (5.14 and 5.15), table 4.7 show maximum peaks obtained in UV-visible spectrum and they were analyzed according to responsible compounds to standard positions.

<table>
<thead>
<tr>
<th>Peak position</th>
<th>Responsible compound</th>
<th>Standard position</th>
</tr>
</thead>
<tbody>
<tr>
<td>469 nm</td>
<td>Chlorophyll a</td>
<td>465 nm&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>666 nm</td>
<td>Chlorophyll a</td>
<td>665 nm&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Tab: 5.7 Peak positions and stranded positions of Chlorophyll

Considering the peak positions and standard positions the precipitate consists of chlorophyll a. The absorption peaks of chlorophyll $a^{19}$ are at 665 nm and 456 nm. According to result there were peaks at 666 nm and 465 nm.
The leaves (fronds) of *Drymoglossum piloselloides* are thick and fleshy and 3 to 10 mm wide. After harvesting the leaves were dried under environmental condition about one month. At that moment the leaves have been not completely dried. The colour was greenish brown. That colour indicated there might be present some amount of chlorophyll. That was the reason UV-visible spectrum showed the corresponding peaks for chlorophyll.

Apart from that there were several peaks but it was unable to explain which are without further investigation.

### 5.5 Determination of UV-Visible spectrum of Hexane extraction

The result, Ultra Violet Visible spectrum of Hexane extraction, was designed as in two charts to get clear idea. They are as followed in Fig: 5.16 and Fig: 5.17.

![UV-visible spectrum of Hexane extraction of *Drymoglossum piloselloides*](image)

**Fig: 5.16 Ultra Violet -visible spectrum of hexane extraction in 195-695 nm**
Fig: 5.17 Ultra Violet -visible spectrum of hexane extraction in 195-695 nm

According to Fig: 5.17 and Fig: 5.16, the peak positions which obtained from spectrum and their responsible compound with standard position were put in table 5:8.

<table>
<thead>
<tr>
<th>Peak position</th>
<th>Responsible compound</th>
<th>Standard position</th>
</tr>
</thead>
<tbody>
<tr>
<td>422nm</td>
<td>α Carotene</td>
<td>422 nm&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td>443 nm</td>
<td>α Carotene</td>
<td>446 nm&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td>464 nm</td>
<td>Chlorophyll a</td>
<td>465 nm&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>471 nm</td>
<td>β Carotene</td>
<td>478 nm&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Tab: 5.8 Peak positions and stranded positions on some responsible compound.

Considering results, there may be present carotinoids.
There is an evidence to prove the result (See Appendix III, page 75-76). Due to the pattern of two figures, which were obtained from book of "plant physiology" and from spectrum, are some extend same. And other reason the colour of hexane extraction was bright yellow. Therefore Drymoglossum piloselloides might be having carotinoids. But there were several sharp peaks. Further studies should be done to identify.
6 CONCLUSION

The results obtained from the phytochemical analysis of the leaf of Drymoglossum piloselloides showed the presence of sterol, triterpen, poly phenol, flavonoid and the absence of alkaloids.

That was confirmed the article written by A. Putradjaja\textsuperscript{25}, August 2009. According to that chemical compound of Drymoglossum piloselloides were aetheric oil, sterol, triterpen, phenol, flavonoid, tannin and glucose.

The study was revealed a lot of ethno-medicinal usages of Drymoglossum piloselloides. According to the study in indigenous medicine of Sri Lank Drymoglossum piloselloides efficacy for treating many debilitating ailments included Ortho-pediatric, Rectal prolepses, reducing pungent feeling, Diarrhoea, Haemorrhoid, Anemia, Reduce swelling, wound healing, Blood formation, Thirsty, Urinary disorders, Hair growing, Pediatric diseases, Subcutaneous lumps, Hair blacking, Dental, Paralysis, Cancer, Diuretics, Antidotes, Eye diseases, Vaginal prolapsed, Blood purification, Jaundice worm disease and Alopecia.

And it considers that Drymoglossum piloselloides may have to a certain extant act for curing Dengue fever because of the plant consist of Rakthasthambana\textsuperscript{21} (Arresting capillary hemorrhages), “Raktashodana\textsuperscript{9}” (Blood purification), “Raktawardaka\textsuperscript{14},” (blood formation) “Shitha Virya\textsuperscript{9,10}” (cooling effect), “Vran ropana\textsuperscript{14}” (Wound healing) and ability to cure Anemia.
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26. www.wildsingapore.com


APPENDIX I

Field Survey for identification of ethno medical Usages of *Drymoglossum piloselloides*

Questionnaire

1. Name:

2. Registration No:

3. Address:

4. Subject:

5. Professional qualification:

6. Duration of service:

7. What is the panampeti?

I. It is an epiphytic fern climbing on trees, leaves are like ancient Ceylon coin called “panama”.

II. It has four leaflets, grow on the ground.

8. Does it have a synonym as kimbulvenna? Yes / No

9. If yes, why is it called as?

10. Are you used this for medicine? Yes / No

11. What are the synonyms?

12. How is it used for medicine? dried/fresh

13. Is it included in “venna hatha”? Yes / No
14. Methods of preparation

1. Mellum
2. Decoction
3. Oil
4. Patthu (poultice)
5. Kenda (lotion)
6. Juice

15. What are the Ethno medical usages?

16. What are the Ayurvedic properties?

17. Is it used as along? Yes / No

18. Write a formula in used?

19. Does it have any action for cure Dengue?
APPENDIX II

MAP OF SRI LANKA
THE MAP OF NORTH WESTERN PROVINCE OF SRI LANKA

THE MAP OF KURUNEGALA DISTRICT OF SRI LANKA
### TABLE I

Absorption values of carotenoids in hexane solution

<table>
<thead>
<tr>
<th>Maxima</th>
<th>Alpha-Carotene</th>
<th>Beta-Carotene</th>
<th>Cryptoxanthol</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ(Å)</td>
<td>α</td>
<td>λ(Å)</td>
<td>α</td>
</tr>
<tr>
<td>4220</td>
<td>182.0*</td>
<td>4230</td>
<td>183.0</td>
</tr>
<tr>
<td>4460</td>
<td>273.0</td>
<td>4500</td>
<td>257.5</td>
</tr>
<tr>
<td>4740</td>
<td>249.0</td>
<td>4780</td>
<td>228.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minima</th>
<th>Alpha-Carotene</th>
<th>Beta-Carotene</th>
<th>Cryptoxanthol</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ(Å)</td>
<td>α</td>
<td>λ(Å)</td>
<td>α</td>
</tr>
<tr>
<td>4280</td>
<td>178.0</td>
<td>4465</td>
<td>206.0</td>
</tr>
<tr>
<td>4615</td>
<td>189.0</td>
<td>4665</td>
<td>205.8</td>
</tr>
</tbody>
</table>

* Values reported in tables I and II are for different samples corresponding to the curves shown in figures 1, 2, and 3.

Table cited in Plant physiology in page 340.
Fig. 2. Absorption spectra of alpha-carotene and cryptoxanthol in hexane solution.

Figure cited in Plant physiology in page 342.
Fig. 1. Absorption spectrum of beta-carotene in hexane solution.

Figure cited in Plant physiology in page 343.
The absorption spectrum of a mixture of chlorophyll a and chlorophyll b in the range of visible light.
APPENDIX IV

Fig: 5.8 TLC analyses for poly phenol using Folin Denis reagent a: - Methanol: Chloroform, 4:6 (v/v), b and c: - Methanol: Chloroform in ratio 6:4 (v/v), d: - Ethyl acetate: Glacial acetic acid: water, 7:1:2 (v/v/v)

Fig: 5.9 TLC Anisaldehyde Sulphuric acid reagent a: - Methanol: Chloroform, 6:4 (v/v) b: - Ethyl acetate: Glacial acetic acid: Formic acid: water, 100:11:11:26 (v/v/v/v)
Fig: 5.10 TLC analyses for Unsaturated Sterols using Liebermann-Burchard reagent in Methanol: Chloroform, 6:4 (v/v)

Fig: 5.11 TLC analyses using KOH reagent in Methanol: Chloroform, 6:4 (v/v)