

**STANDARDIZATION OF AYURVEDIC DRUGS:
CHARACTERIZATION OF DASAMOOLARISTA**

BY

TENNAKON MUDIYANSELAGE SAMANTHA GOME TENNAKON

M.Phil

2002

**I DEDICATE THIS THESIS
TO
MY PARENTS**

DECLARATION

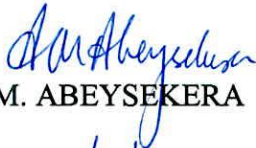
THE WORK DESCRIBED IN THIS THESIS WAS CARRIED OUT BY ME UNDER THE SURPERVISION OF PROF. A.M. ABEYSEKERA AND PROF. K.T.D. DE SILVA OF THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SRI JAYEWARDENEPURA AND A REPORT ON THIS HAS NOT BEEN SUBMITTED TO ANY UNIVERSITY FOR ANOTHER DEGREE.


T.M.S.G. PENNAKOON

DATE: 31/01/02

DECLARATION

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.



PROF. A.M. ABEYSEKERA

DATE:

31/01/02



PROF. K.T.D. DE SILVA

DATE: 31/01/02



**STANDARDIZATION OF AYURVEDIC DRUGS:
CHARACTERIZATION OF DASAMOOLARISTA**

BY

TENNAKON MUDIYANSELAGE SAMANTHA GOME TENNAKON

**Thesis submitted to the University of Sri Jayawardenapura
for the award of the degree of Master of Philosophy in Chemistry.**

January 2002

CONTENTS

	PAGE
LIST OF CONTENTS	i
LIST OF TABLES	ix
LIST OF FIGURES	x
ACKNOWLEDGEMENTS	xiv
ABBREVIATIONS	xvi
ABSTRACT	xvii
1. Introduction	1
1.1 Ayurveda and traditional medicine	1
1.2 Basic concepts of Ayurvedic medicine	1
1.2.1 Tridosha concept	2
1.2.2 Saptha dahtu concept	3
1.2.3 Mala concept	3
1.2.4 Srota concept	3
1.2.5 Agni concept	4
1.3 Pharmacology in Ayurveda	4
1.4 Ayurvedic pharmaceutical preparations	5
1.5 Ayurvedic Pharmacopoeias	6
1.5.1 Comparison between niganthus and modern pharmacopoeias	7
1.6 Traditional medicine(TM) in the world today	9
1.6.1 Barriers to Ayurvedic(AV) drugs	10
1.7 The status of Ayurveda in Sri Lanka	11

1.7.1	Health services and health problems in Sri Lanka	12
1.8	Cultivation of medicinal plants	14
1.9	Need for standardization	14
1.9.1	Problems and methods in the standardization of Ayurvedic drugs	18
1.10	Arista	23
1.10.1	Preparation of decoction	24
1.10.2	Fermentation	25
1.10.3	Sedimentation	26
1.11	Dasamoolarista(DMA)	26
2.0	Materials and Methods	28
2.1.	Spectra	28
2.2	High performance liquid chromatography(HPLC)	28
2.3	Gas liquid chromatography (GLC)	28
2.4	Densitometry	28
2.5	Melting points	29
2.6	Thin layer chromatography(TLC)	29
2.6.1	Chromatographic reagents	29
2.7	Column chromatography	31
2.8	Plant materials and other ingredients	31
2.9	Dasamoolarista (DMA) samples	31
2.10	Solvents	32
2.11	Standard curves	32

2.12	Identity tests	32
2.12.1	Solvents(n-hexane, methylene chloride, chloroform, diethyl ether, ethyl acetate) extract of DMA	32
2.12.2	Extract of the phenolic fraction of DMA	33
2.12.3	Aqueous extracts of plant ingredients of DMA	33
2.12.4	Laboratory scale preparation of the decoction of DMA	33
2.13	Isolation of marker and representative compounds	33
2.13.1	Kaempferol(i) and Quercetin(i)	33
2.13.2	Isoliquiritigenin(iii)	35
2.13.3	Plumbagin(iv) and a 4:1 mixture of Isoshinanolone(v) and Epiisoshinanolone(vi) (ISMIX)	36
2.13.4	Acetylation of ISMIX	38
2.13.5	5-Hydroxymethylfurfural(vii)(5-HMF)	39
2.13.6	Umbelliferone (viii)	40
2.13.7	Gallic acid (ix)	40
2.13.8	Isolation of dehydrocostus lactone(x) and dihydrodehydrocostus lactone(xi)	42
2.13.9	Reduction of dehydrocostus lactone(x) to dihydrodehydrocostus lactone(xi)	43
2.13.10	Alizarin(xii) and Purpurin (xiii)	44
2.14	Quantitative determination of marker and representative compounds	46
2.14.1	TLC-densitometric quantification	46

2.14.1.1 Isoliquiritigenin(iii) by TLC-VIS method	47
2.14.1.2 Gallic acid (ix) by TLC-UV method	49
2.14.1.3 Gallic acid(ix) by TLC-FD method	51
2.14.1.4 5- Hydroxymethylfurfural(vii) (5-HMF) by TLC-UV method	53
2.14.1.5 Costuslactones by TLC-VIS method	55
2.14.1.6 Umbelliferone (viii) by Thin layer chromatography-Fluorescence densitometry (TLC-FD) method	57
2.14.1.7 Mixture of Isoshinanolone(v) and epiisoshinanolone(vi) (ISMIX) by TLC-FD method	59
2.14.2 HPLC quantification	61
2.14.2.1 Gallic acid(ix) in DMA and its decoction	62
2.14.2.2 5-HMF(viii) in DMA and its decoction	64
2.14.2.3 Preparation of <i>Plumbago indica</i> L. root extract for determining the ratio of isoshinanolone(v) and epiisoshinanolone(vi)	66
2.14.3 GLC Quantification	67
2.14.3.1 Dehydrocostus lactone(x)(DHC) and dihydrodehydrocostus lactone(xi) (DDHC) by GLC	68
2.14.3.2 Monitoring of DHC and DDHC contents during industrial scale manufacture of DMA (at Link Natural Products)	71
2.14.3.2.1 Determination of DHC content in decoction	71
2.14.3.2.2 Determination of DHC content in fermenting decoction	71

2.14.3.2.3 Determination of DHC content in fermented decoction (DMA) samples preparation for GLC analysis	72
2.14.3.2.4 Preparation of a mixture of DHC and DDHC extract from <i>Saussurea lappa</i> C.B. Clarke.rhizomes	72
2.15 Preparation of extracts for chromatographic analysis of plant with problems of identity	72
2.15.1 Methanol extract of <i>Acacia catechu</i> Willd, <i>Myroxylon balsamum</i> (L.)Harms heartwoods	72
2.15.2 Hydrodistillation of <i>Nardostachys jatamansi</i> DC.rhizome	73
2.15.3 Chloroform extract of <i>Saussurea lappa</i> C.B. Clarke and <i>Inula racemosa</i> Hook.f.rhizomes	73
2.15.4 Chloroform extract of <i>Nardostachys jatamansi</i> DC and <i>Flickingeria macraei</i> (Lindely) Seidenf.rhizomes	73
2.15.5 Methanol extract of <i>Hemidesmus indicus</i> R.Br. and <i>Cryptolepis buchanani</i> Rome& Shults. roots	74
2.15.6. Diethyl ether extract of bees honey	74
2.16 Development of Fingerprints (FP)	75
2.16.1 GLC fingerprints(GLC-FP)	75
2.16.2 HPLC fingerprints(HPLC-FP)	75
2.16.3 TLC fingerprints(TLC-FP)	75

3.0 Results and Discussion	76
3.1 Development of Identity tests	76
3.1.1 Identity tests based on TLC	77
3.1.1.1 Identity tests for <i>Plumbago indica</i> L.	77
3.1.1.2 Identity tests for <i>Aegle marmelos</i> Correa.	79
3.1.1.3 Identity tests for <i>Saussurea lappa</i> C.B. Clarke.	81
3.1.1.4 Identity tests for <i>Woodfordia fruticosa</i> (L.)Kurz.	82
3.1.1.5. Identity test for <i>Pterocarpus marsupium</i> Roxb.	83
3.1.1.6 Identity tests for <i>Rubia cordifolia</i> L.	84
3.1.1.7 Identity tests for <i>Woodfordia fruticosa</i> (Linn) Kurz. and <i>Glycyrrhiza glabra</i> L.	85
3.1.1.8 Identity test for <i>Eugenia caryophyllata</i> Thumb. and <i>Cinnamomum verum</i> Presl.	85
3.1.1.9 Identity tests for <i>Pterocarpus marsupium</i> Roxb. , <i>Glycyrrhiza glabra</i> L. and <i>Myroxylon balsamum</i> (L.) Harms.	85
3.1.2 Identity tests based on GLC	86
3.1.2.1 Tests for compounds other than marker and compounds in DMA	87
3.1.2.1.1 Gallic acid (ix)	87
3.1.2.1.2 5-Hydroxymethyl furfural(5-HMF) (vii)	88
3.2 Quantitative analysis	88
3.2.1 Quantitative determination of gallic acid(ix)	89

3.2.2 Quantitative determination of 5-HMF (vii)	92
3.2.3 Quantitative determination of Isoshinanolone(v) and Epiisoshinanolone(vi)	94
3.2.4 Quantitative determination of dehydrocostus lactone(x) and dihydrodehydrocostus lactone(xi)	101
3.2.5 Quantitative determination of umbelliferone(viii)	108
3.2.6 Quantitative determination of isoliquiritigenin(iii)	110
3.3 Parameters for process control	112
3.3.1 Boiling process	112
3.4. Adulterants and substitutents	117
3.4..1 <i>Nardostachys jatamansi</i> D.C and <i>Flickingeria macraei</i> (Lindley) Seidenf.	119
3.4.2 <i>Hemidesmus indicus</i> R.Br. and <i>Cryptolepis bunchanaii</i> Roem. & Schult.	120
3.4.3 Bees honey	120
3.5 Chromatographic fingerprints for Dasamoolarista	121
3.6 Conclusion	135
3.7 Draft specifications for Dasamoolarista	137
References	138
Appendix-i Thin layer chromatograms	160

Appendix-ii	185
¹ H Nuclear magnetic resonance spectra(¹ H NMR) and ¹³ C Nuclear magnetic resonance spectra (¹³ C NMR) for marker , representative compounds and compounds other than makers or representatives of Dasamoolarista	
Appendix-iii List of plants used in the preparation of Arista	217
Appendix-iv Structural formulae of markers ,representative compounds and compounds other than markers and representatives	222

LIST OF TABLES

	PAGE
Table-1 TLC densitometer settings	46
Table-2 HPLC settings	61
Table-3 GLC settings	67
Table-4 Gallic acid content of reference samples and commercial samples of DMA determined by TLC-UV, TLC-FD and HPLC methods	91
Table -5 5-HMF content of reference samples and commercial samples of DMA determined by TLC-UV and HPLC methods	93
Table -6 ISMIX content of reference samples and commercial samples of DMA determined by TLC-FD method	97
Table – 7 ISMIX content of <i>Plumbago indica</i> L.root from two locations.	99
Table – 8 Ratio of isoshinanolone and epiisoshinanolone in <i>Plumbago indica</i> L. root determined by HPLC and ¹ H NMR	100
Table -9 Costuslactone content of reference samples and commercial samples of DMA determined by GLC and TLC-VIS methods	105
Table- 10 Umbelliferone content of reference samples and commercial samples of DMA determined by TLC-FD method	109
Table -11 Isoliquiritigenin content of reference samples and commercial samples of DMA determined by TLC-VIS method	111
Table-12 Official subsitutents, unofficial subsitutents and adulterants for some crude drugs used in DMA	118

LIST OF FIGURES

	PAGE
Fig-1 TLC-VIS densitometric standard curve for isoliquiritigenin	48
Fig-2 TLC-UV densitometric standard curve for gallic acid	50
Fig-3 TLC-FD densitometric standard curve for gallic acid	52
Fig-4 TLC-UV densitometric standard curve for 5-HMF	54
Fig-5 TLC-VIS densitometric standard curve for dehydrocostus lactone	56
Fig-6 TLC-FD densitometric standard curve for umbelliferone	58
Fig-7 TLC-FD densitometric standard curve for isoshinanolone	60
Fig- 8 HPLC standard curve for gallic acid	63
Fig- 9 HPLC standard curve for 5-HMF	65
Fig- 10 GLC standard curve for dehydrocostus lactone	69
Fig- 11 GLC standard curve for dihydrodehydrocostus lactone	70
Fig-12 HPLC chromatogram and LC-UV spectra of mixture of isoshinanolone and epiisoshinanolone	95
Fig -13 Stability of fluorescent intensity of ISMIX after treatment with ethanolic aluminium hydroxide and ethanolic paraffin reagent	96
Fig -14 TLC-FD densitometric fingerprint for methanol extract of <i>Plumbago indica</i> L .root	100
Fig-15 Stability of colour complex of mixture of DHC and DDHC with LB spray reagent on TLC	101
Fig -16 TLC –densitogram of chloroform extract of reference DMA - solvent system : hexane :diethylamine (80:20)	103

Fig-17 TLC –densitogram of chloroform extract of reference DMA - solvent system : hexane :diethylamine (99.5: 0.5)	103
Fig –18 Visible spectra of dehydrocostus lactone and dihydrodehydrocostus lactone after treatment of Liebermann Burchard reagent	104
Fig – 19 GLC chromatogram of methylene chloride extract of <i>Saussurea lappa</i> C.B. Clarke rhizome.	107
Fig- 20 HPLC chromatogram(direct injection) for gallic acid and 5-HMF in reference DMA	113
Fig -21 Variation of pH value and temperature in decoction of reference DMA during boiling (R ₁ , R ₂ , R ₃ are selected vessels)	114
Fig -22 Variation of gallic acid content of decoction of reference DMA during boiling	114
Fig -23 Variation of 5-HMF content of decoction of reference DMA during boiling	114
Fig- 24 Variation of dehydrocostus lactone content of decoction of reference DMA during boiling	115
Fig- 25 GLC profile of methylene chloride extract of decoction of reference DMA after 12 hours of boiling	116
Fig-26 Variation of dehydrocostus lactone content in fermenting decoction	116

Fig -27 GLC profile of methylene chloride extract of decoction of reference DMA after 10 days of the fermentation	117
Fig-28 GLC fingerprint of oil of <i>Nardostachys jatamansi</i> DC.	119
Fig-29 Fingerprint-1	126
Fig -30 Fingerprint -2	127
Fig- 31 Fingerprint - 3	127
Fig- 32 Fingerprint - 4	128
Fig- 33 Fingerprint - 5	129
Fig-34 Fingerprint - 6	130
Fig -35 Fingerprint - 7	131
Fig-36 Fingerprint - 8	133
Fig-37 ¹ H NMR spectrum of Kaempferol	186
Fig-38 ¹³ C NMR spectrum of Kaempferol	187
Fig-39 ¹ H NMR spectrum of Quercetin	188
Fig-40 ¹³ C NMR spectrum of Quercetin	189
Fig-41 ¹ H NMR spectrum of isoliquiritigenin	190
Fig-42 ¹³ C NMR spectrum of isoliquiritigenin	191
Fig-43 ¹ H NMR spectrum of Plumbagin	192
Fig-44 ¹³ C NMR spectrum of Plumbagin	193
Fig-45 ¹ H NMR spectrum of mixture of isoshinanolone and epiisoshinanolone	194
Fig-46 ¹³ C NMR spectrum of mixture of isoshinanolone and epiisoshinanolone	195
Fig-47 ¹ H NMR spectrum of isoshinanolone	196

Fig-48 ^{13}C NMR spectrum of isoshinanolone	197
Fig-49 ^1H NMR spectrum of 5-HMF	198
Fig-50 ^{13}C NMR spectrum of 5-HMF	199
Fig-51 ^1H NMR spectrum of Umbelliferone	200
Fig-52 ^{13}C NMR spectrum of Umbelliferone	201
Fig-53 ^1H NMR spectrum of Gallic acid	202
Fig-54 ^{13}C NMR spectrum of Gallic acid	203
Fig-55 ^1H NMR spectrum of dehydrocostus lactone	204
Fig-56 ^{13}C NMR spectrum of dehydrocostus lactone	205
Fig-57 ^1H NMR spectrum of dihydrodehydrocostus lactone	206
Fig-58 ^{13}C NMR spectrum of dihydrodehydrocostus lactone	207
Fig-59 DEPT 45° spectrum of dihydrodehydrocostus lactone	208
Fig-60 DEPT 90° spectrum of dihydrodehydrocostus lactone	209
Fig-61 DEPT 135° spectrum of dihydrodehydrocostus lactone	210
Fig-62 HETROCOSY spectrum of dihydrodehydrocostus lactone	211
Fig-63 HOMOCOSY spectrum of dihydrodehydrocostus lactone	212
Fig-64 ^1H NMR spectrum of the mixture of dehydrocostus lactone and dihydrodehydrocostus lactone	213
Fig-65 ^{13}C NMR spectrum of the mixture of dehydrocostus lactone and dihydrodehydrocostus lactone	214
Fig-66 ^1H NMR spectrum of Alizarin	215
Fig-67 ^{13}C NMR spectrum of Alizarin	216

ACKNOWLEDGEMENTS

I wish to express my most sincere gratitude to Prof . A.M. Abeysekera and Prof. Tuley De Silva, my supervisors , for their guidance and help during my work for this dissertation. Their keenness and the encouragement extended to me with patience, inspired me to proceed with the work to a successful completion, and their advice given through out the project was extremely valuable.

I also wish to express my sincere gratitude to Dr. Devapriya Nugawela, Managing Director, Link Natural Products (Pvt) Ltd, who gave me the opportunity, all facilities and encouragement and co-funded this project along with the University of Sri Jayewardenapure.

I thank the chemistry department of the University of Sri Jayewardenapure for providing me with facilities to carry out my research. The cooperation of the academic and non-academic staff of the chemistry department during my stay there is gratefully acknowledged. Special mention must be made of Mr. Sri Lal Rangoda ,who maintained the research laboratory. I also thank Mr. Janaka Nikawela and Mr. Janaka Abeysinghe who were my research colleagues, for their support.

I wish to thank the Heads , Departments of Chemistry at the , Universities of Colombo and Peradeniya, who extended the facilities of using their analytical instruments. My sincere thanks are also due to Dr. Mrs. Thusita Wijeratne for her valuable assistance in the interpretation of NMR spectra , and to Dr. Dammika Dissanayke who provided me with valuable advice on the analytical techniques of gas chromatography.

I am very grateful Mr. Wimal Pathmasiri who performed my NMR and GC/MS experiments and provided me with literature from the Uppsala University library. His assistance was invaluable throughout my project. I also would like to thank Mr. Jagath Weerasena for sharing with me his valuable experiences in the field of chromatography. I wish to thank the Curator, National Herbarium Peradeniya and Prof. S.S. Handa, RRL, Jammu, India and Dr. A. K. S. Rawat National Botanical Research Institute, Lucknow, India for allowing me to use their herbaria.

I would like to acknowledge the help of the staff of Link Natural Products (Pvt) Ltd who assisted me during the study and I specially thank Miss Yamuna Dasanayake for her kind cooperation during the study of the manufacturing process.

I also wish to thank miss Elani Jayawardena and Miss P.W.N.W. Perera for all their assistance.

I gratefully acknowledge the assistance rendered by Mr. W. Gamagae and Mr. Mallikarachi at thesis preparation.

The assistance given by Mr.K.S. Wijenayake and Mr. Prabath in the documentation of thin layer chromatograms is gratefully acknowledged.

I thank my brother Janaranjana and sisters-in-law, Dilrukshi, Denesha and Deepika for typing my thesis, and their assistance in other ways.

Finally, my deepest thanks goes to my wife Mayurani, for her patience in enduring my many evenings away from home and her ever present encouragement.

ABBREVIATIONS

5-HMF	5-Hydroxymethylfurfural
AA	Arjunarista
ABA	Abeyarista
AKA	Asokarista
AMA	Amurtarista
ASA	Asvagandarista
AT-225	50% Cyanopropylmethyl,50%phenylmethylpolysiloxane
AV	Ayurvedic
BA	Balarista
CDD	Cosmetic Device and Drug Act
DA	Danthyarista
DDHC	Dihydrodehydrocostus lactone
DDMIX	A mixture of dehydrocostus lactone and dihydrodehydrocostus lactone
DHC	Dehydrocostus lactone
DMA	Dasamoolarista
DRA	Draksharista
FD	Fluorescence densitometry
FDC	Food Drug and Cosmetic Act
FP	Fingerprint
GLC-FP	Gas Liquid Chromatography- Fingerprint
HPLC-FP	High performance chromatography- Fingerprint
IS	Internal Standard
ISMIX	A mixture of isoshinanolone and epiisoshinanolone
KA	Kadirarista
LB	Liebermann Burchard reagent
MA	Musthkarista
NA	Not applicable
NBA	Nimbarista
ND	Not detected
NP/PEG	Natural products/polyethyleneglycol reagent
perp. TLC	Preparative Thin Layer Chromatography
Rt	Retention time
SA	Saraswatharista
sh	Shift
TC-1	100 % dimethylpolysiloxane
TLC-FD	Thin Layer chromatography-Fluorescence densitometry
TLC-FP	Thin Layer chromatography-Fingerprint
TLC-UV	Thin Layer chromatography-Ultraviolet spectroscopy
TLC-VIS	Thin Layer chromatography- Visible spectroscopy

**STANDARDIZATION OF AYURVEDIC DRUGS:
CHARACTERIZATION OF DASAMOOLARISTA**

TENNAKOON MUDIYANSELAGE SAMANTHA GOME TENNAKOON

ABSTRACT

Ayurveda plays a significant role in the health care system in Sri Lanka. Within the social context in which Ayurveda is practised in the present day, the standardization and quality assurance of Ayurvedic drugs is urgent and imperative.

Dasamoolarista (DMA)) is a complex drug containing over 60 ingredients. Tests for identity in complex herbal drugs such as DMA can be devised through chromatographic methods to identify specific marker compounds which can be correlated with specific plant ingredients and representative compounds for groups of plant ingredients. A strategy for identifying such marker compounds by comparing the thin layer chromatograms of drugs(Arista) having closely related formulae, was developed. Thin layer chromatographic systems to detected the following plant ingredients through the marker compounds and representative compounds shown in parenthesis were developed; *Aegle marmelos* Correa. (Umbelliferone); *Plumbago indica* L.(Isoshinanolone and Epiisohinanolone); *Saussurea lappa* C.B. Clarke. (Dehydrocostus lactone and Dihydrodehydrocostus lactone); *Glycyrrhiza glabra* L. , *Pterocarpus marsupium* Roxb. , and *Myroxylum balsamum* (L.) Harms. (Isoliquiritigenin); *Rubia cordifolia* L. (Alizarin and Purpurin); *Eugenia caryophyllata* Thumb, and *Cinnamomum verum* Presl (Eugenol);

Woodfordia fruticosa (Linn.)Kurz., *Vitis vinifera* L. and bees honey (Quercetin and Kaempferol).

It is proposed that tests for strength(potency) of drugs such as DMA of unknown pharmacological action, can consist of quantitative measurements of compounds of high biological activity found in the drug and specific marker compounds irrespective of their biological activity. Analytical methods based on TLC densitometry ,HPLC and GLC were used to quantify gallic acid, isoliquiritigenin, umbelliferone, ,dehydrocostus lactone, dihydrodehydrocostus lactone, 5-hydroxymethylfurfural , isoshinanolone and epiisoshinanolone in DMA. The analytical methods were shown to be precise and accurate. These methods were then used to study the variability in composition of different commercial brands of DMA, and of different manufacturing batches of DMA of the same brand. Inter batch and inter brand variability was high, indicating a significant variation in the quality of crude drugs used in the manufacture of the drug. Changes in the level of dehydrocostus lactone, gallic acid and 5-hydroxymethylfurfural during the different stages of manufacture were monitored. It was concluded that levels of gallic acid and 5-hydroxymethylfurfural were more suitable as parameters for process control than those of dehydrocostus lactone.

Finally, eight chromatographic fingerprints covering a wide range of compounds were developed which could be used for routine quality control, and would provide an overall measure of identity and potency.