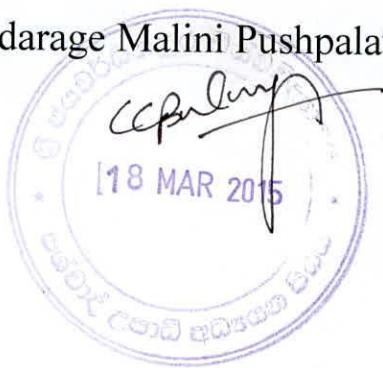


A Pharmacognostic evaluation of different species of Sariva
***Hemidesmus indicus* (L.) R. Br., *Cryptolepis buchananii* Roem.**
Schult. and *Ichnocarpus frutescens* (L.) R. Br.

By

Jeewandarage Malini Pushpalatha



Thesis submitted to the University of Sri Jayewardenepura for the
award of Degree of Master of Philosophy in Botany 30th December

2013

If we certify that by the candidate has incorporated all corrections, additions and amendments recommended by the examiners.

.....
P. L. Hett

Dr. P.L. Hettiarachchi

Date: 18 . 03 . 2015

.....
Amathyselva

Prof. A.M. Abeysekera

Date: 18 . 03 . 2015

Declaration

The work described in the thesis was carried out by me under the supervision of Dr. (Mrs) P. L. Hettiarachchi (Department of Botany, University of Rajarata), Prof. A. M. Abeysekera (Department of Chemistry, University of Sri Jayawardenepura) and Ms. Sudeepa Sugathadasa (Bandarakalika Memorial Ayurvedic Research Institute) and a report on this has not been submitted in whole or in part to any University or any other Institution for other Degree/ Diploma.

.....


J. M. Pushpalatha

.....
18 : 09 : 2015

Date

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

P. L. Hettiarachchi

Dr. P.L. Hettiarachchi

Date: 18 : 03 . 2015

A.M. Abeysekera

Prof. A.M. Abeysekera

Date: 18 : 03 . 2015

S.S. Sugathadasa

Mrs. S.S. Sugathadasa
Date: 18 : 03 . 2015

TABLE OF CONTENTS

	PAGE
TABLE OF CONTENTS	i
LIST OF PLATES.....	ix
LIST OF TABLES.....	xii
LIST OF MAPS.....	xiii
LIST OF FIGURES.....	xiv
ACKNOWLEDGEMENT.....	xv
ABSTRACT	xvii
1.0 INTRODUCTION	
1.1 Historical evidence of herbal medicine	1
1.2 Current global trends	2
1.3 Current status of Medicinal plants in Sri Lanka	4
1.4 Plants as a source of herbal drugs	5
1.5 Standardization of herbal drugs	6
1.6 Pharmacognostic evaluation of crude drugs (raw material)	12
1.7 Members of “Sariva”	14

1.8 Scope /Objectives of the study	15
2.0 LITERATURE REVIEW	
2.1 <i>Hemidesmus indicus</i> (L.) R. Br. (PERIPLOCACEAE)	17
2.1.1 Botanical Classification	17
2.1.2 Distribution	19
2.1.2.1 Local distribution	19
2.1.2.2 .Global distribution	19
2.1.3 Morphological description	19
2.1.4 Phytochemistry	20
2.1.5 Macroscopic characters	21
2.1.6 Microscopic features	21
2.1.6.1 Anatomy	21
2.1.6.2 Powder characteristics	22
2.1.7 Traditional usage	22
2.1.8 Biological activity	24
2.2 <i>Ichnocarpus frutescens</i> R. Br.	25
2.2.1 Botanical Classification	25

2.2.2 Distribution	26
2.2.2.1 Local distribution	26
2.2.2.2 Global distribution	26
2.2.3 Morphological description	27
2.2.4 Phytochemistry	27
2.2.5 Macroscopic characters	27
2.2.6 Microscopic features	28
2.2.6.1 Anatomy	28
2.2.6.2 Powder characteristics	28
2.2.7 Traditional usage	28
2.2.8 Biological activity	29
2.3 <i>Cryptolepis buchananii</i>	30
2.3.1 Botanical Classification	30
2.3.2 Distribution	31
2.3.2.1 Global distribution	31
2.3.2.2 Ecological distribution	31
2.3.3 Morphological description	32
2.3.4 Phytochemistry	32

2.3.5 Macroscopic characters	33
2.3.6 Microscopic features	33
2.3.6.1 Anatomy	33
2.3.6.2 Powder characteristics	34
2.3.7 Traditional usage	34
2.3.8 Biological activity	35

3.0 MATERIALS AND METHODS

3.1 Fresh plant material, raw material, equipment and reagents	36
3.1.1 Fresh plant material and raw material	36
3.1.2 Equipment	39
3.1.2.1 Macroscopic and organoleptic investigation	39
3.1.2.2 Microscopic study.....	39
3.1.2.3 Photomicrography	39
3.1.2.4 Thin Layer Chromatography (TLC).....	39
3.1.2.5 GC/MS analysis	39
3.1.3 Solvents and standards	40
3.1.3.1 Tissue maceration (Prasad and Prasad, 1979).....	40

3.1.3.2 Sample killing and fixation	
(Prasad and Prasad, 1979).....	40
3.1.3.3 Dehydration (Prasad and Prasad, 1979).....	40
3.1.3.4 Tissue staining (Prasad and Prasad, 1979)	40
3.1.3.5 Chromatographic spray reagents (WHO, 1998) ...	41
3.1.3.6 Microscopic measurements (WHO, 1998)	41
3.2 Methods	41
3.2.1 Preparation and authentication of	
voucher herbarium specimens	41
3.2.2 Macroscopic evaluation of genuine “Sariva” species	42
3.2.2.1 External Morphology	42
3.2.2.2 Organoleptic investigation	42
3.2.3 Microscopic evaluation	42
3.2.3.1 Anatomycal study	42
3.2.3.2 Powder microscopical analysis	43
3.2.3.3 Study of macerated root samples	43

3.2.3.4 Calibration of eye piece with ocular Micrometer (WHO, 1998)	44
3.2.4 Macroscopic evaluation of market samples	44
3.2.4.1 External morphology and Organoleptic investigation.....	45
3.2.5 Microscopical evaluation of market samples	45.
3.2.5.1 Anatomical study	45
3.2.5.2 Powder microscopical analysis	46
3.2.5.3 Study of macerated market samples	46
3.2.6 Phytochemical analysis	46
3.2.6.1 TLC analysis of root extracts	46
3.2.6.2 GC/MS analysis of volatile oils	47
4.0 RESULTS AND DISCUSSION	
4. 1 Preparation and authentication of voucher herbarium specimens ...	48
4.2 Macroscopic evaluation of authentic “Sariva” species	48

4.2.1 External morphology of the plants	48
4.2.2 External Morphology and organoleptic properties of roots of three Sariva species	50
4.2.3 Microscopical evaluation of authentic “Sariva” species....	53
4.2.3.1 Anatomical studies of roots of <i>H. indicus</i> and its adulterant/substitutes	53
4.2.3.2 Powder analysis	71
4.2.3.3 Study of macerated root samples.....	73
4.2.4 Macroscopic evaluation of market samples	77
4.2.4.1 External morphology and Organoleptic investigation	77
4.2.5 Microscopic evaluation of market samples	78
4.2.5.1 Anatomical study	78
4.2.5.2 Powder microscopical analysis	81
4.2.5.3 Study of macerated market samples	82
4.2.6. Phytochemical analysis	86
4.2.6.1 TLC analysis of the roots of <i>H. indicus</i> ,	

<i>C. buchananii</i> and <i>I. frutescens</i>	86
4.2.6.2 GC/MS analysis.....	93
5.0 GENERAL DISCUSSION.....	96
6.0 CONCLUSION	98

REFERENCES

APENDICES

LIST OF PLATES

	Page
Plate 1: Habit of <i>Hemidesmus indicus</i> (L.) R. Br.....	18
Plate 2 : Habit of <i>Ichnocarpus frutescens</i> R. Br.	26
Plate 3.: Habit of <i>Cryptolepis buchanani</i> Roem. & Schult.....	31
Plate 4: Polymorphic leaves of <i>H. indicus</i>	50
Plate 5 : T. S. of root of cortex showing prismatic crystals (x400)	52
Plate 6 : T.S. of root showing cork cells x400.....	60
Plate 7 : T. S. root of <i>H. indicus</i> showing cortical cells and medullary rays richly loaded with starch grains	61
Plate 8 : T. S. of root of cortex showing prismatic crystals.....	62
Plate 9 : T. S. of root cortex showing mechanical elements	63
Plate 10 : T. S. root showing phloem zone	64
Plate 11: T. S. of Wood showing the distribution of xylem components	66
Plate 12 : T. S. of root of wood showing apotracheal and	

paratracheal parenchyma	67
Plate 13 : T.L.S. of wood showing medullary ray cells	68
Plate 14 : T.S. of wood showing pith	69
Plate 15 : Powder microscopical view	72
Plate 16 : Elements of <i>H. indicus</i>	74
Plate 17 : Different elements in the macerated root sample of <i>C. buchananii</i>	75
Plate 18 : Different elements in the macerated root sample of <i>I. frutescens</i>	76
Plate 19 : Some sections of market samples	80
Plate 20: Powder microscopical view of market sample	81
Plate 21: Xylem vessel elements	82
Plate 22: Trachieds and stone cells	83
Plate 23 : Laticiferous ducts	83
Plate 24: TLC of ethanol extracts of roots of Sariva.....	86
Plate 25: TLC of oil from Sariva species.....	88
Plate 26 : Ethanolic extract of market samples of “Sariva”	88

Plate 27: TLC of ethanol extract of market samples and genuine	
“Sariva” species	89
Plate 28 : TLC of ethanol extract of market samples and	
authentic “Sariva” species	91
Plate 29 : TLC of ethanol extract of market samples and	
authentic Sariva species	92
Plate 30 : Structures of the major compounds present in Sariva	95

LIST OF TABLES

	Page
Table 1: Diagnostic morphological features of <i>H. indicus</i> and its adulterants/ substitutes	49
Table 2 : External Morphology and organoleptic properties of roots of three Sariva species	51
Table 3 : Anatomical features of roots of <i>H. indicus</i> and its adulterants	57
Table 4 : Organoleptic properties recorded in the powder of Sariva species....	71
Table 5: Measurements of different elements and cells observed in macerated root samples of “Sariva”	73
Table 6 : Morphological and organoleptic analysis of market samples “Sariva”	78
Table 7: Microscopical investigation of market samples of Sariva	79
Table 8 : The composition of market samples	84
Table 9 : Compounds reported from Iramusu	93

LIST OF MAPS

	Page
Map 1 : Map of Sri Lanka showing sampling locations of authentic Sariva species	37
Map 2 : Map of Sri Lanka showing collection of market samples	38

LIST OF FIGURES

	Page
Figure 1 : Transverse Section of roots of H. indicus.....	54
Figure 2 : Transverse Section of roots of I. frutescens	55
Figure 3 : Transverse Section of roots of C. buchananii	56

ACKNOWLEDGEMENT

I express my sincere gratitude and appreciation to my supervisor Dr P. L. Hettiarachchi, Senior Lecturer of the Department of Botany University of Rajarata for her valuable guidance, keen supervision, advice extended towards the successful completion of the thesis through many hardships.

I am grateful to Prof. A. M. Abeysekera, Professor, Department of Chemistry, University of Sri Jayawardenepura, his keen supervision, encouragement, help and critical comments for the completion of the thesis.

My sincere gratitude is also extended to Ms. Sudeepa Sugathadasa, Scientist (Pharmacognocy), BMARI for her encouragement, supervision, comments and valuable guidance throughout the research work.

I must thankful to Prof. K. K. D. S. Ranaweera, Director of BMARI for providing a chance for me to carry out this research work at BMARI and my thanks are also extended to Mr. P. S. K. R. Weerakoon, Commissioner of Ayurveda, for providing financial assistance for the research.

I take this opportunity to express my gratitude to Prof. Nazeera Saleem, the Head and all the academic, technical and non academic staff of the Department of Botany, University of Sri Jayawardenapura

I would like to express my sincere thanks to the Department of Chemistry, for providing facilities for the chemical analysis.

I also take my pleasure in thanking Ms. Achala de Silva, Research officer at BMARI, Nawinna, for her encouragement, invaluable support given to me throughout my research work.

I am grateful to research students in the post graduate laboratory in the department of Chemistry, University of Sri Jayawardenapura for their support given to me in numerous ways.

I extend my special thanks to all the staff members of the Botany Division, BMARI for their encouragement and support in completion of this work.

Finally, I offer my true and cordial gratitude to my father, mother and my husband for their encouragement, understanding and kindness extended to me in various ways to achieve my goal successfully.

A Pharmacognostic evaluation of different species of Sariva *Hemidesmus indicus* (L.) R. Br. , *Cryptolepis buchananii* Roem. Schult. and *Ichnocarpus frutescens* (L.) R. Br.

J. M. Pushpalatha

ABSTRACT

Hemidesmus indicus or true Sariva as it is known in ayurveda is the true medicinal plant supposed to be used in nearly 50 different ayurveda drug preparations. Further this has become one of the ingredients in many other herbal products such as Sherbet and flavoring agents. The plant is neither cultivated in Sri Lanka nor India (from where the raw material is imported to Sri Lanka). Therefore, the entire demand is met with the plant material collected from the wild. Consequently there is a greater chance of the real material been adulterated or substituted by similar looking, cheaper material. Therefore, the present study was launched to carry out a complete pharmacognostic evaluation of *H. indicus* and its possible adulterants / substitutes available in Sri Lanka. *Cryptolepis Buchananii* and *Ichnocarpus frutescens* which are collectively known as “ Sariva” in ayurveda with the view of establish key diagnostic characters that would be used to screen crude drug material at the point of purchasing. Twenty five root samples per species selected form five district and fifty five market sample purchased from six districts were used for the study. Authentic voucher herbarium specimens were prepared and deposited in the institutional herbarium. Macroscopic evaluation of martial were carried out at following WHO