

Evaluation of taxonomic status of *Munronia*
pinnata (Wall.) Theob. (Meliaceae) in
Sri Lanka and its antimalarial properties

By

Rathnayake Mudiyanseelage Dharmadasa



PhD

2011

Evaluation of taxonomic status of *Munronia pinnata* (Wall.)
Theob. (Meliaceae) in Sri Lanka and its antimalarial properties

By

Rathnayake Mudiyanseelage Dharmadasa

Thesis submitted to the University of Sri Jayawardenepura for the
award of the Degree of Doctor of Philosophy in Botany

On 30th December 2011

Declaration

The work described in this thesis was carried out by me under the supervision of Dr (Mrs.) P.L. Hettiarachchi, University of Sri Jayewardenepura and Dr. G. A. S. Premakumara, Industrial Technology Institute and a report of this has not been submitted in whole or in part to any university or any other institution for another degree/diploma.

Signature of the candidate.......... Date 25.1.2013

I/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.

.....

Dr. (Mrs.) P.L. Hettiarachchi
Internal Supervisor,
Senior Lecturer,
Department of Botany,
University of Sri Jayawardanapura,
Nugegoda,
Sri Lanka.
Date.....



.....

Dr.G.A. S. Premakumara,
External Supervisor
Head, Herbal Technology Section,
Industrial Technology Institute
363, Bauddhaloka Mawatha,
Colombo 7,
Sri Lanka.
Date.....

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

P. L. Hett 25/01/2013

Dr. (Mrs.) P.L. Hettiarachchi
Internal Supervisor

G.A.S. Premakumara

Dr. G.A.S. Premakumara
External Supervisor

LIST OF CONTENTS

	Page No.
TABLE OF CONTENTS	i
LIST OF TABLES	x
LIST OF FIGURES	xiii
LIST OF PLATES	xviii
ABBREVIATIONS	xxv
ACKNOWLEDGEMENT	xxvii
DEDICATION	xxix
ABSTRACT	xxx
CHAPTER 1. INTRODUCTION	1
1.1 Use of plants in primary healthcare and herbal medicine	1
1.2 <i>Munronia pinnata</i>	1
1.3 Conservation and systematic survey	3
1.4 Plant taxonomy and numerical taxonomy	5
1.4.1 Sources of taxonomic evidence	6

1.5 Biological activity	15
1.5.1 Brine shrimp (<i>Artemia salina</i>) assay	15
1.5.2 Malaria	16
1.5.2.1 Global scenario of malaria	17
1.5.2.2 Overview of malaria in Sri Lanka	18
1.5.2.3 Use of herbal medicine for malaria control	21
1.5.2.4 Antimalarial potential of <i>Munronia pinnata</i>	22
1.6 Scope of the thesis	23
1.6.1 Overall objective	23
1.6.2 Specific objectives	23
CHAPTER 2. LITERATURE REVIEW	24
2.1 Family Meliaceae	24
2.1.1 Morphology of family Meliaceae	25
2.1.2 Anatomy of family Meliaceae	25
2.1.3 Chemistry of family Meliaceae	28
2.2 Genus <i>Munronia</i>	29
2.2.1 Global distribution of <i>Munronia</i> species	30

2.3 Botanical description of <i>Munronia pinnata</i>	31
2.3.1 Classification of <i>Munronia pinnata</i>	31
2.3.2 Synonyms/ Vernacular names	32
2.3.3 Distribution in Sri Lanka	33
2.4 Systematic surveys on medicinal plants	36
2.5 Plant taxonomy and numerical taxonomy	39
2.5.1 Plant morphology in taxonomy	40
2.5.2 Plant anatomy in taxonomy	43
2.5.3 Plant secondary metabolites in taxonomy	46
2.5.4 Plant proteins and isozymes in taxonomy	49
2.5.5 Plant DNA in taxonomy	52
2.6 Biological activity	53
2.6.1 Brine shrimp (<i>Artemisia salina</i>) assay	53
2.7 Malaria	56
2.7.1 Use of herbal medicine to control malaria	57
2.7.2 Toxicological studies of plant extracts	59

CHAPTER 3 MATERIALS AND METHODS

3.1 Plant material, equipment and chemicals	61
3.1.1 Plant material	61
3.1.2 Equipments and chemicals	62
3.1.3 Laboratory animals	62
3.2 Methods	63
3.2.1 Systematic survey on the geographical distribution and abundance of <i>Munronia pinnata</i> (Wall.) Theob. in Sri Lanka	63
3.2.1.1 Collecting information from available literature	63
3.2.1.2 Island wide survey on the distribution	64
3.2.1.1.2 Collecting data from Grama Niladari Divisions	64
3.2.1.1.3 Field survey to <i>Munronia pinnata</i> growing areas of the country	65
3.2.1.2 Collection and maintenance of collected populations	67
3.2.1.3 Authentication	67
3.2.1.4 Collection of ecological data	68
3.2.1.5 Determination of the stomatal index	68
3.2.1.6 Data analysis	69
3.2.2 Determination of taxonomic status of <i>Munronia pinnata</i> in Sri Lanka based on the morphology	69

3.2.2.1	Selecting and recording morphological characters	69
3.2.2.2	Clearing plant material	70
3.2.2.3	Determination of vein islet number	72
3.2.2.4	Determination of vein termination number	72
3.2.2.5	Cluster analysis	72
3.2.2.6	Construction of a taxonomic key to identify <i>Munronia pinnata</i> populations	73
3.2.3	Comparative anatomical study of <i>Munronia pinnata</i> grown in Sri Lanka	73
3.2.3.1	Preparation of temporary mounts	74
3.2.3.2	Selecting and recording of anatomical characters	74
3.2.3.3	Data analysis	75
3.2.3.4	Construction of a taxonomic key	75
3.2.4	Grouping of <i>Munronia pinnata</i> populations distributed in Sri Lanka using Thin Layer Chromatographic profiles	75
3.2.4.1	Preparation of extracts	76
3.2.4.2	Separation of compounds using Thin Layer Chromatography	76

3.2.4.3 Preparation of spray regents	77
3.2.4.4 Visualization and data analysis	77
3.2.5 Determination of genetic divergence in <i>Munronia pinnata</i> using isozyme profiles and total protein fingerprints	78
3.2.5.1 Preparation of stock solutions and buffers	79
3.2.5.2 Preparation of crude extracts for electrophoresis	79
3.2.5.3 Preparation of polyacrylamide gels and electrophoresis	79
3.2.5.4 Visualization of isozymes by specific staining	81
3.2.5.5 Total protein fingerprinting and Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	81
3.2.5.6 Staining gels with Coomassie brilliant blue	82
3.2.5.7 Data analysis	82
3.2.6 Investigation of the relationship between polyphenol content and cytotoxic potential of <i>Munronia pinnata</i> found in Sri Lanka	83
3.2.6.1 Plant material	83
3.2.6.2 Preparation of extracts for total phenol and flavonoid Content analysis	84
3.2.6.3 Determination of total phenol content	84
3.2.6.4 Determination of total flavonoid content	85
3.2.6.5 Preparation of crude extracts for brine shrimp assay	85

3.2.6.6	Brine shrimp toxicity assay	86
3.2.7	Investigation on anti malarial activity of <i>Munronia pinnata</i>	86
3.2.7.1	Plant material	87
3.2.7.2	Preparation of plant extracts	87
3.2.7.3	Experimental animals	87
3.2.7.4	Parasite isolates (Inoculum)	87
3.2.7.5	Evaluation of blood schizonticidal activity on an early infection	87
3.2.7.6	Toxicological studies	88
3.2.7.7	Data analysis	89
 CHAPTER 4. RESULTS AND DISCUSSION		
4.1	Current distribution and abundance of <i>Munronia pinnata</i> in Sri Lanka	90
4.1.1	Summary	105
4.2	Taxonomic status of <i>Munronia pinnata</i> populations distributed in Sri Lanka based on morphology	107
4.2.1	Morphological variation	107
4.2.2	Sequential indented key based on vegetative morphology	125
4.2.3	Summary	127
4.3	Taxonomic status of <i>Munronia pinnata</i> populations based on anatomy	128
4.3.1	Anatomical variation	128
4.3.1.1	Leaf midrib anatomy	169

4.3.1.2	Stem anatomy	171
4.3.1.3	Petiole anatomy	172
4.3.1.4	Structure of lower and upper epidermis of leaf lamina	174
4.3.2	Sequential indented key prepared using anatomical characters to demarcate populations of <i>Munronia pinnata</i> in Sri Lanka	182
4.3.3	Summary	184
4.4	Taxonomic states of <i>Munronia pinnata</i> (Wall) Theob. based on Thin Layer Chromatographic profiles	185
4.4.1	Summary	197
4.5	Determination of genetic divergence in thirteen populations of <i>Munronia pinnata</i> using isozyme profiles and total protein finger prints	198
4.5.1	Summary	219
4. 5.3	Summary of the overall taxonomic study	221
4.6	Polyphenol content and cytotoxic potential of <i>Munronia pinnata</i>	224
4.6.1	Summary	231
4.7	Investigation on antimalarial activity of <i>Munronia pinnata</i>	232
4.7.1	Evaluation of blood schizonticidal activity on an early infection	232
4.7.2	Toxicological studies	237
5.0	General discussion	240
	Conclusions	245

References	247
Appendix I	273
Appendix II	275
Appendix III	284

LIST OF TABLES

Table 2.1 Distribution of <i>Munronia pinnata</i> in Sri Lanka	34
Table 2.2 Plant species commonly used to treat malaria [Plant species with a potential to develop anti malarial drugs]	58
Table 3.1 Different concentrations and combinations of NaOH and NaOCl used for the clearing of plant material	71
Table 4.1 Detailed description of distribution of <i>Munronia pinnata</i> found in literature and areas surveyed in the present study	91
Table 4.2 Details of geographical and ecological distribution and abundance of <i>Munronia pinnata</i> populations collected in the present survey	93
Table 4.3 Flowering and fruiting performance of 13 populations of <i>Munronia pinnata</i> under greenhouse conditions (Temp. 27 ± 2 °C, normal day length)	100
Table 4.4 Ranks given to the stomat index and to some of the ecological data of areas where <i>Munronia pinnata</i> was collected	102
Table 4.5 Ecological relationship of 13 <i>Munronia pinnata</i> populations as seen in the cluster	103

Table 4.6 Morphological character table of 13 different populations of	
<i>Munronia pinnata</i>	109
Table 4.7 List of morphological characters together with their character	
states recorded in <i>Munronia pinnata</i>	116
Table 4.8 Morphological relationship of 13 <i>Munronia pinnata</i> populations	
as seen in the cluster	122
Table 4.9 Anatomical characters of different parts of 13 populations of	
<i>Munronia pinnata</i>	156
Table 4.10 List of anatomical characters together with their character status	
Recorded in 13 populations of <i>Munronia pinnata</i> used in the	
present study	165
Table 4.11 Anatomical relationship of 13 populations of <i>Munronia pinnata</i> as	
seen in the cluster	179
Table 4.12 Relationship of TLC profiles of 13 populations of <i>Munronia pinnata</i>	
as seen in the cluster	194

Table 4.13 Relationship of SDS-PAGE profiles of 13 populations of <i>Munronia pinnata</i> as seen in the cluster	202
Table 13a. Character table of SDS PAGE and isozyme genotypes of 13 populations of <i>Munronia pinnata</i>	213
Table 13 b. List of SDS-PAGE bands and isozyme genotypes together with their character states of 13 popuations of <i>Munronia pinnata</i>	214
Table 4.14 Relationship of SDS-PAGE and isozyme profiles of 13 populations of <i>Munronia pinnata</i> as seen in the cluster	216
Table 4.15 Total phenol and flavonoid contents of different plant parts (leaf, stem. roots) of <i>Munronia pinnata</i>	224
Table 4.16 LC ₅₀ values obtained for different parts of five morphotypes of <i>Munronia pinnata</i>	225
Table 4.17 <i>In- vivo</i> blood schizonticidal activity, chemosuppresion and survival rate in the 4-day suppressive assay	232

LIST OF FIGURES

Figure 1.1 Price increase of one kilogram of dried material of <i>Munronia</i> <i>pinnata</i> during the last two decades	3
Figure 1.2 Global distribution of malaria endemicity (Source WHO 2005)	18
Figure 1.3 Sri Lankan situation of malaria from year 2003 to 2010	19
Figure 1.4 Microscopically confirmed malaria cases detected during the last 6 months of 2010	20
Figure 2.1 Life cycle of <i>Artemia salina</i>	55
Figure 3.1 Surveyed localities for <i>Munronia pinnata</i> in Sri Lanka	66
Figure 4.2 Dendrogram of ecological relationship of 13 populations of <i>Munronia pinnata</i>	103
Figure 4.3 Dendrogram generated by combining 45 polymorphic vegetative and reproductive morphological characters of 13 populations of <i>Munronia pinnata</i>	121

Figure 4.4 Scatter diagram of PCA analysis on the basis of 45 polymorphic vegetative and reproductive morphological characters of 13 populations of <i>Munronia pinnata</i>	123
Figure 4.5 Dendrogram generated by combining 53 polymorphic anatomical characters of 13 populations of <i>Munronia pinnata</i>	178
Figure 4.6 Scatter diagram of PCA on the basis of 53 polymorphic anatomical characters of thirteen populations of <i>Munronia pinnata</i>	181
Figure 4.7 Dendrogram and scatter diagram generated by analysing TLC profiles of stem, leaf and root extracts of 13 populations of <i>Munronia pinnata</i> observed under UV 366 nm	189
Figure 4.8 Dendrogram and scatter diagram generated by analysing TLC profiles of stem, leaf and root extracts of 13 populations of <i>Munronia pinnata</i> observed after spraying anisaldehyde	190
Figure 4.9 Dendrogram and scatter diagram generated by analysing TLC profiles of stem, leaf and root extracts of 13 populations of <i>Munronia</i> <i>pinnata</i> observed after spraying ferric chloride- sulphuric acid	191

Figure 4.10 Dendrogram and scatter diagramme generated by analysing TLC profiles of stem, leaf and root extracts of 13 populations of <i>Munronia pinnata</i> observed after spraying vanillin sulphate	192
Figure 4.11 Dendrogram and scatter diagram generated by analysing TLC profiles of stem, leaf and root extracts of 13 populations of <i>Munronia pinnata</i> observed under UV366 nm and after spraying different spray regents	193
Figure 4.12 Schematic electrophoresis pattern of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis in leaf extracts of 13 populations of <i>Munronia pinnata</i>	199
Figure 4.13 Dendrogram generated using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) data of 13 populations of <i>Munronia pinnata</i>	201
Figure 4.14 Scatter diagram generated from PCA using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis data of 13 populations of <i>Munronia pinnata</i>	201
Figure 4.15 Schematic electrophoresis pattern of Glutamate dehydrogenase (GDH) loci in leaf extracts of 13 populations of <i>Munronia pinnata</i>	204

Figure 4.16 Schematic electrophoresis pattern of Malate dehydrogenase (MDH) loci of leaf extracts of 13 populations of <i>Munronia pinnata</i>	206
Figure 4.17 Schematic electrophoresis pattern of the Malic enzyme (ME) loci in leaf extracts of 13 populations of <i>Munronia pinnata</i>	207
Figure 4.18 Schematic electrophoresis pattern of the Peroxidase enzyme (POD) loci in leaf extracts of 13 populations of <i>Munronia pinnata</i>	209
Figure 4.19 Schematic electrophoresis pattern of the Superoxide dismutase enzyme (SOD) loci in leaf extracts of 13 populations of <i>Munronia pinnata</i>	211
Figure 4.20 Dendrogram generated by combining SDS-PAGE and isozyme data of 13 populations of <i>Munronia pinnata</i>	215
Figure 4.21 Scatter diagram resulted from PCA on the basis of SDS-PAGE and isozyme characters of 13 populations of <i>Munronia pinnata</i>	215
Figure 4.22 A dendrogram generated by cluster analysis of all morphological, anatomical, micromolecular (Thin Layer Chromatography) and macromolecular (proteins) qualitative and quantitative data of 13 populations of <i>Munronia pinnata</i>	221
Figure 4.23 Scatter diagram generated by the analysis of all morphological, anatomical, micromolecular and macromolecular data	221

Figure 4.24 Variation of total phenol, flavonoid content and LC ₅₀ values of leaf extracts of <i>Munronia pinnata</i>	227
Figure 4.25 Variation of total phenol, flavonoid content and LC ₅₀ values of stem extracts of <i>Munronia pinnata</i>	228
Figure 4.26. Variation of total phenol, flavonoid content and LC ₅₀ values of root extracts of <i>Munronia pinnata</i>	228
Figure 4.27 Variation of cumulative phenol, flavonoid content and LC ₅₀ Values of leaf extracts of <i>Munronia pinnata</i>	229
Figure 4.28 Variation of cumulative phenol, flavonoid content and LC ₅₀ values of stem extracts of <i>Munronia pinnata</i>	229
Figure 4.29 Variation of cumulative phenol, flavonoid content and LC ₅₀ values of root extracts of <i>Munronia pinnata</i>	230
Figure 4.30 Parasitaemia percentage of different treatments from day 3 to day 8	233
Figure 4.31 Body weight changes of the treated mice during the experiment	234
Figure 4.32 Comparison of body temperature changes of treated group vs. control group	234
Figure 4.33 Comparison of body weight changes of the treated group and control group during the toxicological study	235

LIST OF PLATES

Plate 4.1a. *Munronia pinnata* populations collected from different locations of

Sri Lanka [APRG-5- Ritigala (Anuradapura district); BDHM-3-

Haldummulla (Badulla district); GPPW-3, Pallewela (Gampaha district);

GPWP-3-Warakapola (Gampaha district)]

95

Plate 4.1b. *Munronia pinnata* populations collected from different locations of

Sri Lanka [KGKP-5- Kuliapitiya (Kurunegala district); MGDG-3

Dambagalla (Moneragala district) ;MGMD-3 Madulla (Monaragala

district); MGMG-9/11 Monaragala (Moneragala district)]

96

Plate 4.1c. *Munronia pinnata* populations collected from different locations of

Sri Lanka [MGNG-3 Nilgala (Moneragala district); MGWW-7

Wellawaya (Moneragala district); MTKD-3 Kalundewa (Matale

district); MTMM-5 Meemure (Matale district)]

97

Plate 4.1d. *Munronia pinnata* populations collected from different locations of

Sri Lanka [MTNU-5 Naula (Matale district); NEKP-3 Kithulpe (Nuwara eliya district); NEOG-5 Okadagala (Nuwaraeliya district); NEMR-3 Mathurata (Nuwaraeliya district)]	98
Plate 4.2a. Leaves of different morphotypes of <i>Munronia pinnata</i>	114
Plate 4.2b Leaf micro morphological characters of <i>Munronia pinnata</i>	114
Plate 4.2c Micro morphological features of flowers of <i>Munronia pinnata</i>	115
Plate 4.2d Vein islet (A) and vein termination (B) of cleared leaf of <i>Munronia pinnata</i>	116
Plate 4.3a Leaf midrib transverse sections of populations APRG-5 and BDHM-3 of <i>Munronia pinnata</i>	129
Plate 4.3b Leaf midrib transverse sections of populations GPPW-3 and GPWP-3 of <i>Munronia pinnata</i>	130
Plate 4.3c Leaf midrib transverse sections of populations KGKP-5 and MGMD-3 of <i>Munronia pinnata</i>	131
Plate 4.3d Leaf midrib transverse sections of populations MGMG-9 and	

MGNG-3 of <i>Munronia pinnata</i>	132
Plate 4.3e Leaf midrib transverse sections of populations MGWW-7 and	
MTMM-5 of <i>Munronia pinnata</i>	133
Plate 4.3f Leaf midrib transverse sections of populations MTNU-5 and	
NEKP-3 of <i>Munronia pinnata</i>	134
Plate 4.3g Leaf midrib transverse section of populations NEMR-3 of	
<i>Munronia pinnata</i>	135
Plate 4.3h Stem cross sections of APRG-5 and BDHM-3 populations of	
<i>Munronia pinnata</i>	136
Plate 4.3i Stem cross sections of GPPW-3 and GPWP-3 populations of	
<i>Munronia pinnata</i>	137
Plate 4.3j Stem cross sections of KGKP-5 and MGMD-3 populations of	
<i>Munronia pinnata</i>	138
Plate 4.3k Stem cross sections of MGMG-9 and MGNG-3 populations of	
<i>Munronia pinnata</i>	139

Plate 4.3l Stem cross sections of MGWW-7 and MTMM-5 populations of	
<i>Munronia pinnata</i>	140
Plate 4.3m Stem cross sections of MTNU-5 and NEKP-3 populations of	
<i>Munronia pinnata</i>	141
Plate 4.3n Stem cross sections of NEMR-3 populations of <i>Munronia pinnata</i>	142
Plate 4.3o Petiole cross sections of populations APRG-5 and BDHM-5 of	
<i>Munronia pinnata</i>	143
Plate 4.3p Petiole cross sections of populations GPPW-3 and GPWP-3 of	
<i>Munronia pinnata</i>	144
Plate 4.3q Petiole cross sections of populations KGKP-5 and MGMD-3	
of <i>Munronia pinnata</i>	145
Plate 4.3r Petiole cross sections of populations MGMG-9 and MGNG-3	
of <i>Munronia pinnata</i>	146
Plate 4.3s Petiole cross sections of populations MGWW-7 and MTMM-5	
of <i>Munronia pinnata</i>	147

Plate 4.3t Petiole cross sections of populations MTNU-5, NEKP-3 and NEMR-3 of <i>Munronia pinnata</i>	148
Plate 4.3u Leaf upper epidermis of APRG-5, BDHM-3, GPPW-3, GPWP-3, KGKP-5 and MGMD-3 populations of <i>Munronia pinnata</i>	149
Plate 4.3v Leaf upper epidermis of MGMG-9, MGNG-3, MGWW-7 and MTMM-5 populations of <i>Munronia pinnata</i>	150
Plate 4.3w Leaf upper epidermis of MTNU-5, NEKP-3 and NEMR-3 populations of <i>Munronia pinnata</i>	151
Plate 4.3x Leaf lower epidermis of APRG-5, BDHM-3, GPPW-3 and GPWP-3 populations of <i>Munronia pinnata</i>	152
Plate 4.3y Leaf lower epidermis of KGKP-5, MGMD-3, MGMG-9 and MGNG-3 populations of <i>Munronia pinnata</i>	153
Plate 4.3z Leaf lower epidermis of MGWW-7, MTMM-5, MTNU-5 and NEKP-3 populations of <i>Munronia pinnata</i>	154
Plate 4.3za Leaf lower epidermis of <i>Munronia pinnata</i> population NEMR3	155
Plate 4.3zb Special anatomical features of different parts of <i>Munronia pinnata</i>	174
Plate 4.3zc Special anatomical features of different parts of <i>Munronia pinnata</i>	175
Plate 4.3zd Special anatomical features of different parts of <i>Munronia pinnata</i>	176
Plate 4.4a Thin Layer Chromatograms of leaf extracts of 13 populations of <i>Munronia pinnata</i>	185
Plate 4.4b Thin Layer Chromatograms of stem extracts of 13 populations of	

<i>Munronia pinnata</i>	186
Plate 4.4c Thin Layer Chromatograms of root extracts of 13 populations of <i>Munronia pinnata</i>	187
Plate 4.5a SDS-PAGE profiles of leaf extracts of 13 populations of <i>Munronia pinnata</i>	198
Plate 4.5b Electrophoresis pattern of Glutamate Dehydrogenase (GDH) of leaf extracts of 13 populations of <i>Munronia pinnata</i>	204
Plate 4.5c Electrophoresis pattern of Malate Dehydrogenase (MDH) of leaf extracts of 13 populations of <i>Munronia pinnata</i>	205
Plate 4.5d Electrophoresis pattern of Malic Enzyme (ME) loci of leaf extracts of 13 populations of <i>Munronia pinnata</i>	207
Plate 4.5e Electrophoresis pattern of Peroxidase (POD) of leaf extracts of 13 populations of <i>Munronia pinnata</i>	208
Plate 4.5f Electrophoresis pattern of Superoxide dismutase (SOD) in leaf extracts of 13 populations of <i>Munronia pinnata</i>	210
Plate 4.6a Comparison of RBC infection with <i>Plasmodium yoelii</i> in different treatments in 4-day suppressive assay	236

Plate 4.6b Comparison of parasitaemia content of treated mice with T₁ and

positive control at day 4, day 6 and day 8

237

ABBREVIATIONS

ADH	-	Alcohol Dehydrogenase
ANOVA	-	Analysis of Variance
CA	-	Correspondence analysis
DMRT	-	Duncan Multiple Range Test
DSD	-	District Secretariat Division
g	-	Gram
GAE	-	Gallic Acid Equivalents
GDH	-	Glutamate dehydrogenase
GLM	-	General Linear Model
GN	-	Grama Niladhari
HTS	-	Herbal Technology Section
kg	-	Kilo gram
LC ₅₀	-	Lethal concentration at 50% death
MDH	-	Malate Dehydrogenase
ME	-	Malic Enzyme

mg	-	Milligram
mL	-	Milliliter
°C	-	Degree of centigrade
PCA	-	Principle Component Analysis
POD	-	Peroxidase
ppt	-	Parts per thousand
QE	-	Quercetin Equivalents
RBC	-	Red Blood Cells
R _f	-	Relative
SDS	-	Sodium Dodecyl Sulphate
SOD	-	Super oxide dismutase
TLC	-	Thin Layer Chromatography
UPGMA	-	Unweighted Pair Group Method with Arithmetic Means
UV	-	Ultra Violet
WPAEMP	-	Whole plant aqueous extracts of <i>Munronia pinnata</i>
μL	-	Micro liter

ACKNOWLEDGEMENTS

PhD is a long journey of one's life with full of obstacles, problems, barriers and hardships to overcome. Although these are common to everybody, each journey is unique and should have taken individually. Therefore, during this period one must have the strong, enthusiasm and willpower to get a significant output at the end.

The work included in this thesis would have not been achieved without proper planning, strong supervision, tireless correcting and constructive criticism of my internal supervisor Dr. Priyani Hettiarachchi, Senior Lecturer, Faculty of Applied Sciences, University of Sri Jayawardenepura, Sri Lanka. She always backed me in every step of this long journey by giving me an every possible assistance. When there were barriers, obstacles, she cleared my path to achieve the goal of my journey. I humbly appreciate your continues support given during my studies.

I express my heartfelt gratitude to my external supervisor Dr. Sirimal Premakumara, Head, Herbal Technology Section, Industrial Technology Institute for his supervision, correction of the thesis, constructive criticisms and making my working environment comfortable to carry out this study successfully.

I express my sincere gratitude to Dr. A.M. Mubarak, Chief Executive Officer, and Dr. (Miss). Shanthi Wilson, Assistant Director Research and Development, Industrial Technology Institute for providing institutional funds, granting permission, continuous supports and encouragements given at all stages of my study.

I would express my heart-full thanks to all the staff of Herbal Technology Section and all ITI staff, who helped me in various ways to achieve this milestone.

Since this study is multidisciplinary work, I worked in different places with different persons. Especially, I have to mention staff of the Animal House, Medical Research Institute headed by Mrs. Jayasekara for providing me necessary guidance, hands on training and encouragements.

My sincere gratitude also goes to staff of the Department of Zoology; University of Colombo guided by Prof. W.D. Rathnasooriya and Prof. (Mrs.) Preethi Randeniya who helped me in screening of antimalarial activity.

Last but not least I am indebted to my wife and two children (Ravindu and Kavindu) who missed my care and attention during the study.

Dedication

My parents, teachers, brother and my family members for their encouragement,
patience and strength that inspired me to face all the challenges of this
marvelous task

Evaluation of taxonomic status of *Munronia pinnata* (Wall.) Theob.

(Meliaceae) in Sri Lanka and its antimalarial properties

R.M. Dharmadasa

ABSTRACT

Munronia pinnata (Wall.) Theob. (Meliaceae) is a therapeutically important medicinal plant species used for a series of ailments and hence, it has become a very expensive dry herb in traditional medicine in Sri Lanka. Present study describes island wide distribution and conservation measures, systematic identification, brine shrimp toxicity and antimalarial activity of *M. pinnata*. A survey was conducted in 395 Grama Niladari (GN) areas belonging to 68 Divisional Secretariat Divisions (DSD) in seven districts in Sri Lanka revealed that *M. pinnata* is abundant in 53 GN areas while in 217 GN areas, the plant is found in small scale and in 65 GN areas it was rarely found. However, in 8 DSDs it was absent. Ten new localities were also found. A higher diversity was found in Moneragala and Matale districts and populations collected from Madulla (MGMD-3), Nilgala (MGNG-3), Warakapola GPWP-3), Ritigala (APRG-5) and Haldummulla (BDHM-3) can be recommended for ex situ conservation while populations collected from Moneragala (MGMG-9), Wellawaya (MGWW-7), Mathurata (NEMR-3), Meemure (MTMM-5) and Kithulpe (NEKP-3) can be recommended for *in-situ* conservation. Results of the cluster analysis and Principle Component Analysis (PCA) revealed that population APRG-5 is distantly related to all other populations with 100 % distance in morphological,