

Contents

Declaration	ii
Certificate	iii
Acknowledgement	iv-v
Abstract	vi-x
Preface	xi-xiv
List of tables	xv-xvi
List of figures	xvii-xix
Abbreviation	xx-xxii
Chapter - I Introduction	1-4
1.1. Medicinal plants in prevention and treatment of diseases	1
1.2. Heritage of natural remedies in India	2
1.3. Phytopharmacological studies in today's world	2-3
1.4. Prospect of natural remedies in treatment of leishmaniasis and opportunistic fungal infections	3-4
1.5. Objective	4
Chapter - II Ethnobotanical studies on plant species used by tribes of North Bengal	5-16
2.1. Literature review	5-8
2.1.1. The Indian heritage of using medicinal plants	5
2.1.2. Conservation of plant resources	5
2.1.3. Documentation of traditional knowledge	6
2.1.4. Ethnobotanical studies from North East India	7
2.1.5. Ethnobotanical studies in Jalpaiguri, West Bengal	8
2.2. Materials and methods	9-11
2.2.1. Study Area	9
2.2.2. Data Collection	9
2.3. Results	11-15
2.4. Discussion	15-16
Chapter - III Phytochemical content and antioxidant activity of leaf extracts of <i>R. serpentina</i>, <i>M. oleifera</i>, <i>N. arbor-tristis</i> and <i>C. excavata</i>	17-45
3.1. Literature review	17-33
3.1.1. Plants as source of therapeutic compounds	17
3.1.2. Phytochemicals or secondary metabolites	18
3.1.3. Phenolic compounds	20
3.1.4. Flavonoids	20
3.1.5. Lignins, saponins and others	21
3.1.6. Antioxidants from plants	21
3.1.7. Overview of selected medicinal plants	23

3.1.7.1.	Clausenaexcavata	23
3.1.7.1.1.	Traditional use and established therapeutic properties of <i>C. excavata</i>	23
3.1.7.2.	<i>Moringa oleifera</i>	25
3.1.7.2.1.	Traditional use of <i>M. oleifera</i>	26
3.1.7.2.2.	Biological activity of <i>M. oleifera</i>	26
3.1.7.3.	<i>Nyctanthes arbor-tristis</i>	29
3.1.7.3.1.	Traditional uses and biological activities of <i>N. arbor-tristis</i>	29
3.1.7.4.	<i>Rauvolfia serpentina</i>	30
3.1.7.4.1.	Traditional uses and biological activities of <i>R. serpentina</i>	32
3.2	Materials and methods	33-38
3.2.1.	Plant sample collection and extraction	33
3.2.2	Phytochemical analysis	34
3.2.2.1	Qualitative analysis	34
3.2.2.1.1.	Test for tannins	34
3.2.2.1.2.	Test for phlobatannins	34
3.2.2.1.3.	Test for saponins	34
3.2.2.1.4.	Test for flavonoids	34
3.2.2.1.5.	Test for steroids and terpenoids	35
3.2.2.1.6.	Test for cardiac glycosides	35
3.2.2.1.7.	Detection of alkaloids	35
3.2.2.1.8.	Detection of carbohydrates	36
3.2.2.1.9.	Detection of organic acids	36
3.2.2.2.	Quantitative analysis	36
3.2.2.2.1.	Test for total phenol	36
3.2.2.2.2.	Test for flavonoids	37
3.2.3.	Antioxidant activity	37
3.3.	Results	38-43
3.3.1.	Phytochemical analysis (Qualitative)	38
3.3.2.	Phytochemical constituents (quantitative) of leaf extracts	38
3.3.2.1.	Phenolic content	38
3.3.2.2.	Flavonoid content	39
3.3.3.	Antioxidant activity	41
3.4.	Discussion	44-45
Chapter - IV	<i>In vitro</i> antifungal screening of methanolic leaf extracts of <i>R. serpentina</i>, <i>M. oleifera</i>, <i>N.arbor-tristis</i> and <i>C. excavata</i>	46 - 62
4.1.	Literature review	46-57
4.1.1.	Phylogeny and classification	47
4.1.2.	Infection caused by fungus	48
4.1.3.	Epidemiology	50
4.1.3.1.	<i>Candida</i> sp.	53

4.1.4.	Treatment available	53
4.1.5.	Antifungal drug resistance	55
4.1.6.	Prospect of medicinal plants as antifungal agents	55
4.2.	Materials and methods	57-59
4.2.1.	Plant materials	57
4.2.2.	Extraction and purification of excavarin-A from <i>C. excavata</i> leaves	57
4.2.3.	Test organism	58
4.2.4.	Agar cup bioassay	58
4.2.5.	Minimum inhibitory concentration (MIC)	58
4.3.	Results	59-60
4.4.	Discussion	60-62
Chapter - V	<i>In vitro</i> antileishmanial screening of methanolic leaf extracts of <i>R. serpentina</i>, <i>C. excavata</i> and excavarin-A, purified from leaf extract of <i>C. excavata</i>	63 - 95
5.1.	Literature review	63-79
5.1.1.	History	63
5.1.2.	Clinical manifestations and classification of leishmaniasis	64
5.1.3.	Mode of transmission of disease	66
5.1.4.	Epidemiology	66
5.1.5.	Leishmaniasis and AIDS-a vicious cycle	68
5.1.6.	Morphology of parasite	68
	5.1.6.1. The promastigote forms	70
	5.1.6.2. The amastigote forms	70
5.1.7.	Life cycle of parasite and the role of host	70
5.1.8.	Pathogenesis of leishmaniasis and role of immune system in host body	74
5.1.9.	Available line of treatment	76
5.1.10.	Natural remedies	77
5.2.	Materials and Methods	79-83
5.2.1.	Preparation of leaf extract	79
5.2.2.	Propagation of parasites	79
5.2.3.	Maintenance of promastigotes	79
5.2.4.	Model animal	80
5.2.5.	Isolation of amastigotes	80
5.2.6.	Screening for Leishmanicidal activity	80
	5.2.6.1. <i>In vitro</i> inhibitory assay of plant extracts against promastigote proliferation	80
	5.2.6.2. Test of efficacy of crude plant leaf extracts and the purified molecule excavarin-A on intracellular survival of <i>Leishmania</i> -infected hamster macrophages	81
	5.2.6.2.1. Isolation of macrophages	81
	5.2.6.2.2. Antileishmanial activity of plant extracts on amastigotes	82

5.2.6.2.3.	GIEMSA staining	82
5.2.7.	<i>In vitro</i> cytotoxicity assay	82
5.3.	Results	83-91
5.3.1.	<i>In vitro</i> inhibitory assay of plant extracts against promastigote proliferation	83
5.3.2.	Antileishmanial activity of plant extracts on amastigotes	85
5.3.3.	Comparative analysis between effective doses of tested extracts against promastigotes and amastigotes	84
5.3.4.	<i>In vitro</i> cytotoxicity assay	89
5.4.	Discussion	92 - 95
Chapter - VI	<i>In vitro</i> antifungal activity of leaf extracts of <i>R. serpentina</i>, and <i>M. oleifera</i>	96-107
6.1.	Literature review	96-100
6.1.1.	<i>In vivo</i> evaluation of antifungal activity against candidiasis	96
6.1.2.	<i>In vivo</i> evaluation of antifungal activity against other superficial infections	98
6.2.	Materials and methods	100-102
6.2.1.	Preparation of plant extracts	100
6.2.2.	Effect of plant extracts <i>in vivo</i>	100
6.2.2.1.	Infection with <i>C. albicans</i>	100
6.2.2.2.	Treatment with plant extract	100
6.2.3.	Recovery of pathogen from test animals	101
6.2.4.	Analysis of blood sample of test animals	101
6.2.5.	Data analysis	102
6.3.	Results	102-105
6.4.	Discussion	105-107
Chapter - VII	<i>In vivo</i> antileishmanial activity of <i>R. serpentina</i> leaf extract and studies on mechanism of action	108-129
7.1.	Literature review	108-112
7.1.1.	Experimental evidence of antileishmanial activity using plant extracts	108
7.1.2.	Mode of action of leishmanicidal plant extracts	110
7.1.3.	Role of SOD in antileishmanial activity	110
7.1.4.	Antileishmanial medicinal plants change liver function in host	111
7.2.	Materials and methods	112-115
7.2.1.	Preparation of leaf extract of <i>R. serpentina</i>	112
7.2.2.	Parasite isolation	112
7.2.3.	<i>In vivo</i> antileishmanial activity determination	112
7.2.4.	Determination of parasitic burden in liver and spleen	113
7.2.5.	Animal serum enzyme assay	113
7.2.6.	Superoxide dismutase (SOD) assay	113
7.2.7.	Determination of superoxide radical release	114
7.2.8.	Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE)	114

7.2.9.	SOD on-gel activity staining	115
7.2.10.	Statistical analysis	115
7.3.	Results	116-122
7.3.1.	<i>In vivo</i> effect of <i>R. serpentina</i> extract on intracellular amastigotes	116
7.3.2.	Animal serum enzyme assay	119
7.3.3.	Superoxide dismutase (SOD) assay	119
7.3.4.	Impact of <i>R. serpentina</i> on SOD activity and superoxide radical release	119
7.3.5.	SDS-PAGE	122
7.3.6.	SOD activity assay	122
7.4.	Discussion	122-125
	Conclusion	126-129
	Bibliography	130-163
	Appendices	