# DIVERSITY AND PHYTOCHEMISTRY OF PTERIDOPHYTES FROM DIFFERENT RESERVE FORESTS IN MIZORAM

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# DIVERSITY AND PHYTOCHEMISTRY OF PTERIDOPHYTES FROM DIFFERENT RESERVE FORESTS IN MIZORAM

By

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Submitted

In partial fulfillment of the requirement for the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl

# **MIZORAM UNIVERSITY**

(A Central University Established by an Act of Parliament of India)

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# **CERTIFICATE**

This is to certify that the thesis work entitled, "Diversity and Phytochemistry of Pteridophytes from Different Reserve Forests in Mizoram", submitted by R. Vanlalpeka (Regd. No. MZU/Ph. D/671 of 23. 05. 2014) in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany is a record of bonafide work carried out by him under my supervision and guidance.

(Prof. S. K. MEHTA)

Supervisor

#### **DECLARATION**

I, R. Vanlalpeka, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any degree in any other University/Institution.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Botany.

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# Place: Aizawl

(R. Vanlalpeka)

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# Abbreviations

AD	=	Anno Domini (in the Christian era)
ATCC	=	American Type Culture Collection
BC	=	Before Christ
Bot	=	Botany, use in botany
+	=	present; positive reaction
-	=	absent; negative reaction
±	=	more or less
μg	=	microgram
μm	=	micrometer (mile micron)
μl	=	microliter
alt.	=	altitude
ca	=	circa, approximately
CHCl <sub>3</sub>	=	chloroform
cm	=	centimetre
<sup>0</sup> C	=	degree Celsius
diam.	=	diameter
dist.	=	district
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl
DTR	=	Dampa Tiger Reserve
E	=	East

e.g	=	example
ed./ eds.	=	editor/ editors
edn.	=	edition
= (equal sign)	=	taxonomic synonym
et al.	=	et alii/ et aliorum; and others
f.	=	forma (form)
g	=	gram
GAE	=	Gallic Acid Equivalent
g L- <sup>1</sup>	=	gram per litre
- (hyphen)	=	used to link two words/ numeral
Н	=	hour
Ι	=	Inhibition
i.e.	=	<i>id est</i> (that is)
Ι	=	iodine
IC	=	Inhibitory concentration
Κ	=	aqueous solution of Potassium hydroxide
Km.	=	kilometre
lbs	=	libra pondo, pound by weight
М	=	Molarity
m	=	meter
MeOH	=	Methanol

mg	=	milligram
ml	=	mililitre
mm	=	milimeter
min	=	minutes
MNP	=	Murlen National Park
MSL	=	Mean Sea Level
MZU	=	Mizoram University
Ν	=	North
nm	=	nanometer
p./pp	=	prescribed pages/page
ppt	=	precipitate
Pt	=	Pteridophytes, herbarium Code used in Department of Botany, Mizoram University
Pt S	=	
		Botany, Mizoram University
S	=	Botany, Mizoram University South
S SE	=	Botany, Mizoram University South Standard error
S SE sp.	= = =	Botany, Mizoram University South Standard error species
S SE sp. sq.km	= = =	Botany, Mizoram University South Standard error species square kilometer
S SE sp. sq.km T	= = =	Botany, Mizoram University South Standard error species square kilometer Type
S SE sp. sq.km T TAC	-	Botany, Mizoram UniversitySouthStandard errorspeciessquare kilometerTypeTotal antioxidant capacity

TWS	=	Tawi Wildlife Sanctuary
UV	=	Ultra Violet
Vis	=	Visible
Viz.	=	<i>videlicet</i> ; namely
W	=	West
w/v	=	weight by volume

# **CONTENTS**

		<b>Pages</b>
Acknowledgement		i- ii
Abbreviations		iii- vi
List of figures		vii- ix
List of tables		x- xiii
Chapter - I	General Introduction	1-12
Chapter –II	Study Area	13-21
Chapter –III	Materials and Methods	
3.1	Collection of plant materials	22
3.2	Preparation of Herbarium	22-23
3.3	Identification of plants	23
3.4	Preparation of plant extracts	24
3.5	Preparation of reagent and test for	24-28
	Phytochemical analysis	
3.6	Anti-Bacterial Test	28-31
3.7	Determination of Total Phenolic Content	31-32
3.8	Determination of Total Flavonoid Content	33- 34
3.9	Determination of Antioxidant Capacity	34- 35
Chapter –IV	Diversity and Identification of Pteridophytes	

4.1 Introduction	36
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4.2	Materials and methods	37
4.3	Results	37-139
4.4	Discussion	140- 145

Chapter –V	Phytochemical screening and Anti-bacterial activity	
5.1	Introduction	146- 149
5.2	Materials and methods	149- 150
5.3	Results	150- 165
5.4	Discussion	165-168

Chapter –VI	Determination of Total Phenolic, Flavonoid oxidant activity	contents and Anti-
6.1	Introduction	169-172
6.2	Materials and methods	172-175
6.3	Results	175- 183
6.4	Discussion	183-188

Chapter –VII	General Discussion	189- 197
Summary		198-202
References		203-245
Bio data		xiv- xviii

# List of figures

- Figure 1. Map showing Phawngpui National Park one of the study sites in Mizoram.
- Figure 2. Map showing Murlen National Park one of the study sites in Mizoram.
- Figure 3. Map showing Tawi Wildlife Sanctuary one of the study sites in Mizoram.
- Figure 4. Map showing Dampa Tiger Reserve one of the study sites in Mizoram.
- Figure 5. Family distribution of Pteridophytes in PNP, MNP, TWS and DTR.
- Figure 6. Percentage showing their state of habit.
- Figure 7. Family distribution in Phawngpui National Park.
- Figure 8. Percentage showing their state of habit from Phawngpui National Park.
- Figure 9. Family distribution in Murlen National Park.
- Figure 10. Percentage showing their state of habit from Murlen National Park.
- Figure 11. Family distribution in Murlen National Park.
- Figure 12. Percentage showing their state of habit from Murlen National Park.

- Figure 13. Family distribution in Dampa Tiger Reserve.
- Figure 14. Percentage showing their state of habit from Dampa Tiger Reserve.
- Figure 15. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* L. against *Escherichia coli*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 16. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* L. against *Klebsiella pneumoniae*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 17. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* L. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 18. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *E.coli*. The values are mean of three replicates. The vertical bars show  $\pm$  SE.
- Figure 19. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *Klebsiella pneumonia*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 20. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

- Figure 21. The antibacterial activity in the methanolic extract of *Selaginella* bisulcata Spring. against *E. coli*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 22. The antibacterial activity in the methanolic extract of *Selaginella* bisulcata Spring. against *Klebsiella pneumoniae*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 23. The antibacterial activity in the methanolic extract of *Selaginella* bisulcata Spring. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.
- Figure 24. Calibration curve for gallic acid.
- Figure 25. Calibration curve for quercetin.

# List of tables

Table 1.	List of pteridophytes collected along with their habits.
Table 2.	Comparative distribution of Pteridophyte Flora of PNP, MNP, TWS, DTR of Mizoram.
Table 3.	Floristic analysis of Pteridophyte flora Phawngpui National Park.
Table 4.	Floristic analysis of Pteridophyte flora of Murlen National Park.
Table 5.	Floristic analysis of Pteridophyte flora Tawi Wildlife Sanctuary.
Table 6.	Floristic analysis of Pteridophyte flora of Dampa Tiger Reserve.
Table 7.	Phytochemical analysis of the methanolic, aqueous and petroleum ether extracts of <i>Lycopodiella cernua</i> L.
Table 8.	Phytochemical analysis of the methanolic, aqueous and petroleum ether extracts of <i>Diplazium esculentum</i> (Retz.) Sw.
Table 9.	Phytochemical analysis for the methanolic, aqueous and petroleum ether extracts of <i>Selaginella bisulcata</i> Spring.

Table 10.F- and p-values of One-Way ANOVA for the inhibition zone in E.Coli subjected to various concentrations of methanolic extract ofLycopodiella cernua L. The marked effects are significant at  $p \le 0.05$ .

- Table 11. F- and p-values of One-Way ANOVA for the inhibition zone in *Klebsiella pneumoniae* subjected to various concentrations of methanolic extract of *Lycopodiella cernua* L. The marked effects are significant at  $p \le 0.05$ .
- Table 12. F- and p-values of One-Way ANOVA for the inhibition zone in Bacillus subtilis subjected to various concentrations of methanolic extract of Lycopodiella cernua L. The marked effects are significant at  $p \le 0.05$ .
- Table 13.F- and p-values of One-Way ANOVA for the inhibition zone in E.Coli subjected to various concentrations of methanolic extract ofDiplazium esculentum (Retz.) Sw. The marked effects are significantat  $p \le 0.05$ .
- Table 14. F- and p-values of One-Way ANOVA for the inhibition zone in *Klebsiella pneumoniae* subjected to various concentrations of methanolic extract of *Diplazium esculentum* (Retz.) Sw. The marked effects are significant at  $p \le 0.05$ .
- Table 15.F- and p-values of One-Way ANOVA for inhibition zone in Bacillus<br/>subtilis subjected to various concentrations of methanolic extract of<br/>Diplazium esculentum (Retz.) Sw. The marked effects are significant<br/>at  $p \le 0.05$ .
- Table 16.F- and p-values of One-Way ANOVA for inhibitiion zone in *E. Coli*subjected to various concentrations of methanolic extract of

Selaginella bisulcata Spring. The marked effects are significant at  $p \le 0.05$ .

- Table 17.F- and p-values of One-Way ANOVA for inhibition zone in *Klebsiella*<br/>pneumoniae subjected to various concentrations of methanolic extract<br/>of Selaginella bisulcata Spring. The marked effects are significant at<br/> $p \leq 0.05$ .
- Table 18. F- and p-values of One-Way ANOVA for inhibiton zone in *Bacillus subtilis* subjected to various concentrations of methanolic extract of *Selaginella bisulcata* Spring. The marked effects are significant at p≤ 0.05.
- Table 19.Polyphenol contents (mean $\pm$  SD) in mg GAE per g of chloroformextract. Values are mean of three replicates  $\pm$  SD.
- Table 20.Polyphenol contents (mean $\pm$  SD) in mg GAE per g of methanolicextract. Values are mean of three replicates  $\pm$  SD.
- Table 21.Total flavonoid contents (mean± SD) in mg QE per g of chloroform<br/>extract.
- Table 22.Total flavonoid contents (mean± SD) in mg QE per g of methanolextracts of three fern species.
- Table 23.Results of DPPH radical scavenging assay of BHT. Values are mean $\pm$  SD of three replicates.

- Table 24.Results of DPPH radical scavenging assay of chloroform and<br/>methanolic extract of Lycopodiella cernua L. Values are mean  $\pm$  SD<br/>of three replicates.
- Table 25.Results of DPPH radical scavenging assay of chloroform and<br/>methanolic extract of *Diplazium esculentum* (Retz.) Sw. Values are<br/>mean  $\pm$  SD of three replicates.
- Table 26.Results of DPPH radical scavenging assay of chloroform and<br/>methanolic extract of *Selaginella bisulcata* Spring. Values are mean<br/> $\pm$  SD of three replicates.

# **CHAPTER-I**

#### **GENERAL INTRODUCTION**

Pteridophytes, also known as 'vascular cryptogams' and 'ferns and fern allies', comprises about 12,000 species of vascular plants that do not produce flowers or seeds, reproducing instead via spores. Pteridophytes occur in most terrestrial habitats on earth and are also present in some aquatic communities. They typically remain an important part of the ground vegetation in many forest communities and with about one-third of the distinct species growing on the trunks and branches of trees; they constitute also an important part of many epiphytic plant communities. Some species are very beneficial to humans, but the group however retains some of the most important weed species in the world. This group of plants attracts many plant lovers by their graceful and fascinating foliage comprising more than 12,000 species all over the world. Nayar (1957) has accurately reported 191 genera with more than 1,000 species from India. Certain territories of India fall under different biodiversity hot spots of the world.

Pteridophytes unbeatably had a very flourishing past and dominated the vegetation on the earth about 280-230 million years ago (Mehra, 1967; Bir, 1976a, 1987a, 1994b; Khare, 1996). Despite that Pteridophytes have now widely replaced by

the seed-bearing vascular plants in the extant flora of today, they still constitute an equally prominent part of the present-day vegetation of the world. With an extremely variable climate, India has an immense diversity of its flora, Pteridophyte flora greatly contributes to its diversity (Kaur, 1979, 1980, 1989; Bir 1987a, 1987b). Pteridophytes also form an intriguing and conspicuous part of our national flora with their distinctive ecological distributional pattern. 500 species of ferns and 100 species of fern-allies are on record from India (Bir, 1987a). However, according to recent census, the Pteridophyte flora of India makes up 67 families, 191 genera and more than 1,000 species (Dixit, 1984; Dixit and Vohra, 1984). In general, the vascular flora of India has about 15,000 species (Jain, 1984), the ferns and fern-allies form only 5% (Satija and Bir, 1985). Still, due to their abundance as an individual as well as their visibility in epiphytic and terrestrial vegetation along forest margins, roadsides, and forest floors, the valuable contribution of ferns and fern-allies to the vegetational pattern in India rank only next to the flowering plants.

Although Pteridophytes constitute a prominent feature of the native flora of India, it is until the first quarter of the present century that the reliable sources of information on Indian ferns had been the works of R.H. Beddome (1863-1864, 1865-1870, 1876, 1883, 1892), C. B. Clarke (1879, 1880), C. W. Hope (1899, 1990, 1901, 1902, 1903a, 1903b, 1904) and a few others. Fern allies, however, were practically left untouched by all of them. Moreover, the nomenclature of many species described in those published works has become archaic today; above works still remain as the most important reference works on fern flora of India.

Before the comprehensive compilation of classical taxonomic works in the second-half of the 19th century, no significant work has appeared on taxonomy of Indian ferns except for systematic lists of ferns and fern-allies of various regions published time to time (Schwidt, 1857; Blanford, 1888; Macpherson, 1890; Gamble, 1892; Prain, 1903a, 1903b; Blatter, 1908; Stewart, 1917, 1938, 1839, 1944, 1945, 1951, 1957; Blatter and d'Almeida, 1922; Haines, 1924; Majumdar, 1933; Singh, 1931; Mahabale, 1938a, 1938b; Mehra, 1939). It is barely after 1950 research in Peridophyte rejuvenated in almost all the prominent fields in India. Comprehensive reviews on works done on Pteridology in India have been provided by Mehra and Chowdhury (1957), Maheswari and Kapil (1963), Chowdhury (1973a), Bir (1987b) and Bir (1976a, 1976b, 1977a, 1977b, 1979, 1983). Kaur and Raza (1983) prepared a detailed bibliography of Indian Pteridology till the end of 1980 followed by scholarly publications of Vasudeva and Bir (1994a) for the period 1983-1993 and Bir (1994a) for the period 1981-1994.

Last three decades have perceived a renewed interest in the plant systematic, distribution, ecology, phytogeography and taxonomy of Indian Pteridophytes. Bir (1976a) have published an account on the complex taxonomy of Indian Pteridophytes by carefully reviewing the comparative literature till the end of 1973. Nayar and Kaur (1974) provided the nomenclature equivalents to the species described together with the correct identity to the names used by Beddome in *Handbook to the Ferns of British India, Ceylon and the Malaya Peninsula*. Chandra and Kaur (1987) have published the nomenclatural equivalents of the names used by Beddome for the illustrations of *Ferns of Southern India* and *Ferns of British India* giving correct identity to nearly all the names used there. Nair and Dixit (1981) published a list of Indian fern taxa not included in Beddome's *Handbook to the Ferns of British India, Ceylon and the Malaya Peninsula* and the supplement to the above work. All the above-mentioned works not only provided a sound basis for workers on Indian Pteridophytes but also rendered relevancy to the historical works of Beddome even after more than one hundred years of publications of these works.

Through intensive explorations during the last few decades, botanists from several parts of India revived the floristic studies. These comprehensive studies have resulted in several floristic systematic accounts at regional, district or even sub-division level along with investigative reports of new taxa, new records, and taxonomic and nomenclature notes (Jain, 1984). Nair and Dixit (1981) and Chandra (1981) published a compiled list of new taxa added to the Indian fern flora. It is presently possible to work out the status of Indian Pteridophytes as Pterologists have adequately explored more and more areas. Chandra (1982) enlisted 96 ferns as endemic to India and then Chandra and Kaur (1984) and Kaur and Chandra (1994) added 41 and 40 species, respectively,

to the exhaustive list of endemic ferns of India. Bir (1987a, 1993) identified region-wise rare and endangered Pteridophytic elements in India with effective strategies for their proper conservation. Embodying relevant information on distribution, use and other specific information of the taxa Dixit (1982) has published a conspectus of the families and genera of Indian Pteridophytes. Dixit (1984) published a census of the Indian Pteridophytes wherein he has listed 191 genera and more than 1,000 species of Pteridophytes. Dixit and Vohra (1984) also published a comprehensive dictionary of Pteridophytes of India.

During the last three decades a reasonable number of comprehensive works have on top appeared for various parts of India with the revival of considerable interest in floristics of Pteridophytes in India. Of the important floristic accounts, mention can be made on the work(s) of Kachroo (1953, 1975), Panigrahi (1960, 1968), Panigrahi and Patnaik (1961a), Panigrahi and Chowdhury (1961, 1962), Ghose and Biswas (1977), Rao and Hajra (1980), Chandra (1980), Dutta *et al.*, (1980), Baishya and Rao (1982), Chandra and Chandra (1983), Jamir and Rao (1988), Bir *et al.*, (1989), Kachroo *et al.*, (1989) and Vasudeva *et al.*, (1990) on different parts of North Eastern India; Mehra and Bir (1964) on Darjeeling and Sikkim; Bir and Shukla (1966,1968,1971), Bir (1968a, 1968b) and Bir *et al.*, (1986) on Simla hills; Dhir (1980) on North Western Himalayas; Dhir and Dutta (1977a, 1977b) on Dharmasala hills; Chandra (1979) on Kedemath, Madhyamaheswar and Tunganath; Loyal and Verma (1960) and Verma and Khullar (1980) on Nainital; Dhir and Sood (1981) on Mussoorie hills; Bir *et al.*, (1983) on Garhwal Himalaya; Pande and Pande (1990, 1994) and Pande (1990) on Kumaun Himalaya; Mehra and Dhir (1968) on Dalhousiae hills; Bir and Verma (1961, 1963) on Mt. Abu; Sharma *et al.*, (1969) on Gorakhpur, Pande (1973) on Ranikhet; Mehta (1956) on Parasnath of Bihar; Chowdhury and Raizada (1961) and Chowdhury (1973b) on Upper Gangetic Plains; Bir and Vasudeva (1973) and Vasudeva and Bir (1994b, 1994c, 1994d) on Pachmarhi hills of Central India; Agashe (1968) on Kolhapur, Maharastra; Subramanyam *et al.*, (1960), Bir and Basudeva (1971), Manickam and Ninan (1976), Manickam and Irudayaraj (1990, 1992) and Dixit and Mondal (1994) on different parts of South India; Nayar and Srivastava (1962) on Great Andaman and Ellis (1987) on Andaman and Nicobar Islands.

Alteration, revisionary and critical taxonomic studies in respect of certain families and genera have seamlessly stepped towards a complied and modern Pteridophytic flora of India (Nayar, 1955, 1956, 1957, 1961a, 1961b, 1961c, 1961d, 1962a, 1962b, 1963, 1964a; Gupta, 1962; Panigrahi and Dixit, 1966a, 1966b, 1967, 1968, 1969a, 1969b; Dixit and Panigrahi, 1969; Nayar and Kazmi, 1962, 1963; Nayar and Kaur, 1963a, 1963b, 1964a, 1964b; Nayar and Chandra, 1965; Nayar and Chandra, 1968; Bir and Devi, 1968; Bir and Trikha, 1968a, 1968b, 1969, 1974; Chandra, 1971; Bir *et al.*, 1974; Dixit and Das, 1979, 1981; Dixit 1980, 1981; Panigrahi, 1981; Satija *et al.*, 1983; Bir, 1986). In addition to the above works taxonomic notes and critical

studies on nomenclature of certain Indian taxa published during the last three decades have equally contributed to a modem Pteridophytic flora of India (Nayar, 1954; Nair, 1968, 1969; Bir, 1962a, 1962b; Bir and Trikha, 1973; Panigrahi, 1975c; Chakravarty, 1981; Dixit and Balkrishna, 1993). Based on the studies on materials in Indian and foreign herbaria, Satija and Bir (1985) have carefully compiled a taxonomic account of Polypodiaceous ferns of India. Similarly, Dixit (1988) has compiled family Lycopodiaceae in India.

Of relevance and applicable to the Pteridophytic flora of India comprise the works on revisions, monographs and critical taxonomic interpretations published from time to time (Ching, 1931, 1935, 1938; Holttum, 1965, 1969, 1971, 1972, 1973a, 1973b, 974a, 1974b, 1976a, 1976b, 1977, 1983,1985; Sledge 1967; Nair, 1972; Dixit and Nair, 1974; Panigrahi, 1975a, 1975b, 1975d; Nair and Ghosh, 1975; Hennipman, 1977; Khullar and Sharma, 1980; Ghosh, 1983; Khullar *et al.*, (1983); Stewart, 1984; Fraser-Jenkins, 1984, 1989, 1991; Fraser-Jenkins and Khullar, 1985 and others). The works of Ching (1931, 1935, and 1938) on Sino-Himalayan members of *Vittaria, Pyrrosia* and *Dryopteris,* and of Ching *et al.*, (1983) on *Lepisorus clathratus* in the Sino-Himalayan region have greatly clarified the taxonomic confusions about several species of the Indian region. Fraser-Jenkins (1984) provides a chronological review of fem study of the region in addition to the information about the adjoining region.

Of relevance and use in the floristic works in India, particularly in the Northeastern India and taxonomic revision of Indian members are on Nepal-Darjeeling -Sikkim region (Hara, 1966, 1971; Ohashi, 1975), on East Himalayas (Wu, 1983), Malaysia (Holttum, 1954, 1982) and on Thailand (Iwatsuki, 1979, 1985).

Mizoram in the Eastern Himalaya is a biodiversity centre of Pteridophytes. The combination of various key factors like climate, temperature, edaphic, precipitation, humidity, altitude, forest type etc. have favoured a lush and luxuriant vegetation of Pteridophytes in Mizoram. The pteridophytes are a unique group of plants as they are strictly habitat-specific, shade and moisture-loving and any disturbances to the microclimate cause threat to the existence. At present microclimate is altered by anthropogenic activities like population growth, deliberate destruction of the habitat particularly forest, urbanization, intensive agriculture, slash and burn agricultural practice, clear-felling practice together with monoculture infrastructure development. Consequently, an altered microclimate leads to loss of habitat and destabilization of traditional management system causing tremendous threat to the existing Pteridophytes.

India has a rich plant diversity including Pteridophytes. Accordingly, the rich plant diversity is endowed with medicinal properties. Various phytochemical compounds such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites are associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases. The Greek botanist Theophrastus (ca. 372–287 BC) wrote about the medicinal value of pteridophytes. Dioscorides (ca. 50 AD) in his *de Materia Medica* has mentioned medicinal values of *Pteridium aquilinum* and *Dryopteris filix-mas*. In ancient Indian medicine systems, several ferns were used to cure many human ailments. Sushruta (ca. 100 AD) and Charaka (ca. 100 AD) recommended the medicinal use of some ferns in their *Samhitas*. Unani physicians used several ferns in India and Western Asia (Banerjee and Sen, 1980).

It is of utmost importance to screen pteriodophyte diversity for medically beneficial compounds for treating humans and plants diseases. Traditionally people used Pteridophytes as medicine and anti-bacterial agents. Many Pteridophytes were used for medicinal purposes by the ancient Greeks and Romans and through the Median ages. In recent years, the search for phytochemicals with antioxidant, antimicrobial, or antiinflammatory properties has been on the rise due to their potential use in treating various chronic and infectious diseases (Halliwell, 1996). Spider brake fern (*Pteris multifida*) has various bioactive flavonoids with heat-clearing, antipyretic, detoxification, antibiotic, anti-inflammatory, and anti-mutagenic activity (Lee and Lin, 1988). The entire plant of *Pteris multifida* has various bioactive compounds such as luteolin-7-*O*glucoside (Murakami and Machashi, 1985; Lu *et al.*, 1999), 16-hydroxy-kaurane-2- $\beta$ -Dglucoside (Liu and Qin 2002), luteolin, palmitic acid, apigenin 4-*O*- $\alpha$ -L-rhamnoside (Lu *et al.*, 1999; Qin *et al.*, 2006), quercetin, hyperin, isoquercitrin, (Lu *et al.*, 1999; Hoang

and Tran, 2014), and apigenin-7-O-β-D-glucoside (Lu et al., 1999; Hoang and Tran, 2014). Also, the cytotoxic bioactive compounds like pterosin and sesquiterpenes have been isolated from Pteris multifida (Shu et al., 2012). Bioactive terpenoids, especially the monocyclic sesquiterpene  $\alpha$ -caryophyllene, were found in an ethanolic extract of Pteris tripartita (Baskaran and Jeyachandran, 2010). Sesquiterpenes constitute a significant group of secondary metabolites and have a varied range of medicinal activities including anti-cancer, anti-inflammatory, cytotoxic, plant growth-regulatory, and antimicrobial properties (Baruah et al., 1994). Chen et al., (2005) have reported antioxidant activities in several fern species, namely, Polystichum semifertile, Nothoperanema hendersonii, Brumea insignis, Several studies have explored the antimicrobial activity of ferns such as *Nephrolepis acuminata* (Jimenez et al., 1979), Davallia sodila, Lygodium reticulatum (Cambie and Ash, 1994), Marattia fraxinea (de Boer et al., 2005), Sphenomeris chinensis (Sengupta et al., 2002), Adiantum caudatum, Adiantum peruvianum, Adiantum incisum, Adiantum latifolium, and Ampelopteris prolifera (Banerjee and Sen, 1980; Lakshmi et al., 2006; Lakshmi and Pullaiah, 2006; Singh et al., 2008a), P. aquilinum (Francisco and Driver, 1984), Nephrolepis sp. (Basile et al., 1997), Adiantum lunulatum (Reddy et al., 2001), E. arvense (Joksic et al., 2003; Radulovic et al., 2006), S. tamariscina (Woo et al., 2005), A. capillus-veneris (Guha et al., 2004, 2005; Besharat et al., 2008), Athyrium pectinatum (Parihar et al., 2006), P. vittata (Singh et al., 2008b), P. multifida (Hu et al., 2008; Hum et al., 2008), Mecodium exsertum (Maridass 2009), Selaginella involvens, Selaginella inaequalifolia (Haripriya

et al., 2010), Selaginella pallescens (Rojas et al., 1999), Asplenium scolopendrium, Cystopteris fragilis, Pteris vulgare (Soare et al., 2012b), A. caudatum, A. evecta, Pteris confusa, Pteris argyraea, Lygodium microphyllum (Gracelin et al., 2012), Pteris biaurita (Dalli et al., 2007; de Britto et al., 2012), D. crassirhizoma (Lee et al., 2009), and various species (Maruzzella, 1961; Mc Cutcheon et al., 1995). Furthermore, several studies (May, 1978; Dixit and Vohra, 1984; Dixit, 1992; Verma and Singh, 1995; Manandhar, 1996; Das, 1997; Singh, 1999; Benjamin and Manickam, 2007) have reported the antiviral, antibacterial, and antifungal activity of *Equisetum ramosissimum*, Drynaria quercifolia, Psilotum nudum, Parahemionitis arifolia, Helminthostachys zeylanica, Ophioglossum gramineum, Tectaria caodunata, S. involvens, Selaginella delicatula, Hypodematium crenatum, Leucostegia immersa, and Solvinia molusta. Singh (1999) documented antibacterial and antifungal activity of *Botrychum lanuginosum*, Dryopteris cochleata, Nephrolepis cordifolia, Polystichum molluscens, Polystichum squarrosum, Salvinia radicata, Sphaerostephanos unitus, Selaginella palustris, Pteris cretica and Pteris vittata. Although the antimicrobial activities have been independently reported by several leading researchers, an in-depth study to properly estimate minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) have not yet been carried out for several ferns.

Although record of the higher group of plants about their diversity, bioactive compounds, antioxidant and their anti microbial activities are available from the Mizoram, attempt had not been made so far to objectively assess the diversity, bioactive compounds, antioxidant and antimicrobial activities of Pteridophytes of North-East India particularly Mizoram. Given the above gap in knowledge, the present study is focused on diversity and phytochemical analysis of medicinally important species of Pteridophytes growing in ecologically important forest sites of Mizoram. Also, the present work invariably includes the correct nomenclature, synonyms and distribution of Pteridophyte species from North-Eastern region of India along with their vivid descriptions and illustrations.

#### **OBJECTIVES:**

The following prime goals were set to carry out the proposed work -

- To collect and accurately identify the species of Pteridophytes in selected sites of Mizoram.
- 2) To analyze the phytochemical diversity and antioxidant activities of selected species of Pteridophytes.
- 3) To discover precisely the anti-bacterial activities of selected species of Pteridophytes.

# **CHAPTER -II**

# **STUDY AREA**

# **2.1. LOCATION**

Mizoram lies between  $21^{\circ}56$  N –  $24^{\circ}31$  N latitudes and  $92^{\circ}16$  E –  $93^{\circ}26$  E longitudes. Sandwiched between Bangladesh and Myanmar, location of Mizoram is of strategic significance geographically and politically and shares a total common international boundary of about 585 km with these two countries. In terms of size, it may not be significant as total area of the Mizoram constitutes only 0.64 per cent of the total area of India.

The tropic of cancer, i.e. 23°30′N latitude, cuts across the region in Aizawl District at the Southern periphery of Aizawl traversing places like Champhai, Chhawrtui, Darlung and Phuldungsei, etc. This imaginary line divides the region into two almost equal parts. Mizoram is bounded on the North by Cachar District of Assam and the State of Manipur; on the East and South by Chin Hills of Myanmar; on the West by Chittagong Hill tracts of Bangladesh and the State of Tripura. Mizoram has a total geographical area of 21,087 square km. Its largest dimensions – North to South is 285 km and East to West is 115 km.

# **2.1.1. CLIMATE**

Despite its tropical location, Mizoram enjoys a moderate climate. This is mainly due to its high elevation. It is neither very hot nor too cold throughout the year. The region falls under the direct influence of the South-West monsoon. The region receives an adequate amount of rainfall. Climate is humid tropical and characterized by short winter, long summer with heavy rainfall.

### **2.1.2. TEMPERATURE**

Since Mizoram has no proper observatory stations, the comprehensive information about the temperature conditions of the region is not available. The analysis is based mainly on the observations and data generated by the scholar with meagre temperature records available on the region.

In Mizoram temperature does not fluctuate much throughout the year, except in the low-lying valley sites. However, it is observed that there has been a steady increase in the annual temperature, at par with the global phenomenon. The hottest months are May, June and July. Thereafter, the onset of the monsoon brings down the temperature. The temperature continues to fall with the break of the monsoon rains and it minimized in December and January. In autumn, the temperature is usually between 18°C and 25°C, while winter temperature records normally between 11°C and 23°C. The summer temperature is usually between 25°C to 34°C. During the last 20 years or so, a steady

increase in temperature has been noticeable as felt in the global context, mainly due to the large-scale degradation of vegetation and mismanagement of the environment.

During winter, the lowest temperature is felt at places having high altitudes such as Champhai, Zote, Ngur, etc. in the East; Bualpui (Ng) and Phawngpui mountainous area in the South. The highest temperature in summer is observed at relatively lower places such as Kanhmun, Zawlnuam, Bairabi, Vairengte, etc. in the Northern part; Tlabung, Chawngte, Tuipang, Tuipuibari, etc. in the South and West-end. Places at higher altitude experience lower diurnal range of temperature; while places at lower altitudes have higher temperature ranges.

#### 2.1.3. RAINFALL

The entire state of Mizoram is under the direct influence of the monsoon. It rains heavily from May to September. The average rainfall is 257 cm per annum. The northwestern portion of the State receives highest rainfall i.e. more than 350 cm per annum. The rainfall also increases southward with an increase in humidity. While Aizawl at 23°44′N and 92°43′E receives about 208 cm annual rainfall, Lunglei (22°53′N and 92°45′E) records as high as 350 cm. The study of the available rainfall data reveals the highest monthly rainfall (602.60 cm) in Mizoram during July 1983. Precipitation is heavy in summer, normally from May to September, and lasts till late

October. Normally July and August are the rainiest months, while December and January are the driest months.

# **2.1.4. SEASON**

Depending on the variation in temperature and general weather conditions, three seasons are observed in Mizoram. They are -(1) The cold season or winter (2) Warmseason or spring (3) Rainy season or summer.

# The Cold or Winter Season

The season starts from November and lasts till February. The temperature is comparatively lower (11 to 23°C), but not too low to make human habitation difficult. The diurnal temperature varies from 8 to 24°C during this season. The season receives very less rainfall originated from Northeast retreating monsoon. The season is very pleasant with clear blue sky in the absence of cloud covering. Morning mists are common upon the valleys during the season; which gives an enchanting view resembling a wide stretch of ice-sheets.

## The Warm-Season or spring

The warm season begins from March and lasts till the first part of May and merges with rainy season. The temperature has risen up to a range of 19 to 29°C being aggravated by rainless days. The early part of this season has bright sunshine and clear

sky with little or no cloud till it is disrupted by the coming of pre-monsoon showers. Maximum diurnal temperature sometimes reaches as high as 32°C. Due to little or no cloud cover, maximum insulation is received during this period and this is the hottest season in Mizoram.

#### The Rainy Season or summer

This is the longest season in Mizoram; hold out for nearly six months from the second part of May till late October. The season starts with violent storms which swept the State from South-west through the Bay of Bengal, marking the beginning of monsoon rains. Rainfall is heavy from May to September while July-August receives about 40 per cent of the annual rainfall. The heavy outpour which starts normally in the morning is sometimes associated with hailstorms and thunder. This is the season when cyclonic rains are often felt. The temperature remains high but is kept down to a considerable extent by the usual rains.

#### 2.1.5. SOILS

Loose sedimentary formations mainly dominate the soils of Mizoram. Soils in Mizoram are generally young, immature and sandy. Derived soils with red, loamy texture are also found with a high level of laterite. The soil acidity is high; low in potash and phosphorus. But in an un-eroded soil, the content of nitrogen is quite high fostered by an accumulation of organic matters. Rainwater brings down soil from high altitudes in valleys; consequently, the soils in the valleys are heavier. In respect of soil formation, the soils of different physiographic units are homogenous. They are mainly derived from sandstones, shales and siltstones. The hill slopes and valleys have the soils order of Ultisols and Entisols with combinations of Inceptisols. The type of soils found at sub-order level is Udults, Ochrepts and Orthents.

The surface soils of the hilly terrain are dark, highly leached and poor in bases, rich in iron and low in pH values ranging from 4.5 to 5.5 (highly acidic). They are welldrained, deep to very deep, rich in organic carbon, low in available phosphate content and high in available potash. The surface soil texture is loam to clay loam with clay content increasing with depth. The percentages of clay, silt and sand within 50 cm of the surface in most cases is 20 to 30 per cent, 35 to 45 per cent and 25 to 45 per cent, respectively. The pH and organic carbon content decrease and clay content increase with the depth. They provide substantial oxygen supply for plant growth and can keep moisture and keep up its supply throughout the growing seasons of most crops. The soils on the top of the barren ridges and escarpments, however, are mostly shallow or underlain by weathered rocks and have a thin solum depth.

Soils of the valley flatlands are brown to dark brown, poor in bases, moderately acidic with pH ranging from 5.5 to 6.0, medium to high in organic carbon content, low available phosphate and medium to high available potash. They are deep to very deep but moderately to poorly drained. The texture of the soil is mostly sandy loam to clay loam. The percentage of clay, silt and sand in the upper 50 cm ranges 15 to 35 per cent and 40 to 75 per cent, respectively. Soil is yet on its way to pedogenic horizon development as clay content does not increase with depth and argillic horizon is absent.

# 2.1.6. VEGETATION

The natural factors which influence the geographical distribution of forest in Mizoram are latitude, elevation, rainfall and nature of the soil. There is a marked difference between the vegetation of the western and eastern part of the state. The influence of altitude, soil and moisture is obvious.

There is an abundant growth of vegetation in Mizoram. Out of the total geographical area (21,087 sq.km), the 15,935 sq.km is covered by vegetation which accounts for about 75% of the area of the state. Its tropical location, which furnishes conducive climatic condition such as an adequate rainfall, moderate temperature, etc. favors the luxuriant growth of vegetation. The type of vegetation in Mizoram ranges from tropical trees to sub-Tropical trees. They include valuable species of timber, lumber, medicinal herbs and domestic resources.

On a broad scale, Mizoram has wooded forests in the higher altitudes and bamboo forests in the lower ridges – normally below 600 m, including riverine low lands. However, according to vegetation, there are six types of forest in Mizoram – (a) Tropical Evergreen Forest (b) Sub-Himalayan Semi-Evergreen Forest (c) Sub-Tropical Pine Forest (d) Sub-Tropical Hill Forest (e) Mixed Forest (f) Overlapping Bamboos.

# **2.2. SITES SELECTED**

### **2.2.1. PHAWNGPUI NATIONAL PARK**

Phawngpui National Park is located in Lawngtlai district, Mizoram, India. It is 250 km away from District headquarter Aizawl with an area of 50 sq. km. The temperature ranges from 2 to 30°C with an average annual rainfall of 2,500 mm per year. Villages like Sangau, Sentetfiang, Thaltlang, Vawmbuk, Archhuang, SiachangKawn and Cheural surround the sanctuary. The geo-coordinate of Phawngpui National Park is 23°35'-22°40' North Latitude and 93°03'- 93°05' East longitude having an altitude of 1000 to 2157 m above MSL.

## **2.2.2. MURLEN NATIONAL PARK**

Murlen National Park is located in Champhai district, Mizoram, India. Situated 240 km away from District headquarter Aizawl with a total area of 100 sq. km. The temperature ranges from 20 to 30°C with an average annual rainfall of 2200 mm per year. The sanctuary is surrounded by villages like North Khawbung, Rabung, Murlen, Vapar, Ngur, Hmunhmeltha, Tualpui and Khualen. The geo-coordinate of Murlen National Park

is 23°32'-23°42' North Latitude and 92°13'-92° 27' East longitude having an altitude of 400 to 1900 m above MSL.

#### 2.2.3. TAWI WILDLIFE SANCTUARY

Tawi Wildlife Sanctuary is located in Aizawl district, Mizoram, India and is situated 100 km away from district headquarter Aizawl with a total area of 35 sq. km. The temperature ranges from 20 to 30°C in summer and 16 to 20°C in winter with an average annual rainfall of 2000 to 2500 mm. The sanctuary is surrounded by villages like Hualtu, Hmuntha, Maite, Lenchim, Tawizo, and Mualpheng. The geo-coordinate of the Tawi Wildlife Sanctuary is 23°30' North Latitude and 93°00' East longitude with an altitude of 680 to 1,890 m above MSL

#### 2.2.4. DAMPA TIGER RESERVE

Dampa Tiger Reserve is located in Mamit district, Mizoram, India. It is situated 127 km away from District headquarter Aizawl with a total area of 500 sq km (core) and 488 sq km (buffer). The temperature ranges from 20 to 30°C in summer and 16 to20°C in winter with an average annual rainfall of 2,000 to 2,500 mm. The sanctuary is surrounded by villages like W. Phaileng, Teirei, Damparengpui, Tuipuibari, Lallen, Saithah, Serhmun, Phuldungsei, Pukzing, Khawhnai, West Phulpui and Silsuri. The geo-coordinate of Dampa Tiger Reserve is 23°32'42"- 23°41'36" North Latitude and 92°13'12"-92°27'27" East longitude with an altitude of 200 m to 1200 m above MSL

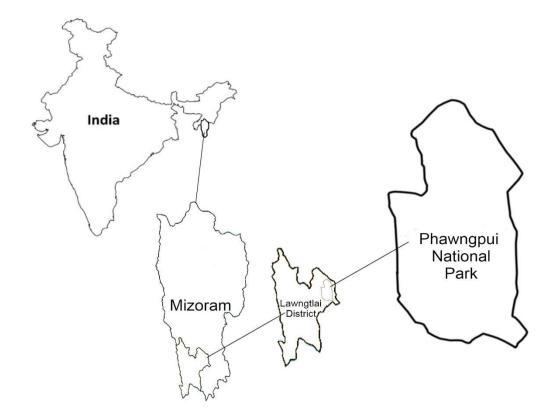


Figure 1. Map showing Phawngpui National Park one of the study sites in Mizoram.

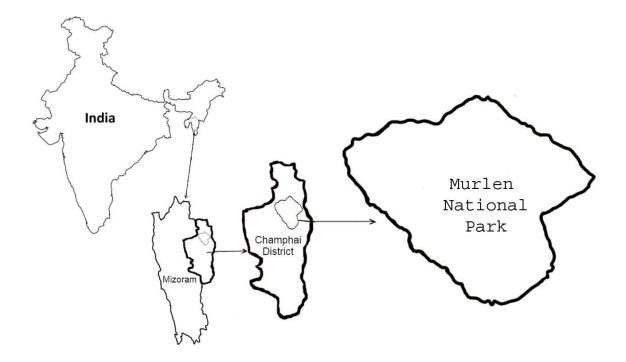


Figure 2. Map showing Murlen National Park one of the study sites in Mizoram.

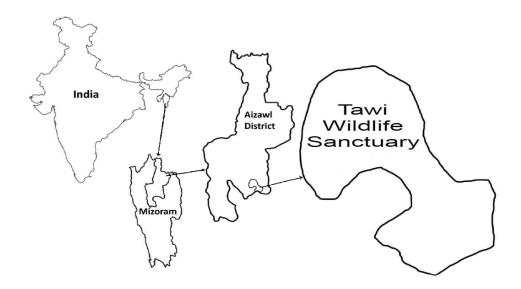


Figure 3. Map showing Tawi Wildlife Sanctuary one of the study sites in Mizoram.

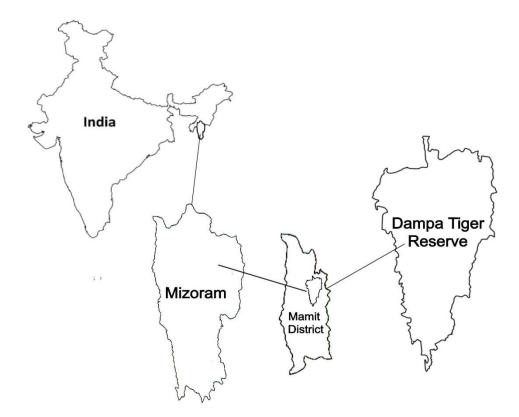


Figure 4. Map showing Dampa Tiger Reserve one of the study sites in Mizoram.

## **CHAPTER-III**

# **MATERIALS AND METHODS**

# **3.1. COLLECTION OF PLANTS MATERIALS**

Pteridophytes having medicinal properties known to the locals were collected during their thriving season of July to August from 2013 to 2016 from the Murlen National Park, Phawngpui National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve. Collected plants were identified with the available literature and get confirmed in Pteridology Lab of National Botanical Research Institute, Lucknow, India.

# **3.2. PREPERATION OF HERBARIUM**

The preparation of the specimens was done using the methods described by Manilal and Kumar (1998).

# **3.2.1. COLLECTION**

The specimens collected were put in a collection bag and attached of any insects, spiderwebs and foreign bodies were removed. Next, the specimens were mounted in 42 cm x 29 cm ( $16\frac{1}{2}$ " x  $11\frac{1}{2}$ ") blotting paper. Moreover, plant specimen larger than the specimen blotting paper was mounted in V or N or M shape to accommodate the entire plant material in the above size. The leaves were ordinarily mounted on dorsi-ventral position.

# **3.2.2. POISONING AND DRYING OF SPECIMEN**

Poisoning kills the plants and prevents the formation of abscission layer. Accordingly, the poisoning was done by spraying the plant with a saturated solution of mercuric chloride in ethyl alcohol (20 g of mercuric chloride is added to a 1 L of alcohol). The plant was again placed between the blotters in the presser till it gets completely dried.

# **3.2.3. MOUNTING**

The dried plant specimen was mounted on a standard herbarium sheet, 28 cm (breadth) x 42 cm (length) using a natural glue.

### **3.3. IDENTIFICATION OF PLANTS**

The enumerated species are presented under families as per the system of classification proposed by Pichi-Sermolli (1977) and added literature (Hovenkamp *et al.*, 1998; Fraser-Jenkins 2008). For the correct citation of the author of the species, Pichi Sermolli (1996) has been followed. Moreover, the specimens collected were deposited in Herbarium Collection, the Department of Botany, Mizoram University.

# **3.3. PREPARATION OF PLANT EXTRACTS**

The dried plants of the aerial parts of *Lycopodiella cernua* (L.) Pic. Serm, *Diplazium esculentum* (Retz.) Sw. *and Selaginella bisulcata* Spring. were macerated to powder form using a hand-held grinder. The plant powder was subsequently subjected to a sequential continuous hot extraction in a Soxhlet apparatus using Petroleum ether, Chloroform, Methanol and Water as the solvent. Extraction was run for 72 h. The extracts were concentrated in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The plant extracts produced in the form of semi-solid mass were refrigerated at 4°C until further use.

# 3.4. PREPARATION OF REAGENT AND TEST FOR PHYTOCHEMICAL ANALYSIS

The following methods and tests as described by Kokate *et al.*, (2013) were used for the screening of phytochemicals in the selected species of Pteridophytes.

# 3.4.1. ALKALOID

1. Wagner's Reagent

Mercuric chloride (1.35 g) was dissolved in 60 ml of distilled water and next potassium iodide (5.0 g) was dissolved in 10 ml of distilled water. The two solutions were mixed and made up to 100 ml with distilled water.

To a few ml of the filtrate, 1-2 drops of Wagner's Reagent were added in a test tube. A reddish-brown precipitate confirmed the test as positive.

2. Hager's Reagent

The 1.0 g of picric acid was dissolved in 100 ml of distilled water.

To a few ml of filtrate, 1-2 ml of Hager's reagent was added. A prominent yellow colour indicates the test as positive.

# **3.4.2. CARBOHYDRATES**

1. Fehling's Solution

Fehling's A: Copper sulphate (34.7 g) was dissolved in 500 ml of distilled water.

Fehling's B: Potassium sodium tartarate (173.0 g) and sodium hydroxide (50.0 g) was dissolved in 500 ml of distilled water.

1.0 ml of the filtrate was boiled in a water bath with 1.0 ml of each Fehling's solution A and B. A red ppt indicated the presence of sugar.

2. Benedict''s Reagent

Sodium citrate (173.0 g) and Sodium carbonate (100.0 g) were dissolved in 800 ml distilled water and boiled to make it clear. Copper sulphate (17.3 g) was dissolved in 100 ml of distilled water.

To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 minutes. A characteristic coloured ppt indicated the presence of sugars.

# **3.4.3. PROTEINS**

Ninhydrin test:

Crude extract when boiled with 2.0 ml of 0.2% solution of Ninhydrin, the violet colour appeared suggested the presence of proteins.

# **3.4.4. TANNINS**

Ferric chloride test:

Crude extract was mixed with 2.0 ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black colouration indicated the presence of tannins.

# **3.4.5. TERPENOIDS**

1. Salkowski's test:

5.0 ml of each extract was added to the 2.0 ml of chloroform and 3.0 ml of conc. Sulphuric acid. The formation of a monolayer of reddish-brown colouration of the interface indicates the presence of terpenoids.

# 2. Chloroform test

Crude extract was dissolved in 2.0 ml of chloroform and evaporated to the dryness. To this, 2.0 ml of concentrated sulphuric acid was added and heated for about 2.0 minutes. A greyish colour indicated the presence of terpenoids.

# **3.4.6. PHENOLS**

Ferric chloride test:

The extract (50 mg) was dissolved in 5.0 ml of distilled water. To this, a few drops of 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

#### **3.4.7. GLYCOSIDES**

### Keller-Kilani's test

Crude extract was mixed with 2.0 ml of glacial acetic acid containing 1-2 drops of 2.0% of ferric chloride. The mixture was then poured into another test tube containing 2.0 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of glucosides.

#### **3.4.8. SAPONINS**

Crude extract was mixed with 5.0 ml of distilled water in a test tube and shaken vigorously. The formation of stable form was taken as an indication for the presence of saponins

# **3.4.9. PHYTOSTEROLS**

Salkowski's test:

The extract was treated with Salkowski's reagent. The yellowish colour with green fluorescence appearance indicates the presence of phytosterols.

#### **3.4.10. FLAVANOIDS**

Alkaline reagent test

The crude extract was mixed with 2.0 ml of 2% solution of NaOH. An intense yellow colour was formed which turns colourless on the addition of a few drops of diluted acid which indicates the presence of flavonoids.

# **3.5. ANTIBACTERIAL TEST**

# **3.5.1. MEDIA PREPARATION AND ITS STERILIZATION**

Antimicrobial susceptibility was tested on solid media in Petri plates using agar-well diffusion method (Murray *et al.*, 1995) later modified by Olurinola (1996). For bacterial

assay nutrient agar (40 gm L<sup>-1</sup>) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) was determined by serial micro-dilution assay. The suspension culture of bacterial cells was raised by preparing 2% (w/v) Lauria Broth for evaluation. All the media prepared were then sterilized by autoclaving at (121°C) for 20 min.

# **3.5.2. NUTRIENT AGAR PREPARATION**

Nutrient agar was prepared using the following compositions

Ingredients	g L-1
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Agar	15.0
Final pH (at 25°C)	7.4±0.2

The 28.0 g of nutrient agar was suspended in 1.0 L distilled water and heat to boiling to dissolve the medium completely followed by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The sterilized medium was cooled to 45-50°C and poured into sterile Petri plates.

# **3.5.3. LAURIA-BROTH PREPARATION**

Lauria-Broth was prepared using the following compositions

Ingredients	g L-1
Casein enzymic hydrolysate	10.0
Yeast extract	5.0
Sodium chloride	5.0
Final pH (at 25°C)	7.0± 0.2

The 20.0 g of Lauria-Broth was suspended in 1.0 L distilled water. It was then heated to boiling to dissolve the medium completely followed by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

#### **3.5.4. AGAR-WELL-DIFFUSION METHOD**

Agar-well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hours old broth culture of respective bacteria. Wells (7 mm diameter and about 2 cm apart) were made in each of these plates using a sterile cork borer. The stock solution of each plant extract was prepared at a concentration of 100.0 mg ml<sup>-1</sup> and was diluted to different concentration viz 20, 40, 60 and 80 mg ml<sup>-1</sup> using a serial-dilution method of methanol

extracts of different plants. About 70.0  $\mu$ L of different concentrations of plant extracts were added into the well by using a sterile syringe and allowed to diffuse at room temperature. Control experiments comprising inocula without plant extract were set up. The plates were incubated at 37°C for 18 to 24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured, and the activity index was also calculated. Triplicates were maintained and the experiments were repeated thrice. For each replicate, the readings were taken in three different fixed directions and the average values were recorded.

# 3.5.5. PREPARATION OF INOCULUM TEST FOR ANTIBACTERIAL ACTIVITY

The antibacterial assay was carried out by the microdilution method against the bacteria namely *Escherichia coli (ATCC - 10536), Klebsiella pneumoniae (ATCC - 10031), Bacillus subtilis (ATCC- 11774).* The inocula were prepared and stored at 4°C until use. Dilutions of the inocula were cultured on the solid medium to verify the absence of contamination and to check the validity of the inoculum.

#### **3.6. DETERMINATION OF TOTAL PHENOLIC CONTENT**

Total phenolic contents of methanolic extracts (MeOH) of *Lycopodiella cernua* (L.) Pic. Serm., *Diplazium esculentum* (Retz.) Sw. *and Selaginella bisulcata* Spring.were determined by using the method of Mc Donald *et al.*, (2001) with slight modifications.

The extract of the plants was prepared by taking 10.0 mg of dried powder in a test tube, and 10 ml of methanol was added, followed by 10-folds dilution. The 1.0 ml of diluted plant extract (50 µg ml<sup>-1</sup>) was mixed with 5.0 ml of FCR. After 3.0 min, 4.0 ml of sodium carbonate solution (0.7 M) was added, and the mixture was allowed to stand for 1.0 hour at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer (Model TM-200). The 1.0 ml extract (50 µg ml<sup>-1</sup>) was also mixed with the reagents above and after 1.0 hour the absorbance was measured to find total plant phenolic content. With the help of the calibration curve, the phenolic concentrations in the extracts were determined and expressed as milligrams of Gallic acid equivalent (GAE) per g of the dried plant material.

# 3.6.1. PREPARATION OFF STANDARD GALLIC ACID AND CALIBRATION CURVE

The 10.0 mg of gallic acid was taken in a test tube, and 10.0 ml of methanol was added followed by 10-folds dilution After dilution, 1.0 ml of standard gallic acid (50  $\mu$ g ml<sup>-1</sup>) was mixed with 5.0 ml of FCR. After 3.0 minutes 4.0 ml of sodium carbonate solution (0.7 M) was added, and the mixture was allowed to stand for 1.0 h at room temperature. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Thermo Scientific Evolution, Model TM-200). A standard calibration curve at different concentrations (10, 20, 40, 60, 80, and 100  $\mu$ g ml<sup>-1</sup>) for gallic acid was prepared.

# **3.7. DETERMINATION OF TOTAL FLAVONOID CONTENT**

Total flavonoid contents of the extracts were determined by the aluminium chloride method (Chang *et al.*, 2002). The extract of the plants was prepared by taking 10.0 mg of dried powder in a test tube and 10.0 ml of methanol was added followed by the 10-fold dilution. After dilution 1.0 ml of the extract (50  $\mu$ g ml<sup>-1</sup>) was mixed with 2.0 ml of distilled water. After 5 minutes, 3.0 ml of 5% sodium nitrite (NaNO<sub>2</sub>) and 0.3 ml of 10% aluminium chloride (AlCl<sub>3</sub>) were added. After 6 minutes of incubation, 2.0 ml of NaOH (1.0 M) was added, and the volume was made up to 10.0 ml with distilled water followed by 1.0 h incubation at room temperature. The absorbance was taken at 510 nm in a UV-Vis spectrophotometer (Thermo Scientific, Evolution, Model TM -200). As described below, a standard calibration curve at different concentrations (10, 20, 40, 60, 80, and 100  $\mu$ g ml<sup>-1</sup>) of quercetin was prepared. From the calibration curve, the total flavonoid content of the samples was determined and expressed as milligrams of quercetin equivalent (mg QE) per g of extract.

# 3.7.1. PREPARATION OF STANDARD GALLIC ACID AND CALIBRATION CURVE

The 10.0 mg of quercetin was taken in a test tube and 10.0 ml of methanol was added followed by 10 times dilution. The 1.0 ml of diluted quercetin (50  $\mu$ g ml<sup>-1</sup>) solution was mixed with 2.0 ml of distilled water. After 5 minutes incubation, 3.0 ml of 5% sodium

nitrite (NaNO<sub>2</sub>) and 0.3 ml of 10% aluminium chloride (AlCl<sub>3</sub>) were added. After 6 minutes, 2.0 ml of NaOH (1.0 M) was added, and the volume was made up to 10.0 ml with distilled water. It was then incubated at room temperature for 1 h and absorbance was taken at 510 nm in a UV-Vis spectrophotometer (Thermo Scientific Evolution TM - 200). A standard calibration curve at different concentrations (10, 20, 40, 60, 80, and 100  $\mu$ g ml<sup>-1</sup>) for Quercetin was prepared.

## **3.8. DETERMINATION OF ANTIOXIDANT CAPACITY**

# **3.8.1 DPPH RADICAL SCAVENGING ACTIVITY**

DPPH (2,2-diphenyl-1-picrylhydrazil) free radical scavenging assay was carried out according to the method described by Kim *et al.*, 2017. A standard stock solution of the plant extracts was made by dissolving 10.0 mg of dried powder in 10.0 ml of distilled water. A solution of 0.0005, 0.001, 0.005, 0.01, 0.025 and 0.05 mg ml<sup>-1</sup> of each plant species were prepared. The 3.0 ml of plant extract was then mixed with 1.0 ml of 0.1 mM DPPH solution (in MeOH) and the solution was made 6.0 ml with distilled water in a test tube. It was then vortexed and incubated at 37 <sup>o</sup>C for 30 minutes. The absorbance of the solution was then measured at 517 nm using Thermo Scientific EVOLUTION 200 UV-Visible spectrophotometer. The percentage inhibition was calculated by comparing the absorbance values of the test samples with those of the controls (not treated with

extract). The inhibition percentage (I) was calculated as radical scavenging activity as follows:

I (%) = 
$$\left(\frac{\text{Control absorbance - sample absorbance}}{\text{Control absorbance}}\right) X 100$$

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the extract was expressed as  $IC_{50}$ . The  $IC_{50}$  value was defined as the concentration (µg ml<sup>-1</sup>) of extract that inhibits the formation of DPPH radicals by 50%.

# **3.9. STATISTICAL ANALYSIS**

All the experiments were performed in triplicates and the mean values with  $\pm$  SE were calculated. One Way ANOVA was performed for each experiment. Statistical significance at P  $\leq$  0.05 was considered.



Photo plate 1:

- a. Field visit at Phawngpui National Park
- b. Areas of Phawngpui National Park
- c. Field visit at Tawi Wildlife Sanctuary
- d. Areas of Tawi Wildlife Sanctuary



Photo plate 2:

- a. Field visit at Dampa Tiger Reserve
- b. Areas of Dampa Tiger Reserve
- c. Field visit at Murlen National Park
- d. Areas of Murlen National Park



Photo Plate 3:

- a. With Dr. Ajit Singh, Head, Pteridology lab, NBRI
- b. Herbarium specimen in NBRI
- c and d. Inside campus of NBRI

#### **CHAPTER-IV**

## **DIVERSITY AND IDENTIFICATION OF PTERIDOPHYTES**

## **4.1. INTRODUCTION**

The Eastern Himalaya with Mizoram is a biodiversity center of Pteridophytes. The combination of different factor like climate, temperature, edaphic, precipitation, humidity, altitude, forest type favors the growth of rich and luxuriant vegetation of pteridophytes in Mizoram. The Pteridophytes are unique group of plants as they are strictly habitat specific, shade and moisture loving and any disturbances to the micro climate causes treat to the exist. At present anthropogenic activities like population growth, destruction of habitat particularly forest, urbanization, agriculture, slash and burn agricultural practice, clear felling practice together with mono-culture infrastructure development result in the alteration of the micro climate leading to habitat lost, destabilization of traditional management system cause tremendous threat to the existence of Pteridophyte in the state. So, the present Chapter 4 aims at assessing the diversity and identify the Pteridophytes collected from four reserve forests area (Phawngpui National Park, Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve ) with a view to fill the record gap of these lesser known plants in Mizoram, so that a conservative steps will be taken in the future for their survival.

## **4.2. MATERIALS AND METHODS:**

# **4.2.1. IDENTIFICATION OF PLANTS**

The enumerated species are presented under families as per the system of classification proposed by Pichi-Sermolli (1977) and literatures (Hovenkamp *et al.*, 1998, Freser-Jenkins 2008) have been followed and for correct citation of the author of the species, Rodolfo E.G. Pichi Sermolli (1996) has been followed. The specimen collected is deposited in the Department of Botany, Mizoram University.

#### 4.3. RESULTS

#### **4.3.1. DOCUMENTED PTERIDOPHYTES WITH DESCRIPTION**

# 1. Adiantum phillipense L.

Sp. Pl. 2: 1094. 1753; Jamir and Rao, Ferns of Nagaland, 162. 1988.

**Description:** Rhizome short, erect or suberect, ca 2.5 cm thick, scaly, ovate-lanceolate, acuminate at apex, entire, brown with deep brown central region. Stipes naked and polished, scaly at base, deep brown to black. Lamina ca  $20 \times 5 - 6$  cm, simple pinnate, either terminated by an apical pinna or apex of rachis prolonged bearing a vegetative bud, broad lobes, each lobe may be further lobed; pinnae pale-green, glabrous; texture-herbaceous; rachis similar to the stipe. Veins distinct above and below, dichotomously branched, free, reaching the margin. Sori continuous along the margin of the lobe which

is slightly depressed from the general outline, cresent-shaped, upto 2mm wide; indusia coriaceous, entire, brownish; sporangia stalked. Spore triangular, smooth.

Specimens examined: Mizoram, Champhai district, MNP, 1423 m alt., Dt. 13. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00001.

#### 2. Adiantum caudatum L.

Mant.308. 1771; Bedd. Ferns South India, T. 2. 1864; Handb. Ferns Brit, India, 83. T. 44. 1883; Clarke, Trans. Linn. Soc. Lond. II. Bot. 1: 453. 1880 (pro parte); Jamir and Rao, Ferns of Nagaland, 162. 1988.

**Description:** Rhizome short, erect, ca 0.5 cm thick, densely covered by scales; scales ca 3 x 0.2 mm, lanceolate, apex acuminate, base broad, entire, dark-brown at the centre, gradually become pale towards the margin. Stipes ca  $4-15 \times 0.1$  cm, rounded below, grooved above, densely covered by long, multicellular pale-brown hairs all over, dark-brown. Lamina ca  $15-20 \times 2-3$  cm, simple pinnate with a terminal pinna or with the rachis much prolonged and bearing an apical vegetative bud, oblong-lanceolate or linear-oblong-lanceolate; pinnae numerous, alternate, sessile or sub-sessile, upper pinnae gradually smaller, largest pinnae in the middle portion of the lamina, venation distinct on the upper surface, dichotomously branched, reaching the margin. Sori on the apices of the lobes, roundish-oblong, about 1 mm in diameter, indusia cordate, round, entire; sporangia are small. Spores deep-brown, granulated.

Specimens examined: Mizoram, Aizawl district, TWS, 1200 m alt., Dt. 16. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00002.

3. Aleuritopteris formosana (Hayata) Tagawa.

Acta Phytotax. Geobot, 14: 191. 1952; S. K. Wu, Mem. Fac. Sci. Kyoto Univ. Biol, 8: 155. 1983.

**Description:** Rhizome short, erect to ascending, scaly; scales black in centre, margins paler, narrowly ovate. Stipes 5-12(-23) cm long, dark purplish to castaneous, polished, scaly at base; scales lanceolate, thin, blackish in centre and brownish at marginal portion. Laminae bipinnate, 5-10(-17) by 2-7 cm, oblong to lanceolate, covered with white or yellow waxy powder on lower surface; pinnae 4–6 pairs. Indusia free, not or slightly lacerate at margin.

Specimens examined: Mizoram, Champhai district, MNP, 1450 m alt., Dt. 16. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00003.

4. Aleuritopteris dubia (C. Hope) Ching.

Hong Kong Naturalist, 10: 200. 1941.

**Description:** Rhizomes erect, short; scales bicolorous, black with narrow brown margins, lanceolate. Fronds clustered. Stipe dark brown or black, lustrous, usually noticeably shorter than lamina, 5-15 cm x 1-2 mm, densely scaly and often with

multicellular hairs; scales concolorous or somewhat bicolorous with lighter margins, narrowly lanceolate to hairlike. Lamina oblong to deltoid, 6-25 x 4-10 cm, pinnatebipinnatifid, papery when dry, with white or pale yellow farina abaxially, also with hairlike to narrowly lanceolate scales along costae and midveins abaxially, glabrous or rarely sparsely hairy adaxially; pinnae 4-12 pairs, distinct along rachis, basal pair of pinnae ovate-deltoid, inequilateral, bipinnatifid; basal basiscopic segments larger than adjacent acroscopic ones; second and upper pairs of pinnae lanceolate, pinnatifid. Sori consisting of several sporangia, confluent at maturity. False indusia interrupted, with fimbriate margins.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1817 m alt., Dt. 23. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00004.

5. Angiopteris evecta (G. Forst.) Hoffmann.

Comm. Soc. Reg. Gott, 12: 29, T. 5. 1796. Beddome, Handbook Ferns Brit. India, 460. 1883. Clarke, Trans. Linn. Soc. London 2 Bot. 1: 585. 1880.

**Description:** Rhizome short, erect, very thick, sometimes up to 60 cm in diameter. Stipes 1-1.5 m long or longer, green, scaly, and hairy; scales brown; hairs usually present in the young stages but deciduous later on; rachis sparsely scaly and hairy like the stipe. Lamina 2- pinnate, 1.5 to 6 m long, ca 2 m broad, texture herbaceous to subcoriaceous, glabrous; pinnae 30-100 cm long, lowest pinna the largest; pinnae costae swollen at their bases; pinnae numerous, 10-30 cm long, 2-4 cm broad, 2-3 cm distant, opposite, very shortly petiolate with swollen petioles, linear-lanceolate, unequal at their bases, basiscopic side round, acroscopic side usually cuneate, margin entire or minutely toothed specially at the apex which becomes prominently toothed; veins simple, almost parallel, free, forked; recurrent veins present; costa, costules and veins glabrous. Sori exindusiate, short, a little distance away from the marginSpores tetrahedral, trilete, sometimes bilateral, monolete, smooth or variously granulose, yellowish brown.

Specimens examined: Mizoram, Champhai district, MNP, 1315 m alt., Dt. 12. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00005.

6. Angiopteris helferiana C. Presl.

Suppl. Tent. Pteri., 22. 1845.

**Description:** Fronds 2-3 m; stipes smooth. Laminae bipinnate; pinnae  $60-80 \times 20-30$  cm, with 15-20 pairs of pinnules; pinnules  $10-20 \times 2-6$  cm, bases cuneate, margins serrate to sharply serrate, apices acuminate to caudate. Veins sparse, ca. 10 per cm; false veins present, rarely absent. Sori 2-3 mm from margin, 1.5-3 mm, composed of 14-26 sporangia.

Specimens examined: Mizoram, Champhai district, MNP, 1417 m alt., Dt. 20. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00006.

#### 7. Asplenium cheilosorum Kunze ex Mett.

Abhandl. Sanckenb, Naturforsch, Ges., 3: 177, T.5. F. 12. 13.1859.

**Description:** Rhizome long-creeping, thin, 0.2-0.3 cm, scaly, scales dark-brown, linear, margin entire, apices acute, stipes 18-20 cm long, dark-purple, thin, dia. 0.1-0.15 cm, stiff, erect, glabrous, glossy. Stipes base scaly, scales as on rhizome; rachis glabrous, glossy, lamina pinnate, 15-30 cm long or longer, 3-5 cm broad, linear, texture thin, membranaceous, herbaceous, glabrous; pinnae numerous, veins forked with two branches one going to each tooth, glabrous; costa and costules glabrous. Sori indusiate, short, 0.2-0.4 mm, borne along the apical part of the veins invariably on the distal part of each lobe, indusia brown, thin, margin entire. spores dark-brown.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1717 m alt., Dt. 30. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00007.

#### 8. Asplenium unilaterale Lam.

Flora. Indo-China, 7(2): 224. 1940, p.p.; C.Chr. Sledge, Bull. Brit. Mus. (Nat. Hist.) Bot. 3: 246. 1965.

**Description:** Rhizome creeping; rhizome scales 2-3 mm long, dark brown, lanceolate, margins entire. Fronds spaced apart, erect, herbaceous. Stipe 7.5-22 cm long, stipe and rhachis glabrous, dark brown to almost black. Lamina 17-30×5-10 cm, pinnate, narrowly elliptic to oblong in outline, lowest pinnae hardly reduced but often more lobed, apex

decrescent and tapering to a point. Pinnae 12-20 pairs, glabrous, narrowly triangularrhombic or shaped like a parallelogram, costa forming the lower margin for about 1/2 to 3/4 of the pinna length. Sori 2-10, oval-linear, set along the veins towards the apex of the pinnae, 2.5-3 mm long; indusium narrowly oblong, membranous, entire, 0.8 mm wide.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1417 m alt., Dt. 10. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00008.

9. Asplenium yoshinagae Makino.

Phan. Pter. Jap. Icon., T. 64. 1900.

**Description:** Plants 20-45 cm tall. Rhizome erect, short, scaly; scales dark to reddish brown, narrowly triangular, entire, apex acuminate. Fronds caespitose; stipe grayish brown or grayish green to stramineous, 10-20 cm, adaxially grooved longitudinally, with small scales similar to rhizome scales, subglabrous when old; lamina narrowly triangular. Veins obvious, grooved adaxially, basal acroscopic vein multi-forked, other veins 1 or 2 forked. Fronds subleathery, grass-green to stramineous when dry, adaxially with wrinkles above veins, small, brown, narrowly triangular and apically filiform scales on abaxial surface and pinna stalks, subglabrous when old; rachis (when dry) abaxially triangular and apically filiform scales, adaxially longitudinally valleculate, gemmae or juvenile plants near rachis on pinna stalks. Sori linear, 4-8 mm, from near costa running

nearly to margin, distal sori close to rachis; indusia grayish brown, linear, entire, opening toward costa or first acroscopic vein.

Specimens examined: Mizoram, Mamit district, DTR, 785 m alt., Dt. 03. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00009.

#### 10. Asplenium laciniatum D. Don.

Prodr. Fl. Nepal, 8. 1825; Khullar. Fern Fl. W. Himalaya, 418. 1994: Boonkerd and Pollawat, Pterid. Thailand, 179. 2000.

**Description:** Lithophytic. Rhizome short, erect, bearing fronds in a tuft; scales narrowly elliptic, gradually narrowing towards apex, about 3.5 by 0.5mm, dark brown, clathrate, entire. Stipes castaneous, or stramineous upwards, glabrous or scaley, grooved above, 2–3 cm long. Laminae oblong-subdeltoid, acute at apex, about 5 by 3 cm at base, bipinnatifid; rachis green, grooved above, glabrous; pinnae shortly stalked, with 3–6 segments below indistinctly dissected apical portion, about 2 by 1 cm; ultimate segments spathulate, round and toothed at apex, cuneate at base, herbaceous, green, glabrous; veins visible, each entering a tooth of ultimate segments. Sori 2 to 5 for each segment, up to 2 mm long, hardly confluent to each other; indusia enrolling the sori, thin but firm.

Specimens examined: Mizoram, Champhai district, MNP, 1411 m alt., Dt. 08. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00010.

#### 11. Asplenium nidus L.

Boonkerd and Pollawatn, Pterid. Thailand. 110, 142. 2000.

**Description:** Epiphytic or lithophytic. Rhizome short, erect or ascending, stout, bearing a rosette of fronds, usually with a mass of roots on which are growing various epiphytes, scaly; scales brown to darker, membranous, up to 2 cm long, 3 mm broad, clathrate. Stipes stramineous to dark, 2–5 cm long, scaly at base. Frond simple, up to 110 cm or more long, 12–30 cm broad (but occasionally narrower, about 6 cm broad in soriferous ones, broadest at middle, gradually narrowing towards both apex and base, coriaceous, grass-green when living, paler below; midrib raised on upper surface, flat below, veins once or rarely twice forked, the first forking near midrib and then running parallel, uniting at apex to form submarginal veins about 0.5 mm inside leaf margin. Sori elongate along veins, extending from near midrib half-way to the margin, usually on every vein; indusia about 0.5 mm broad, with a space of 0.5 mm or wider between. It was epiphytic and found on *Gmelina arborea* Roxb.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1303 m alt., Dt. 20. 07. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00011.

12. Athyrium attenuatum (Wall. ex Clark) Tagawa.

Acta Phytotax. Geobot., 16: 177. 1956.

**Description:** Rhizome short, erect to sub-erect, scaly, covered with persistent leaf bases. stipes short, 5.0-10.0 cm long, light-brown stramineous, scaly, base without a tuft of large scales but otherwise densely scaly, scales brown, distal part of scales dark-brown, large,0.5-1.0 cm long, 0.2-0.3 cm broad, lanceolate, margin entire, apex acuminate, higher up stipe sparsely scaly becoming glabrous, slightly muricated due to the fallen bases of scales. Rachis brown or stramineous, succulent, appearing grooved when dry, very sparsely scaly, scales few, scattered, brown distal park dark-brown, lanceolate, margin entire, apex acute. lamina 2-pinnate, veins 5-7 pairs in a pinnule (depending upon the length of the pinnule); costae sparsely scaly, scales light-brown, lanceolate, margin entire, apex acuminate, grooved (like the rachis); costules glabrous.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1223 m alt., Dt. 09. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00012.

Anisocampium cuspidatum (Bedd.) Yea C. Liu, W. L. Chiou and M. Kato.
 Taxon, 60: 829. 2011.

**Description:** Plants terrestrial, medium-sized. Rhizomes short or shortly creeping with ascending apex, woody, densely clothed with brown linear scales at apex. Fronds caespitose, with radial phyllotaxis; fronds up to 1 m; stipe upward pale purple or brownish, 30-65 cm, base up to 5 mm in diameter. Veins free, visible on surfaces, lateral veins pinnate, 3-6 pairs, simple, catadromous, except basal basiscopic veins branched

directly from costa, not reaching sinuses between teeth, acroscopic veins branched from base of lateral veins, ending halfway. Lamina papery when dry, dark green, adaxially glabrate, costae occasionally with few brown small scales abaxially; costae grooved adaxially, protuberant abaxially. Sori dense, cinnamon-colored, small, orbicular, abaxial near base or in proximal, occasionally medial places of veins; indusia brown, small, orbicular-reniform and thinly membranous.

Specimens examined: Mizoram, Aizawl district, TWS, 1322 m alt., Dt. 07. 07. 2015 R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00013.

# 14. Athyrium falcatum Bedd.

Ferns Southern India, T. 151.1863.

**Description:** Rhizome short, erect, thick, scaly. stipes 3.5-10.0 cm long, stramineous, thin, fragile, scaly, base densly scaly, scales light-brown, concolorous, linear-lanceolate, margin entire, apex acuminate, higher up stipe sparsely scaley becoming glabrous. Rachis stramineous, sparsely scaley, scales as on stipe. lamina pinnate, 9.0-20.0 cm long; generally auricled on both the sides (in lower pinnae). Veins 10-12 pairs (depending upon length of the pinnae), in groups of 2-4 corresponding to the pinna lobe, costae globrous. Sori indusiate, medical (close to the costa, away from the margin near the middle of the pinna); indusial yellowish-brown; straight or j-shaped.

Specimens examined: Mizoram, Mamit district, DTR, 607 m alt., Dt. 02. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00014.

### 15. Blechnum orientale L.

Jamir and Rao, Ferns Nagaland, 403. 1988; Manickam and Irudayaraj, Pterid. Fl. West. Ghats. India, T. 229. 1992.

**Description:** Rhizome erect, ca 1.2 cm thick, densely scaly, massive; scales ca 2 x 0.3 cm, linear-lanceolate, apex acuminate, hair-ponted, margin entire, shining, dark-brown. Stipes  $15-75 \times 1$  cm, tufted, erect, scaly at base, glabrous above, reddish-brown at the base, grey-brown above, tubercles present along the stipe. Lamina ca  $20-175 \times 15-75$  cm, ovate to linear-lanceolate, apex acute base subtruncate or broadly cuncate, simple pinnate; pinnae numerous, spreading, sessile or adnate at lower side, free above, alternate, upto 4 cm apart. Veins slightly distinct, simple or forked once or two times, free; texture coriaceous; lamina pale-green, glabrous above and below, glossy. Sori linear along either side of the costa, continuous nearly to the apex, dark-brown; indusia narrow, firm with entire margin. Spores round to oval, translucent, yellowish-brown.

Specimens examined: Mizoram, Champhai district, Murlen National Park, 1512 m alt., Dt. 09. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00015.

16. Christella dentata (Forssk.) Brownsey and Jermy.

Brit. Fern Gaz., 10.338. 1973; Bhir, Ferns N.W. Himalayas, 103. 1980.

**Description:** Rhizome short creeping, ca 1 cm thick, apex sparsely scaly; scales ca 1 x 0.1 cm, linear-lanceolate, apex acuminate, margin entire, hairy, pale-brown. stipes ca  $15-45 \ge 0.4 \text{ cm}$ , scaly at base, glabrous above or pubscent, pale-brown to dark-brown. lamina ca 75 x 25 cm, oblanceolate, simple pinnate; pinnae about 15 - 25 pairs, subopposite or alternate, sessile, rachis and costae densely covered by long acicular hairs on upper surface, sparsely on costules and veins. Veins 6-9 pairs, basal one pair anastomosing to form an excurrent vein reaching the base of sinus, acroscopic vein to the side of sinus membrane, others free; texture thin but firm; intervenal area with few minute hairs above and below. Sori median on veins, arranged in two rows, one on either side of the costule; indusia large entire, hairy, brown. spores planoconvex or reniform.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1447 m alt., Dt. 20. 08. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00016.

17. Christella evoluta (C. B. Clarke and Baker) Holttum.

Beddome Handb. Ferns Brit. India, 208. 1974.

**Description:** Plants 60-120 cm tall. Rhizomes shortly to long creeping, including stipe bases with dark brown lanceolate scales. Stipes 15-40 cm, dark stramineous; laminae 40- $80 \times 20$ -40 cm, bases abruptly narrowed, apices caudate with a large apical pinna; lateral pinnae 10-20 pairs, proximal 1-5 pairs abruptly shortened, proximal pair triangular-

auriculate, ca.  $3 \times 2$  cm or longer, reflexed, sometimes hastate. Veinlets 7-9 pairs per segment, proximal 1.5-3 pairs anastomosing, next 1-3 pairs running to sinus membrane. Laminae papery, grayish green when dried, subglabrous or glabrous on both surfaces (rarely shortly hairy). Sori orbicular, inframedial or medial; indusia glabrous or shortly hairy. Sporangia bearing large reddish orange spherical glands on stalks. Spores thickly cristate.

Specimens examined: Mizoram, Mamit district, DTR, 677 m alt., Dt. 23. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00017.

18. Coniogramme fraxinea (D.don) Fee ex Diels.

Engler and Prantl, Nat. Pflanzenfam, 1(4): 262. 1899.

**Description:** Rhizome creeping, thick, densely covered with scales. Stipes stramineous, up to 1 m or more long, 1 cm, usually smaller, densely scaly at base, sparsely clad with minute scales upwards. Laminae bipinnate, up to 80 by 60 cm; rachis and pinna-rachis like the upper part of stipe, stramineous, minutely scaly, grooved on upper surface, grooves decurrent; lateral pinnae 5-8 pairs, lower 3-5 pairs bipinnate, upper ones simple; pinnules of larger pinnae like the upper simple lateral pinnae and apical ones, the lowest anterior pinnules rarely trifoliate or pinnate; leaflets oblong or narrower or oblong-lanceolate, usually slightly falcate, caudate at apex, cuneate at stalked base, dentate at margin, 20-30 by 3- 4.5 cm; the apical pinnae or pinules sometimes larger,

herbaceous, thin, glabrous; costa distinctly raised on lower surface, grooved on upper surface; veins a few times forked, hydathodes placed below the bottom of sinus. Sori extending along veins, exindusiate.

**Specimens examined:** Mizoram, Mamit district, DTR, 587 m alt., Dt. 29. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00018.

19. Cyclosorus aridus (D. Don) Ching.

Bull. Fan Mem. Inst. Biol., Bot. 8: 194. 1938.

**Description:** Plants 50-100 cm tall. Rhizomes long creeping including stipe bases with sparse brown lanceolate scales. Fronds distant; stipes 10-35 cm; laminae (20-)40-80(-120) x (10-) 15-35 cm, bases abruptly or gradually narrowed, apices caudate to acuminate; pinnae linear-lanceolate, (5-) 10-18 x 1.2 cm, bases truncate, lobed to 1/3 toward costae or sometimes only dentate, apices long acuminate; segments 20-40 pairs on middle pinnae, triangular 1-3 x 3mm, entire, acute or pointed at apices; veinlets 6-12 pairs, muvh oblique, proximal 2 or 3 pairs anastomosing, nect 1 or 2 pairs running to sinus membrane. Laminae papery to somewhat leathery, brownish green or yellowish green when dried, adaxially subglabrous except for several short acicular hairs along costae, abaxial surface with short acicular hairs along costae and veins, also with yellow or orange clavate glands along veins. Sori orbicular, medial; indusia glandular,

sometimes hairy. sporangia bearing yellow or orange clavate glands on stalks. spore with long wings or ridged folds.

**Specimens examined:** Mizoram, Mamit district, DTR, 417 m alt., Dt. 29. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00019.

20. Cyclosorus parasiticus (L.) Farwell.

Amer. Midl. Naturalist, 12: 259. 1931.

**Description:** Plants 30-100 cm tall. Rhizomes shortly to long creeping, including stipe bases with dark brown lanceolate scales. Fronds approximate to distant. Stipes 10-40 cm, stramineous. Laminae (20-)30-50(-60)  $\times$  (8-)15-25(-35) cm, bases not narrowed (sometimes slightly narrowed), apices caudate-acuminate; lateral pinnae 10-15(-20) pairs, proximal 1 or 2 pairs reflexed; veinlets 5-8 pairs, simple (ca. 10 pairs on basal acroscopic segment, occasionally forked), proximal pair anastomosing, sometimes next vein running to sinus membrane. Laminae herbaceous, brownish green or yellowish green when dried, with thin acicular hairs throughout on both surfaces, and reddish orange glands throughout abaxially. Sori orbicular, medial; indusia densely hairy. Sporangia bearing reddish orange glands on stalks.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1917 m alt., Dt. 11. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00020.

# 21. Cyathea chinensis Copel.

Philipp. J. Sci., 3: 355. 1909; Holttum, Kew Bull. 19:466. 1965; Boonkerd and Pollawatn, Pterid. Thailand, 113. 2000.

**Description:** Trunks up to 5 m or more tall. Stipes about 50 cm long, dark purplish near base, brownish upwards, with rather dense short spines throughout, warty; scales linear, to 3.5 cm long, 1.5 mm broad, shining dark brown, stiff, the edges narrow, paler, ferrugineous, soon abraded; main rachis smooth, glabrescent, light brown; largest pinnae about 50 cm long, 13 cm wide; costae and costules hairy throughout on lower surface, scales few, pale, more or less convex, hardly bullate; texture papyraceous, light green, paler beneath; veins simple or forked. Sori close to costules; receptacles large; indusia in mature sori reflexed as broad pale brown scales, irregular and abraded at margin.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1487 m alt., Dt. 03. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00021.

### 22. Cyclosorus falcilobus (Hook.) Panigrahi.

Res. J. Pl. Environ., 9: 66. 1993. Tagawa and K. Iwats., Fl. Thailand, 3: 422. 1988; Boonkerd and Pollawatn, Pterid. Thailand, 230. 2000.

**Description:** Rhizome short, erect; scales up to 4 by 2 mm, pale brown, thin, hairy at margin. Stipes up to 30 cm long, with reduced pinnae nearly to base. Laminae oblong-lanceolate, acute at apex, up to 44.5 by 21.5 cm, 17–28 pairs of full-sized primary

pinnae; lower pinnae reduced to mere auricles, middle pinnae linear-lanceolate, gradually narrowing towards long-acuminate apex, cuneate at sessile base. Veins pinnate, veinlets simple, hairy, basal anterior ones running to callous-membrane in sinus between segments. Sori round, medial; indusia persistent, glandular.

Specimens examined: Mizoram, Champhai district, MNP, 1217 m alt., Dt. 20. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00022.

23. Cystopteris fragilis (L.) Bernhardi.

Neues J. Bot., 1(2): 27. 1805.

**Description:** Stems creeping, not cordlike, internodes short, beset with old petiole bases, hairs absent; scales tan to light brown, lanceolate, radial walls thin, luminae tan. Petiole dark at base, mostly green to straw-colored distally, shorter than or nearly equaling blade, base sparsely scaly. Blade lanceolate to narrowly elliptic, (1-2)-pinnate-pinnatifid, widest at or just below middle, apex acute; rachis and costae lacking gland-tipped hairs or bulblets; axils of pinnae lacking multicellular, gland-tipped hairs. Pinnae usually perpendicular to rachis, not curving toward blade apex, margins serrate to sharply dentate. Veins directed mostly into teeth. Indusia ovate to lanceolate, without gland-tipped hairs.

**Specimens examined:** Mizoram, Mamit district, DTR, 417 m alt., Dt. 20. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00023.

24. Deparia boryana (Willd.) M. Kato.

Bot. Mag. (Tokyo), 90: 36. 1977.

**Description:** Rhizomes creeping, ascending or suberect at apex. Fronds subcaespitose. Fertile fronds 1.2-2 m; stipe upward pale brown-stramineous, (35-)40-95 cm, up to 1 cm in diam. at base. Pinnae (10-)12-15 pairs, alternate, stalked, slightly ascending, oblonglanceolate. Veins pinnate in segment, lateral veins simple or forked. Lamina herbaceous when dried, yellow-green, with sparse short pale hairs adaxially, rachis, costae, and costules with sparse brown lanceolate small scales and scalelike vermiculate hairs. Sori small, orbicular, abaxial, medial or subcostular; indusia brown, orbicular-reniform, membranous, subentire or irregularly toothed, frequently abortive or fugacious.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1477 m alt., Dt. 10. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00024.

25. Deparia petersenii (Kunze) M. Kato.

Bot. Mag. Tokyo, 90: 37. 1977.

**Description:** Plants evergreen. Rhizome slender, creeping, dark brown, 2-5 mm in diam., apex with dense red-brown broadly lanceolate scales; fronds distant to approximate, variable, smallest ca.  $6 \times 1$  cm, large fronds up to  $1 \text{ m} \times 25$  cm; stipe usually dark brown at base, upward stramineous, 2-40(-50) cm, 1-3 mm in diam. at base, with sparse pale brown to red-brown (rarely dark castaneous). Veins hairy with many

red-brown or yellow-brown to light gray-brown, long, nodose hairs, lamina between veinlets glabrous or with pale white nodose hairs, sometimes with few, brown, lanceolate scales; adaxial side of rachis, costae, and veinlets with short pointed nodose hairs. Sori shortly linear or linear-oblong, rarely J-shaped.

Specimens examined: Mizoram, Champhai district, MNP, 1717 m alt., Dt. 30. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00025.

26. Dicranopteris linearis (Burm.F.) Underw.

Bull. Torrey Bot. Club, 34: 249. 1907; Tagawa and K. Iwats., Fl. Thailand, 3: 55. 1979; Boonkerd and Pollawatn, Pterid. Thailand, 33: 78. 2000.

**Description:** Rhizome creeping, hairy; hairs dark-brown, straight, few branched, similar hairs present on the apices of dormant buds. stipes long, brownish, +0.2 cm thick, glabrous; rachis repeatedly pseudodichotomously forked with a dormant bud between the forks, lamina ultimate branches with a pinnatifid lamia, 9-15(-18) cm long, 2.0-3.5(-7) cm broad, lanceolate, texture thin, lower surface almost glabrous, pale, glaucous; lobes many, 1.5-2.0 cm long, 0.4-0.5 cm broad; veins usually forked at least twice; slightly rised on both surfaces of the same colour as the lamina surface, glabrous; costules 0.3-0.4 cm apart. sori exindusiate, round, medial, one on each vein group, usually on an acroscopic branch, never at veins-ends. spores tetrahedral, trilete, 24-28 x 32-35 um, exine smooth.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1567 m alt., Dt. 10. 07. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00026.

27. Dicranopteris splendida (Hand. Mazz.) Tagawa.

Acta Phytotax. Geobot., 8: 164. 1939.

**Description:** Terrestrial. Rhizome dichotomously branched, near the apex protected by peltate scales, long creeping, about 4 mm in diameter, densely hairy with shining brown stiff hairs. Fronds of mature plants usually with indefinite growth in length, bearing primary branches in pairs. Stipes about 50 cm long, stramineous or brown, glabrescent. Pinnae twice forked; ultimate lobes bearing no accessory branches, narrowly oblong, 30–45 cm long, up to 17 cm wide; ultimate segments linear, round to moderately acute at apex, entire and usually flat at margin, to 10 cm long, 1 cm broad; costules 1–1.3 cm apart; veins pinnate, distinct on both surfaces, texture rigid, green, glabrous, lower surface glaucous. Sori more than one row at each side of costules.

Specimens examined: Mizoram, Aizawl district, TWS, 1657 m alt., Dt. 25. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00027.

28. Didymochlaena truncatula (Sw.) J. Sm.

J. Bot. Hooker, 4: 196. 1841-1842.

**Description:** Rhizome erect, forming a short caudex up to 25 cm in diameter; rhizome scales up to 20 mm long, pale to dark brown, subentire. Fronds tufted, erect to arching, firmly herbaceous to coriaceous, up to 2.5 m long. Stipe up to 60 cm long, straw-coloured, set with reddish-brown, twisted scales up to 1 cm long. Lamina up to  $2 \times 0.5$  m, bipinnate, oblong-elliptic in outline, with a pinnate apical segment. Pinnae alternate, spaced apart, shortly petiolate, up to  $25 \times 4$  cm, narrowly oblong, pinnate into 26 pairs of pinnules. Pinnules dimidiate, almost rectangular in outline, shortly petiolate, articulated, apex rounded to truncate, lower margin entire, upper margin irregular to toothed, hairless, deep glossy green above, paler below. Rachis and secondary rachis straw-coloured with pale brown scales. Sori 1-6 per pinnule, broadly elliptic, sometimes deeply sunk into the lamina.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1417 m alt., Dt. 24. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00028.

29. Asplenium bantamense (Blume) Baker.

Syn. Fil., 231.1867.

**Description:** Rhizome short, erect or ascending, bearing a few fronds at apex; scales narrow, up to 13 by 1.2 mm, concolorous, dark brown, minutely toothed at margin. Stipes 17.5–65 cm long, usually brown, occasionally stramineous, darker in lower portion, grooved on adaxial surface. Frond imparipinnate, ovate in outline, 19–60 by 12–

29cm; rachis grooved on upper surface, minutely hairy, often gemmiferous at junction with costa; costa raised below, grooved with minute hairs on upper surface. Veins several times forked, all free. Sori elongate along veins, longest on basal acroscopic veinlets, usually on both sides of veins; indusia thin, brown, margin entire to lacerate.

Specimens examined: Mizoram, Champhai district, MNP, 1337 m alt., Dt. 21. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00029.

# 30. Diplazium dilatatum Blume.

Enum. Pl. Javae, 194. 1828, Tagawa and K. Iwats., Fl. Thailand, 3: 464. 1988, Boonkerd and Pollawatn, Pterid. Thailand, 191. 2000.

**Description:** Rhizome massive, erect; scales to about 20 by 2 mm, more commonly narrower, dark brown, black-margined, distinctly toothed. Stipes 40–100 cm or higher, densely scaly near base, dark brown to stramineous. Lamina 65–200 by 40–100 cm, bipinnate to tripinnatifid; pinnae 8–14 pairs, narrowly oblong, acuminate at apex, veins pinnate with 5–9 pairs of simple or forked veinlets. Sori along veinlets about 5 mm long.

**Specimens examined:** Mizoram, Mamit district, DTR, 417 m alt., Dt. 30. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00030.

31. Diplazium uraiense Rosenstock.

Hedwigia, 56: 336. 1915.

**Description:** Plants evergreen, medium-sized to large. Rhizome erect, brown, ca. 1.5 cm in diam., apex like base of stipe, densely scaly; scales dark brown, linear-lanceolate, ca. 1 cm, membranous, margin black, narrow, toothed. Fronds caespitose; fertile frond up to 1.4 m; stipe brown at base, veins pinnate, visible abaxially, not prominent adaxially, veinlets 4 or 5 pairs per pinnule lobe, simple, ascending. Lamina papery, brown-green when dry, glabrous on both surfaces; rachis brown-stramineous, slightly quadrangular, glabrous, shallowly grooved adaxially; costules abaxially with sparse hairlike red-brown scales. Sori linear,  $\pm$  falcate, 2-5 pairs per lobe, inframedial from costule to 1/2 or more to margin, single or double on basal acroscopic veinlets; indusia light brown, linear, membranous, opening acroscopically, persistent.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1717 m alt., Dt. 03. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00031.

### 32. Diplazium esculentum (Retz.) Sw.

Schrad. Journ. Bot., 1: 312. 1803; Dhir, Jamir and Rao, Ferns Nagaland, 311. 1988.

**Description:** Rhizome erect, ca 4 cm thick, apex densely covered by scales; scales ca 1 x 0.1 cm, linear- lanceolate, apex long acuminate, margin finely toothed, dark-brown. Stipes ca  $30-60 \ge 0.5-1$  cm, stout, erect, sparsely scaly at base, glabrous above, purplish glands scattered throughout the stipe and rachis, dark-brown at the base, pale-brown above. Lamina ca  $100 \ge 50$  cm, bipinnate at base, simple pinnate at apex, rarely simply

pinnate, deltoid, apex acuminate, base truncate, basal pair of pinnae slightly reduced; pinnae upto seven pairs, basal one or two opposite or subopposite, others alternate; rachis and costa glabrous or pubescent beneath with numerous hairs and scales; costa slightly raised above and below, grooved above, flattered below; veins fine, forked, copiously branched with distinct costules, veins in the unlobed part of the adjacent groups joining to form an irregular excurrent vein reaching the base of sinus; texture herbaceous ; lamina dark-green, glabrous. Sori upto 8 mm long, linear, continuous along veins on both.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1447 m alt., Dt. 20. 08. 2017, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00032.

# 33. Diplazium polypodioides Blume.

Enum. Pl. Javae., 194. 1828; Bedd., Handb. Ferns Brit. India, 184, F. 89. 1883; Tagawa and K. Iwats., Fl. Thailand, 3: 465. 1988; Boonkerd and Pollawatn, Pterid. Thailand, 194. 2000.

**Description:** Rhizome massive, erect; scales narrow, about 30 by 1.8 mm, medium brown, black-margined, toothed. Stipes stout, about 1.5 cm diam. near base, up to 1 m long, densely scaly near base, surface prickly due to scars of fallen scales. Frond usually more than 1 m long, 70 cm wide, bipinnate-tripinnatifid; lower pinnae about 50 by 20 cm, acute at apex; larger pinnules oblong with acuminate apex and subtruncate base,

sessile or shortly stalked, up to 10 by 2 cm, lobed nearly to costule; lobes oblong to subquadrangular, oblique, rounded to obtuse at apex, sharply serrate, about 4 mm broad, up to 1 cm long; papyraceous, deep green, paler below; veins pinnate, veinlets 5–9 pairs, mostly forked. Sori usually close to costules, less than 2 mm long; indusia thin.

Specimens examined: Mizoram, Champhai district, MNP, 1017 m alt., Dt. 20. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00033.

34. Pseudodrynaria coronans (Wall. ex Mett.) Ching.

Sunyatsenia, 5: 262. 1940; Holttum, Dansk Bot. Ark., 23: 231. 1965

**Description:** Epiphytic or lithophytic. Rhizome creeping, thick, 1.5–3 cm diam., densely scaly throughout; scales pseudopeltate, brown, linear, (5–)10–15(–20) by 0.5–1 mm, sharply toothed at margin. Fronds sessile, 70–170 by 20–45(–60) cm, lobed almost to rachis, lobes continuing with wings less than 1 cm broad, veins raised on both surfaces, venation drynarioid, or reticulate, main areoles quadrangular, smaller areoles with free included veinlets; coriaceous, green, glabrous. Sori one, or very rarely two, row(s) between main veins, more or less elongate, or sometimes uniting longitudinally, but rarely continuous beyond cross veins. It was epiphytic and found on *Terminalia chebula* Retz.

**Specimens examined:** Mizoram, Mamit district, DTR, 417 m alt., Dt. 11. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00034.

### 35. Drynaria propinqua (Wall. ex Mett.) J. Sm.

Journ. Bot., 4: 61. 1842; Bedd. Ferns Brit. India, T. 160. 1866 Jamir and Rao, Ferns Nagaland, 131. 1988.

**Description:** Epiphytes. Rhizome creeping, ca 1 - 1.5 cm thick, branched, fleshy, densely scaly; scales ca  $0.8 \ge 0.1$  cm, lanceolate, acuminate hair- pointed apex, base peltate, margin hairy, reddish-brown. Lamina dimorphic ; nest leaves sessile, ca  $8 - 15 \ge 10 - 12$  cm, obovate to cordate-ovate, deeply pinnatifid nearly reaching the costa ; lobes ca  $6 \ge 1$  cm, lanceolate, apex blunt, faintly constricted base, margin obscurely crenate ; veins finely reticulate ; texture coriaceous, thin, lamina glabrous, glossy ; pale-green when young, but soon turning dark-brown. Sori ca 2 mm across in two rows one on either side close to the midrib of the lobes. It was epiphytic and found on *Shorea robusta* Gaertn.

Specimens examined: Mizoram, Lawngtlaii district, PNP, 1117 m alt., Dt. 03. 07. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00035.

# 36. Dryopteris cochleata (D.don) C. Chr

Index Filic., 258. 1905; Tagawa & K. Iwats. Fl. Thailand, 3: 347, 1988; Boonkerd and Pollawatn, Pterid. Thailand, 202. 2000.

**Description:** Rhizome short creeping; scales light brown, linear, entire, about 10 by 1 mm. Stipes stramineous, up to 30 cm in sterile and 50 cm in fertile fronds, densely scaly

at base, sparsely clad with linear scales. Frond bipinnate, distinctly dimorphic; sterile lamina oblong-subdeltoid, acuminate at apex, up to 45 by 30 cm; rachis glabrescent or minutely scaly, grooved above; lateral pinnae up to 10 pairs, basal pinnae the largest or slightly smaller than the next above, slightly falcate, oblong-lanceolate, caudately acuminate at apex, narrowing towards stalked base, veins pinnate, veinlets simple or forked. Sori in one row between midrib and margin; indusia large, up to 2 mm diam., very close to each other or slightly imbricate, glabrous.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1237 m alt., Dt. 30. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00036.

37. Dryopteris sparsa (D. Don) Kuntze,

Revis. Gen. Pl., 2: 813. 1891.

**Description:** Plants 50-70 cm tall. Rhizome erect or obliquely ascending, short, rhizome and stipe base with abundant lanceolate, entire, brown scales. Fronds caespitose; stipe pale castaneous-brown or dark stramineous or amber above, 20-40 cm, scales absent from top of stipe and rachis; lamina ovate-oblong or deltoid-ovate, 30-45  $\times$  15-25 cm, bipinnate to tripinnate below in large plants, not narrowed to base, apex long acuminate; pinnae 7-9 pairs, opposite or subopposite, slightly oblique, shortly stalked, basal pair largest, deltoid-lanceolate, slightly falcate. Lamina sub papery, both surfaces glabrous. Sori on middle of veinlets; indusia orbicular-reniform, entire.

Specimens examined: Mizoram, Champhai district, MNP, 1447 m alt., Dt. 10. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00037.

38. Dryopteris stenolepis (Baker) C. Chr

Index Filic., 294. 1905.

**Description:** Plants up to 1 m tall. rhizome erect or obliquely ascending, apex with dense, linear to broadly lanceolate, entire, brown scales. fronds caespitose; stipe ca. 40 cm, ca. 7 mm in diam., base gray-brown, distally pale brown, with dense lanceolate, brown or dark brown scales; lamina oblong-lanceolate, up to  $70 \times$  ca. 28 cm, once pinnate, base not shortened, apex acuminate; pinnae 30-40 pairs, lower ones opposite, upper ones alternate, narrowly lanceolate, ca.  $15 \times 2$  cm, base rounded, margin coarsely dentate or lobed, apex caudate-acuminate. lamina nearly papery, subglabrous adaxially, clothed with subulate, serrulate, brownish black scales along costa abaxially; veins pinnate, lateral ones simple, distinct on both surfaces. Sori in 3 or 4 rows on each side, nearer to costa than margin; indusia brown, membranous, readily deciduous.

Specimens examined: Mizoram, Champhai district, MNP, 1417 m alt., Dt. 10. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00038.

## 39. Egenolfia appendiculata (Willd.) L. Sm.

Ferns Brit and For., 111. 1886; Jamir and Rao, Ferns Nagaland, 372. 1988

Specimens examined: Mizoram, Lawngtlai district, PNP, 1917 m alt., Dt. 23. 07. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00039.

40. Goniophlebium lachnopus (Wall. ex Hook.) Bedd.

Ferns Brit. India, 2: 163. 1868; Rödl-Linder Blumea, 34: 393. 1990; Boonkerd and Pollawatn, Pterid. Thailan, 284. 2000.

**Description:** Rhizome long creeping, 2.5–3 mm diam., dark brown to nearly black, densely covered with scales; scales thin, ovate with long tails, about 1 mm diam. with tails about 5 mm in length, irregularly toothed at margin, light brown, shining, clathrate. Stipes stramineous, 6–7 cm long, densely scaly at base, densely hairy upwards. Laminae linear-lanceolate, round at base, acuminate at apex, 25–50 by 6–12 cm, pinnatifid to pinnatisect; rachis stramineous, densely hairy throughout, scaly beneath; lobes up to 40 pairs, the upper ones gradually becoming smaller; veins reticulate to form a single row of large costal areoles along each side of costa. Sori round, terminal on the included simple veinlets, thus in a single row along each side of costa, medial or closer to costa, up to 1.5 mm diam., superficial. It was epiphytic and found on *Ficus hirta* Vahl.

**Specimens examined:** Mizoram, Mamit district, DTR, 717 m alt., Dt. 05. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00040.

### 41. Lepisorus contortus (Christ) Ching.

Bull. Fan Mem. Inst. Biol., 4: 90. 1933; Tagawa and K. Iwats., Fl. Thailand, 3: 513, F.51.3. 1989; Boonkerd and Pollawatn, Pterid. Thailand, 273. 2000.

**Description:** Rhizome creeping, about 2.5 mm diam., bearing fronds less than 0.5 cm apart, densely scaly throughout; scales dark brown, slightly clathrate, minutely toothed at margin, oblong-subdeltoid, gradually narrowing towards attenuate apex, up to 2 by 0.7 mm. Stipes very short, indistinct. Laminae linear, attenuate towards both ends, in

mature large laminae about 15 cm by 0.7 cm, the margin more or less recurved; coriaceous; veins hardly visible, copiously anastomosing. Sori round, medial, oblong. Spores monolete. It was epiphytic and found on *Mangifera sylvatica* Roxb.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1517 m alt., Dt. 09. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00041.

42. *Lepisorus excavatus* (Bory ex Wild) Ching.

Bull. Fan. Mem. Inst. Bio., 4: 68. 1933; Baishya and Rao, Fern and Fern-Allies Meghalaya, 64. 1982.

**Description:** Rhizome 0.3 - 0.7 cm wide, rather stout, compressed, densely covered with pale-brown scalest; scales clathrate with a slightly erose margin, narrowly lanceolate, acuminate, about 0.2 - 0.7 cm long. stipes 0.5 - 2.5 cm long, pale-brown to yellowish- green, articulate to rhizome, glabrous or slightly stramineous; lamina 10 - 50 x 1.5 - 3 cm, ovate-lanceolate, gradually decurrent at the stipe, narrowly lanceolate-acute at apex; margin entire, broadest somewhat below the middle; texture thin, membranous, pale-brown to greenish when dry, sparsely small scaly on the lower surface along the costal; scales yellowish-brown, thickened in the centre, deciduoud; veins prominent. Sori 0.2 - 0.5 cm wide, closer to the midrib than to the margins, deeply sunk, forming pustules on the dorsal surface of the lamina. It was epiphytic and found on *Bischofia javanica* Blume

Specimens examined: Mizoram, Champhai district, MNP, 1317 m alt., Dt. 20. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00042.

43. Lepisorus nudus (Hook.) Ching.

Bull. Fan Mem. Inst. Biol., 4: 83. 1933; Tagawa and K. Iwats., Fl. Thailand, 3: 512, F.51.4. 1989; Boonkerd and Pollawatn, Pterid. Thailand, 274. 2000.

**Description:** Rhizome long creeping, 1.5–2 mm diam., green on surface, bearing fronds rather remotely, scaly throughout; scales ovate-oblong with gradually narrowing attenuate apex, up to 4 by 1 mm, concolorously light brown, clathrate, entire. Stipes 2–7 cm long, stramineous, castaneous or dark, winged on the upper part, scaly at base. Laminae linear, broadest usually at middle portion, linear-lanceolate, gradually narrowing towards both long-attenuate ends, up to 32 by 2.4 cm, entire or a little revolute at margin; coriaceous, minutely and sparsely scaly underneath. Sori medial, round or oblong or a little obliquely elongate, up to 4.5 mm broad, more or less raised, hollowing on upper surface. Spores monolete. It was epiphytic and found on *Artocarpus lakoocha* Roxb.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1877 m alt., Dt. 26. 07. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00043.

44. Lepisorus oligolepidus (Baker) ching

Bull. Fan Mem. Inst. Biol., 4: 80.1933.

**Description:** Rhizome creeping, thick, dia. 0.5 – 0.8 cm, densely scaly; scales darkbrown, bicolorous (central broad region with blackish-brown cell walls, luminae opaque, very narrow, not distinct, thick-walled, narrowly linear subulate, base broad, margin toothed with long teeth like projections, apex acuminate stipes short, 0.5-2.0 cm long. lamina simple, less than 12.0 cm long, 2.0 cm broad, broadest shortly below the middle, lanceolate, base gradually attenuated and decurrent on stipe, apex acuminate, margin entire, texture coriaceous, lower surface scaly, scales black, ovate-lanceolate, margin toothed, apex long acuminate, deciduous; veins abscure, anastomosing to form 4-5 areolae between margin and rachis, areolae with free simple or forked included veinlets. Sori round, large (dia. 0.5-0.7 cm). Spores light-yellow. It was epiphytic and found on Dipterocarpus *turbinatus* C.F. Gaertn

**Specimens examined:** Mizoram, Mamiti district, DTR, 417 m alt., Dt. 20. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00044.

# 45. Lepisorus thunbergianus (Kaulf) Ching.

Bull. Fan Mem. Inst. Biol., 4: 88. 1933.

**Description:** Plants 8-20 cm tall. Rhizomes creeping, 1.5-2.5 mm in diam., densely scaly when young, later naked; scales brown, lanceolate,  $2-4 \times 0.4-1$  mm wide, margin usually denticulate, opaque except for marginal 1 or 2 rows of transparent lumina. Fronds 0.5-2 cm apart; stipe straw-colored, veinlets obscure. Sori restricted to distal 1/2

of lamina, orbicular or elliptic, 1.5-3 mm in diam., nearly confluent after maturity; paraphyses brown, orbicular, 0.3-0.5 mm in diam., lumina small, central ones thickened, opaque or transparent. Spore surface with large and shallowly reticulate ornamentation. It was epiphytic and found on *Betula cylindrostachys* Wall. ex Diels

Specimens examined: Mizoram, Champhai district, MNP, 1657 m alt., Dt. 22. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00045.

46. Leucostegia immersa (Wall.ex) C. Presl.

Tent. Pterid., 95, T. 4. F. 11. 1836; Bedd., Handb. Ferns Brit. India, 51.1883; Boonkerd and Pollawatn, Pterid. Thailand, 172, 173, 236. 2000.

**Description:** Rhizome long creeping, bearing fronds remotely; hairs rather dense, golden-yellow, multicellular, wooly; scales narrowly lanceolate, up to 4 by 0.4 mm, light brown, membranous, entire at margin. Stipes stramineous or brownish on lower surface, scaly at base, glabrescent upwards, up to 39 cm long. Laminae oblong, acuminate at apex, quadripinnatifid, up to 40 by 25 cm; pinnae more than 10 pairs, pinnules oblong to subdeltoid on stalks in larger ones, secondary pinnules oblong or narrower, with 1–6 segments. Sori terminal on veinlets, one to each segment; indusia circular, attached only by base, entire, 1.3–2 mm broad, white to pale brown, glabrous. It was epiphytic and was found on *Callicarpa arborea* Roxb.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1317 m alt., Dt. 01. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00046.

47. Leucostegia truncata (D. Don) Fraser-Jenk.

Taxon. Revis. Indian Subcontinental Pteridophytes, 348. 2008.

**Description:** Rhizome long creeping, bearing fronds remotely; hairs rather dense, golden-yellow, multicellular, wooly; scales narrowly lanceolate, up to 4 by 0.4 mm, light brown, membranous, entire at margin. Stipes stramineous or brownish on lower surface, scaly at base, glabrescent upwards, up to 39 cm long. Laminae oblong, acuminate at apex, quadripinnatifid, pinnae more than 9 pairs, pinnules oblong to subdeltoid on stalks in larger ones, secondary pinnules oblong or narrower, with 1–6 segments; ultimate segments circular to oblong or terminal ones spathulate, coarsely dentate at margin; thin herbaceous, light green, glabrous. Sori terminal on veinlets. It was epiphytic and found on *Garuga pinnata* Roxb.

Specimens examined: Mizoram, Champhai district, MNP, 1217 m alt., Dt. 10. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00047.

# 48. Lindsaea ensifolia Sw.

Schrad. J. Bot., 1800(2): 77. 1801; Tagawa and K. Iwats., Fl. Thailand, 3: 131. 1985; Boonkerd and Pollawatn, Pterid. Thailand, 92. 2000 **Description:** Rhizome creeping, 3–5 mm diam., bearing fronds close together or up to 2 cm apart, brown to darker, scaly at least apically; scales linear, up to 2.5 mm long, 0.3 mm broad, brown, slightly shining. Stipes stramineous or castaneous at least at base. Laminae simply pinnate or rarely simple, ovate to oblong-lanceolate in outline, narrowly ovate with acuminate apex when simple, veins anastomosing forming 2–4 rows of areoles at each side of costa, distinct beneath. Sori continuous along margin; indusia firm, nearly reaching the edges.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1417 m alt., Dt. 03. 07. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00048.

49. Lindsaea lobata Poir.

Encycl., Suppl., 3(2): 448. 1814.

**Description:** Rhizome creeping. Stipes stramineous at base. Laminae simply pinnate or rarely simple, ovate to oblong-lanceolate in outline, narrowly ovate with acuminate apex when simple, lateral pinnae 3–6 pairs, linear-lanceolate, caudately acuminate at apex, cuneate, rounded or subtruncate at base, very shortly stalked, entire at margin, up to 20 cm long, 2 cm broad, rather variable, smaller ones about 5 mm broad; terminal pinnae like lateral ones, subcoriaceous; veins

anastomosing forming 2–4 rows of areoles at each side of costa, distinct beneath. Sori continuous along margin.

**Specimens examined:** Mizoram, Mamit district, DTR, 817 m alt., Dt. 04. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00049.

50. *Loxogramme involuta* (D. Don) C. Presl.

Tent. Pterid., 215. 1836; Bedd., Handb. Ferns Brit. India, 393, F. 228. 1883; Tagawa and K. Iwats., Fl. Thailand, 3: 577. 1989; Boonkerd and Pollawatn, Pterid. Thailand, 278. 2000.

**Description:** Rhizome short, ascending to creeping, about 1.5 mm diam., bearing a tuft of fronds near apex, densely covered with scales or dark on older portion; scales subdeltoid with acuminate apex, up to 7 by 4 mm, thin but stiff, entire, greyish brown, clathrate. Stipes indistinct, or very short with wings. Laminae caudately long-acuminate at apex, attenuate at base and decurrent to narrow wings of stipes nearly to the base, lanceolate, up to 35 by 4 cm, deep green on upper surface, paler beneath; midrib more or less raised beneath, usually flat on upper surface, stramineous or pale green; veins all obscure, anastomosing with free included veinlets; thick and fleshy, glabrous on both surfaces. Sori linear, to form angles of about 80° to midribs, continuous from near margin to near midrib, up to 3 cm long, about 1.5 mm broad, superficial.

Specimens examined: Mizoram, Aizawl district, TWS, 1717 m alt., Dt. 20. 08. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00050.

# 51. Loxogramme porcata M. G. Price.

Amer. Fern J., 80: 6. 1990; Boonkerd and Pollawatn, Pterid. Thailand, 278. 2000.

**Description:** Rhizome short creeping, phyllopodia approximately 3 mm apart, to 3 mm high, not functionally articulate. Scales linear-lanceolate,  $5-10 \ge 0.5-1.2$  mm, dark greyish-brown, aging to nearly black, not rhizoidal, thin, flat or slightly revolute, apex long attenuate, contorted. Laminae narrowly elliptic to oblanceolate, apex gradually acuminate, evenly narrowed to base,  $10.5-61 \ge 0.8-6$  cm, coriaceous, light olive-brown to deep brown, surface slightly to deeply wrinkled abaxially, smooth adaxially. Costa adaxially prominent, to 0.5 mm high, abaxially planate to slightly raised. Primary lateral veins slightly evident adaxially, some areoles with free included simple veinlets, also a few free submarginal veinlets. Sori to 6 cm long, 2-5 mm apart, up to 4 on each side of costa overlapping.

Specimens examined: Mizoram, Champhai district, MNP, 1617 m alt., Dt. 06. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00051.

52. Lycopodiella cernua (L.) Pic. Serm.

Webbia, 23(1): 166 1968.

**Description:** Plants medium-sized to large; aerial shoots erect, up to 60 cm tall, terete, 1.5-2.5 mm in diam. at middle, glabrous, dichotomously branched with well-differentiated lateral branchlets uch branched; leaves of aerial shoots spirally arranged,

sparse, subulate to linear, ca.  $4 \times 0.3$  mm, straight or slightly involute, papery, midrib indistinct, base rounded, decurrent, sessile, margin entire, apex acuminate. Lateral branches ascending, dichotomously branched with well-differentiated lateral branchlets much branched, pubescent or glabrous; leaves of lateral branches and branchlets spirally arranged, dense, slightly bent upward, subulate to linear,  $3-5 \times ca$ . 0.4 mm, papery, longitudinally furrowed on surface, glabrous, midrib indistinct, base decurrent, sessile, margin entire, apex acuminate. Strobilus solitary, terminal on small branches, often pendulous when mature, pale yellow, shortly terete,  $3-10 \times 2-2.5$  mm, sessile; sporophyll ovate-rhombic, imbricate, ca.  $0.6 \times 0.8$  mm, margin membranous, with irregular teeth, apex acute, caudate. Sporangia enclosed.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1417 m alt., Dt. 11. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00052.

# 53. Lygodium flexuosum (L.) Sw.

Schrad. J. Bot., 1800(2): 106. 1801; Bedd., Handb. Ferns Brit. India, 457, F. 283. 1883; Tagawa and K. Iwats., Fl. Thailand, 3: 62. 1979; Boonkerd and Pollawatn, Pterid. Thailand, 82. 2000.

**Description:** Rhizome short, densely covered with dark brown hairs. Fronds climbing, usually several metres tall; stipes 50 cm or more long, stramineous with dark brown basal portion, minutely hairy or glabrescent, narrowly winged on the upper part; rachis

winged throughout, puberulous on the upper surface between the wings, stramineous; primary rachis-branches very short, up to 3 mm, the apex dormant, covered with downy pale brown hairs; secondary rachis-branches pinnate to bipinnate, oblong to subdeltoid in outline, usually with acute apex, 10–25 cm long, 7–12 cm wide, lamina below between veins with minute glandular hairs but appearing glabrous or with the glandular hairs and larger stiff hairs, veins with the larger hairs. Sporangia-bearing lobes protruding at margin of tertiary leaflets, up to 1 cm long, 1.5 mm broad; indusia glabrous.

**Specimen examined:** Mizoram, Mamit district, DTR, 817 m alt., Dt. 21. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00053.

# 54. Lygodium salicifolium C. Presl.

Suppl. Tent. Pterid., 102. 1845; Tagawa and K. Iwats., Fl. Thailand, 3: 64. 1979; Boonkerd and

Pollawatn, Pterid. Thailand, 35: 83. 2000.

**Description:** Rhizome shortly creeping, densely covered with blackish brown hairs. Fronds very large, climbing, to several. metres tall; stipes stramineous, minutely pubescent, very narrowly winged or with a distinct line at both sides; rachis like the upper part of stipes, 1.5–2.2 mm diam.; primary rachis-branches very short, up to 4 mm long, the apex dormant, covered with brown hairs; secondary rachis-branches pinnate (rarely basal branches slightly bipinnate with two small free lobes and one large terminal lobe), with about 4 pairs of leaflets and a terminal usually deeply lobed one; lamina herbaceous to soft papyraceous, fresh green, almost glabrous on both surfaces except the hairy margin; every axis higher than the secondary rachis-branches with narrow but distinct wings, pubescent throughout, somewhat swollen at every junction. Sporangia-bearing lobes protruding at margin of tertiary leaflets, 2–5(–10) mm long, about 1.2 mm broad.

Specimens examined: Mizoram, Champhai district, MNP, 1217 m alt., Dt. 07. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00054.

# 55. Macrothelypteris torresiana (Gaudich.) Ching.

Acta Phytotax. Sin., 8: 310. 1963; Boonkerdand Pollawatn, Pterid. Thailand, 224. 2000.

**Description:** Rhizome short creeping to suberect; scales narrow, brown, up to 20 by 1.5 mm, hairy on dorsal surface as well as margin, the base often a few cells thick. Stipes 35–100 cm long, about 1 cm diam. near base, terete when fresh but grooved when dry, sometimes spiny in lower part. Laminae oblong to oblong-ovate, deeply tripinnatifid, 32–90 by 15–75 cm; pinnae 12–15 pairs, oblong, acuminate at apex, 9–40 by 5–20 cm; larger pinnules sessile, oblong subdeltoid, acuminate at apex, up to 10 by 4 cm; ultimate segments oblong, oblique, rounded to moderately acute at apex, lobed to 3/4 way towards midrib; lobes oblong to subdeltoid, pinna-rachis hairy on upper surface,

costules winged throughout, hairy; veins and surfaces hairy with unicellular or multicellular hairs; texture herbaceous to softly papyraceous, green but often brownish when dried. Sori round, usually close to midrib of ultimate segments; indusia small, often covered by mature sporangia, round-reniform, with long hairs.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1227 m alt., Dt. 20. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00055.

56. *Microlepia pilosula* (Wall.) Presl.

Tent. Pterid., 125. 1836; Baishya and Rao, Ferns and Fern-allies Meghalaya, 91.1982; Jamir and Rao, Ferns Nagaland, 211. 1988

**Description:** Rhizome semi-erect, short, ca 0.5 cm, thick stout, apex covered by hairs; hairs small, pale-brown. Stipes ca  $25-40 \ge 0.3$  cm, abaxially rounded, adaxially grooved, densely wooly tomentose, dark-brown at base, pale-brown above. Lamina ca  $30-60 \ge 20-30$  cm, tripinnatifid, deltoid, apex acuminate; lateral pinnae upto 15 pairs, alternate, shortly stalked; largest pinnae ca  $15 \ge 5$  cm, oblong, apex short acuminate or acute; pinnules ca  $3 \ge 1.5$  cm, sessile, alternate, oblique, delto-lanceolate, apex rounded or subacute, margin deeply cut down to midrib at base, pinnatifid at apex; lobes ca  $0.7 \ge 0.3$  cm, ablong, apex rounded, entire or slightly crenate; veins free, forked; rachis, costae, costules and veins covered with hairs on both surfaces; texture coriaceous;

lamina pale-green. Sori superficial on veinlets, 1-2 sori on each acroscopic aubmargin of the lobes; indusia halfcup-shaped, surface sparsely hairy, margin undulating.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1117 m alt., Dt. 28. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00056.

57. *Microlepia hallbergii* C. Chr.

Index Filic Suppl. Tertium pro Annis, 3:127 1934.

**Description:** Plants terrestrial, medium-sized. Rhizome creeping, siphonostelic, covered with multicellular grayish stiff hairs, without scales. Fronds medium-sized to large, stipe base without articulation, hairy, vertically grooved above; lamina 1-4-pinnately compound, oblong to ovate-oblong; pinnules or lobes slightly oblique, acroscopic pinnule at base larger than basiscopic, usually parallel to rachis or pinna rachis, mostly triangular, rarely lanceolate, usually grayish hispid or with soft hairs, especially on rachis and pinna rachis. Veins free, pinnately branching, veinlets not reaching margin. Sori orbicular, intramarginal (slightly farther from margin), terminal on one veinlet, usually near notch; indusium hemitelioid, fixed at base and both sides, opening toward margin, truncate above, or indusium orbicular-reniform, basifixed; stalk short; annulus erect, cut off by sporangiophore at base. Spore tetrahedral, glabrous or slightly verrucate.

Specimens examined: Mizoram, Champhai district, MNP, 1417 m alt., Dt. 23. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00057

58. *Microlepia calvescens* (Wall. ex Hook.) C.Presl.

Epimel. Bot., 95. 1851; Boonkerd and Pollawatn, Pterid. Thailand, 39, 40, 86. 2000.

**Description:** Rhizome long creeping, about 4 mm diam. densely covered with bright blackish-brown hairs of 2 mm or sometimes more. Stipes 2–5 cm apart, stramineous, hairs at base like those on rhizome, minutely pubescent upwards, grooved on upper surface, 50 cm or more long; lamina oblong-lanceolate, acuminate at apex, 50–70 cm long, up to 30 cm wide, pinnate to bipinnatifid; rachis grooved on upper surface, densely pubescent throughout, stramineous or darker beneath; costa densely pubescent; veins pinnate, main veins usually zigzag. Sori terminal on veinlets, 1–1.5 mm from the margin of lobes; indusia cup-shaped, hairy prominent, adaxially obscure, lateral veins slender, pinnate, not reaching margin. Sori orbicular, near acroscopic notch at pinnule bases; indusium gray-brown, nearly reniform, membranous, occasionally hairy.

Specimens examined: Mizoram, Champhai district, MNP, 1517 m alt., Dt. 30. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00058.

59. *Microlepia rhomboidea* (Hook) C. Presl ex Prantl.

Arbeit Konigl.Bot. Gart.Breslau, 1:31.1892

**Description:** Plants terrestrial, ca. 1.4 m tall. Rhizome stout, ca. 5 mm in diam., densely long brownish acicular hairy. Fronds distant; stipe 60-70 cm, ca. 4 mm in diam., base with long brownish hairs, hairs abscising distally to leave scabrous marks and only sparse, short, brown hairs; rachis and rachillae with dense, short, brown hairs; lamina green when dried, 2- or 3-pinnate, oblong in outline, ca.  $70 \times 40$  cm, herbaceous, both surfaces and veins with sparse, long, acicular hairs, base gradually narrowed, almost half as wide as widest part, apex acuminate; pinnae ca. 15 pairs, alternate, Veins obvious on both surfaces, pinnate at lobules, veinlets furcate. Sori small, at base of lobules; indusium greenish, sparsely hairy.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1217 m alt., Dt. 05. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00059.

60. *Microlepia speluncae* (L.) T. Moore.

Index Filic, 93. 1857; Bedd., Handb. Ferns Brit. India, 67. 1883; Tagawa and K. Iwats., Fl. Thailand, 3: 118, F.7.7 and 7.8.1979; Boonkerd and Pollawatn, Pterid. Thailand, 88. 2000.

**Description:** Rhizome long creeping, almost naked in the older part, deep brown, more than 7 mm diam. Stipes stramineous or brownish, pubescent or glabrescent, 50–70 cm long; lamina large, tripinnate to quadripinnatifid, up to 70 cm long, 50 cm wide; rachis stramineous to brownish, grooved on upper surface, more or less hairy; larger pinnae

oblong-subtriangular, broadly cuneate at base, broadest at lower second or third pinna; costa grooved on upper surface, more or less hairy, upper pinnae gradually reducing in size; larger pinnules oblong-subtriangular or oblong-lanceolate, gradually narrowing towards apex, unequally cuneate at base; veins pinnate, veinlets once or twice forked, indistinct on both surfaces, variously hairy. Sori a little within the margin of lobes, small; indusia cup-shaped, hairy.

Specimens examined: Mizoram, Mamit district, DTR, 1417 m alt., Dt. 08. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00060.

61. Microlepia strigosa (Thunb.) C.Presl.

Epimel. Bot., 95. 1851; Bedd., Handb. Ferns Brit. India: 67. 1883; Tagawa and K. Iwats., Fl. Thailand, 3: 116, F. 8.2. 1979; Boonkerd and Pollawatn, Pterid. Thailand, 89. 2000.

**Description:** Rhizome long creeping, about 5 mm diam., densely covered with yellow brown setose hairs about 2 mm long. Stipes stramineous or brownish, densely pubescent especially in the grooves on upper surface or glabrescent in older ones, up to 40 cm long; lamina bipinnate, or tripinnatifid in larger fronds, 40–70 cm long, 25–35 cm wide, ovate-oblong to oblong-lanceolate, acuminate at apex; rachis like the upper part of stipes, distinctly grooved on upperside, the groove not joined to that of pinna-rachis, densely pubescent below; lateral pinnae sometimes more than 20 in pairs, a few lower

ones a little reduced or not, the upper ones gradually reducing in size, the largest ones straight, ascending, distinctly stalked, linear-subtriangular, gradually narrowing towards long-caudate acuminate apex, cuneate at base, up to 20 cm long, 4 cm wide.Sori between the crenae of lobes, submarginal; indusia rather broadly cup-shaped, small, less than 1 mm broad, hairy.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1447 m alt., Dt. 09. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00061.

62. Colysis insignis (Blume) J. Sm.

Hist. Filic, 101. 1875.

**Description:** Rhizome 2-11 mm in diam., dorsiventrally flattened or subcylindrical, not white waxy, closely attached to substrate. Scales pseudopeltate, apically densely set, otherwise sparsely set, appressed or distinctly spreading. Fronds not or slightly dimorphic. Stipe 0-10 cm, terete or carinate, lamina decurrent to its base. Lamina simple or pinnatifid, thinly herbaceous, base narrowly decrescent, stipe winged for a considerable part, margin entire, apex acute to acuminate. Veins prominent and distinct, smaller veins  $\pm$  sunken and indistinct, variously anastomosing, free veinlets simple or once forked. Sori separate, mostly irregularly scattered, sometimes forming 2-8 irregular rows between veins or some connate, elongate along veinlets, orbicular or elongate, superficial or slightly sunken.

Specimens examined: Mizoram, Champhai district, MNP, 1217 m alt., Dt. 20. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00062.

#### 63. *Microsorum membranaceum* (D. Don) Ching.

Bull. Fan Mem. Inst. Biol., 4(10): 309. 1933.

**Description:** Rhizome creeping, thick, 3-10 mm in diamter, dorsiventrally flattened or subcylindrical, not white waxy. Scales pseudopeltate, ovate or triangular,  $1.5-9 \times 1-3$  mm, margin entire, apex acute. Fronds not or slightly dimorphic. Stipe up to 15 cm, 3-5 mm in diam. Lamina simple, ovate to elliptic or narrowly so to linear, (5-) 25-110 × (1-)5-15 cm, membranous, base narrowly decrescent, stipe winged for a considerable part, margin entire, apex acuminate. Veins prominent and distinct. Sori separate, on whole surface of lamina, sometimes forming 2-8 irregular rows between veins or some connate, elongate on veinlets, orbicular or elongate, 1-2 mm in diam., or length ca. 2.5 mm, superficial or slightly sunken.

Specimens examined: Mizoram, Aizawl district, TWS, 1127 m alt., Dt. 29. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00063.

64. *Tricholepidium superficiale* (Blume) Fraser-Jenk.

Taxon. Revis. Indian Subcontinental Pteridophytes, 69. 2008.

**Description:** Rhizome very long creeping, scandent on tree, 1-5 mm diam., usually scaly throughout, sometimes naked in older sections; scales narrowly oblong-subtriangular, gradually narrowing towards apex, irregularly round at base, entire, 1-6.5 by 0.5-2.5 mm, brown, consisting of smaller cells with distinct internal walls. Stipes 10-20 cm long, winged only on the upper portion, scaly at base, green, stramineous or dark in basal portion. Laminae lanceolate, broadest at middle, gradually narrowing towards both ends, acuminate at apex, attenuate at base, entire and flat at margin, (3-)20-40(-60) by (0.5-)3-5(-6) cm; midrib distinctly raised beneath, veins more or less visible, copiously anastomosing; thin chartaceous. Sori round, punctate, at junction of veinlets, scattered on the whole of the under surface of laminae, up to 2 mm diameter. It was epiphytic and found on *Toona ciliate* (F. Muell.) Harms

Specimens examined: Mizoram, Champhai district, MNP, 1317 m alt., Dt. 30. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00064.

65. Neocheiropteris zippelii (Blume) Bosman.

Leiden Bot. Ser., 14: 123 1991.

**Description:** Rhizome shortly creeping, cylindrical, thick, 4-5 mm in diam., not white waxy. Scales  $4-6 \times 1-2$  mm, margin denticulate to dentate, central region glabrous. Fronds not or slightly dimorphic; stipe 0.8-8 cm, 0.8-3.2 mm in diam., winged for a considerable part; lamina simple, narrowly elliptic to narrowly obovate,  $40-65 \times 6-8$  cm,

herbaceous (to firmly herbaceous), dichotomously branched near margin, connecting veins 3-7 between adjacent secondary veins, catadromous, smaller veins  $\pm$  sunken and indistinct, or prominent and distinct, variously anastomosing, free veinlets simple to once or twice forked. Sori separate, in 2 irregular rows between each pair of veins (occasionally in part confluent) over surface of lamina.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1417 m alt., Dt. 20. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00065.

66. Nephrolepis auriculata Trimen.

J. Linn. Soc., Bot., 24: 152.1887.

**Description:** Plants terrestrial or epiphytic. Rhizome erect, short, dictyostelic, producing long wiry stolons and sometimes tubers giving rise to new plants. Stipe tufted; lamina pinnate; pinnae sessile, articulate to rachis, lanceolate or falcate, base usually asymmetrical, often auriculate on upper side, margin crenate. Sori orbicular, terminal on a veinlet; indusia orbicular-reniform, often with a narrow sinus or lunulate, with broad sinus. It was epiphytic and found on *Podocarpus acuminatus* de Laub.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1617 m alt., Dt. 11. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00066.

## 67. Nephrolepis cordifolia (L.) C. Presl.

Tent. Pterid., 79. 1836.

**Description:** Plants terrestrial or epiphytic. Rhizome erect, short, covered with yellowish brown, narrowly lanceolate scales; stolons bearing scaly tubers (1-1.5 cm in diam.). Stipe 5-15 cm, densely covered with same scales as on rhizome; lamina linear-lanceolate or narrowly lanceolate,  $25-75 \times 3-6$  cm, pinnate; pinnae 40-120 pairs, approximate, lanceolate,  $1.5-2.5 \times 0.6-1.2$  cm, unequal at base, margin serrulate to crenate, auricle acute, lower pinnae obtuse, gradually shorter upward; rachis with sparse, fibrillar scales. Sori lunulate or rarely orbicular-reniform; indusia brown, elongate.

Specimens examined: Mizoram, Champhai district, MNP, 1417 m alt., Dt. 26. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00067.

68. Odontosoria chinensis (L.) J. Smith.

Bot. Voy. Herald., 10: 430. 1857.

**Description:** Rhizomes shortly creeping, densely scaly; scales dark brown, ca. 2 mm, narrow, 1 or 2 cells wide at base, acicular at apex, stiff. Stipe stramineous to dark stramineous, 20-30 cm, abaxially sulcate except at base; lamina ovate-oblong to lanceolate,  $20-50 \times 5-15$  cm, firmly herbaceous to papery, 3- or 4-pinnate, widest at middle, base broadly cuneate, apex acuminate; pinnae 15-20 pairs, eins visible on

abaxial surface, forked in ultimate lobes. Sori terminal on 1 or uniting 2 or 3 vein ends; indusia basally and partially adnate laterally, denticulate to erose, rarely repand, distinctly shorter than or rarely coterminous with adaxial lamina.

Specimens examined: Mizoram, Champhai district, MNP, 1447 m alt., Dt. 14. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00068.

69. Odontosoria biflora (Kaulf) C. Chr.

Index Filic, 207. 1905.

**Description:** Rhizomes shortly creeping, densely scaly; scales dark brown, stiff, 2-3 mm, 3-6 cells wide at base, acicular at apex. Stipe stramineous or castaneous, 15-30 cm, abaxially sulcate except toward base; lamina triangular-ovate,  $10-20 \times 10-15$  cm, thickly papery to leathery, 3- or 4-pinnate, base broadly cuneate to rounded, apex acuminate; pinnae 8-10 pairs, alternate, slightly to distinctly ascending, lanceolate or narrowly triangular, 2- or 3-pinnate at base. Sori terminal on 2-4 vein ends; indusia basally and entirely adnate laterally, denticulate to erose, rarely repand, distinctly shorter than or rarely coterminous with adaxial lamina.

**Specimens examined:** Mizoram, Mamit district, DTR, 717 m alt., Dt. 18. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00069.

## 70. Odontosoria krameri Fraser-Jenk.

Taxon. Revis. Indian Subcontinental Pteridophytes, 152. 2008.

**Description:** Rhizome short creeping, 2–4 mm diam.; scales reddish brown to castaneous, acicular, entirely uniseriate, or the base often biseriate, less often the extreme base tri- or quadriseriate, to 4 mm long. Fronds clustered. Laminae usually over 20 cm long, tripinnate or more throughout; segments cuneate, suddenly spathulate-broadened at the sorus, slightly narrowed at the rounded apex, the apical margin sometimes erose, the sides often corniculate; at the base often 0.5 mm wide, slightly broadened to the apex, 1–1.25 mm wide at the sorus, of varying length; sori sometimes two together in a segment, mostly uninerval, if binerval mostly on two connivent veinends; indusium even in binerval sori with distinctly convex base. Spores mostly 55–60 mm long.

Specimens examined: Mizoram, Aizawl district, TWS, 1557 m alt., Dt. 19. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00070.

#### 71. Oleandra undulata (Willd.) Ching.

Lingnan Sci. J., 12: 565. 1933; Tagawa and K. Iwats., Fl. Thailand, 3: 180. 1985.

**Description:** Rhizome long creeping, 3–5 mm diam., bearing distant fronds, or rather closely on some portions, densely scaly throughout; scales appressed, oblong, round to moderately acute at basal edge, acuminate at apical edge, up to 7 by 1.3 mm, entire,

brown, dark near attached points, long downy hairy. Fronds simple. Stipes on tall phyllopodia 1.5–12 cm high, stramineous, hairy, up to 20 cm or more long including phyllopodium. Laminae narrowly lanceolate, gradually narrowing towards both ends, up to 30 by 4.5 cm, the margin entire but more or less undulate, herbaceous to softly papyraceous; veins once or twice forked near midribs, densely hairy beneath, glabrous to densely hairy above, glabrous to densely hairy at margin of lamina. Sori in one regular row close to costa or rather irregularly arranged near costa, dorsal on acroscopic veinlets. It was epiphytic and found on *Phoebe haineshana* Merr.

Specimens examined: Mizoram, Aizawli district, TWS, 1317 m alt., Dt. 30. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00071.

## 72. Peranema aspidioides (Blume) Mett.

Fil. Lechl., 2. 33. 1859. Tagawa and K. Iwats., Fl. Thailand, 3: 330, F. 28.3–28.7. 1988; Boonkerd and Pollawatn, Pterid. Thailand, 202. 2000.

**Description:** Rhizome short, ascending, covered with scales; scales linear-subtriangular, up to 10 by 2 mm, entire, glabrous, brown. Stipes dark stramineous to brown, deep brown on abaxial surface, polished, up to 40 cm long, scaly throughout. Laminae oblong-subdeltoid, about as long as stipe, tripinnate to quadripinnate; rachis minutely scaly throughout; pinnae more than 10 pairs, basal ones the largest, up to 20 by 12 cm; pinnae papyraceous, green to deep green, sparsely hairy on veins, hairs articulated. Sori

dorsal on veinlets, round; indusia glabrous, green when young, turning dark brown to black with age.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1497 m alt., Dt. 23. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00072.

#### 73. *Plagiogyria pycnophylla* (Kunze) Mett.

Farngatt. II: 272, T. 4, F. 22. 1858; Bedd., Handb. Ferns Brit. India, 129. 1883.

**Description:** Rhizome short, erect or ascending, bearing a tuft of fronds, naked. Sterile fronds: stipes 23–30 cm or more long, dark brown, laminae oblong-lanceolate, 69–80 cm long, c.30 cm wide; pinnae 30–40 in pairs, sessile, middle ones patent, straight, lanceolate, gradually narrowing towards attenuate apex, round to broadly cuneate at base, distinctly toothed at margin, to 15 cm long, 1.5 cm broad, lower pinnae a little reduced in size, reflexed, widely spaced, falcate upper ones gradually becoming smaller; texture subcoriaceous, deep green, veins forked, all free, each veinlet ending at apex of marginal tooth.Sporangia along veins, covering the whole under surface of fertile pinnae except for the midribs and thin edges, protected when young by the reflexed edges.

Specimens examined: Mizoram, Champhai district, MNP, 1177 m alt., Dt. 20. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00073.

74. Polypodiodes amoena (Wall ex Met) Ching.

Acta Phytotax. Sin., 16(4): 27. 1978.

**Description:** Rhizome 5-7 mm in diam., densely covered with scales; scales darkbrown, lanceolate, base broad, margin denticulate, apex acuminate. Stipe straw-colored or castaneous, (5-)30-40 cm, glabrous. Lamina pinnatisect, ovate-lanceolate in outline,  $(11-)40-50 \times (4-)20-25$  cm, base slightly narrowed, apex acuminate. Veins forming 1 or 2 rows of areoles along each side of rachis and costa. Lamina thickly papery, green, both surfaces glabrous or pubescent, abaxial surface scaly. Sori orbicular, medial.

**Specimens examined:** Mizoram, Mamit district, DTR, 917 m alt., Dt. 10. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00074.

75. Polypodiodes lachnopus (Wall ex Hook) Ching.

Acta Phytotax. Sin., 16(4): 27. 1978.

**Description:** Rhizome long creeping, 5-6 mm in diam., densely covered with scales; scales broad and brown at base with ciliate margin, upper part black, subulate with entire margin. Fronds remote. Stipe straw-colored, 5-8 cm, glabrous. Lamina pinnatipartite, linear-lanceolate in outline,  $40-60 \times 5-7$  cm, base cordate, apex acuminate. Veins reticulate to form 1 row of narrow areoles along each side of rachis and 1 row of large areoles along each side of costa, Lamina papery or herbaceous, green, glabrous or

subglabrous except rachis covered with hairs, abaxial surface sparsely scaly. Sori orbicular, terminal on included veinlets, in 1 row on each side of costa, medial.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1517 m alt., Dt. 02. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00075.

76. Polystichum obliquum (D. Don) T. Moore.

Index Filic, 87. 1858.

**Description:** Plants evergreen. Rhizome erect, short, densely scaly apically; scales reddish brown, membranous, central part of scales hardened and bright castaneous, ovate-lanceolate, ca.  $3 \times 1$  mm, margins shortly fimbriate or with dense and short cilia, apices acuminate. Fronds 6-32 cm; stipe light stramineous, 0.5-12 cm, 0.5-1 mm in diam., sparsely scaly; scales dimorphic. Lamina 1-pinnate, green or grayish green when dry, adaxially darker. Pinnae 7-15 pairs, alternate or nearly opposite, shortly stalked, distant to contiguous, sometimes hardly imbricate, most pinnae attached at right angles with rachis or slightly angled acroscopically; frond texture thinly leathery; venation pinnate, slightly distinct abaxially, visible adaxially; lateral veins nearly reaching pinna margin. Sori small, medial on each side of midrib of pinna.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1557 m alt., Dt. 29. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00076.

#### 77. Polystichum lentum (D. Don) T. Moore.

Index Filic, 86: 95. 1858.

**Description:** Plants evergreen. Rhizome erect, short, densely covered with large scales; scales narrowly ovate or ovate-lanceolate, middle part thickened, castaneous, with broad brown margins, densely serrulate. Fronds 40-100 cm; stipe stramineous, light brown, or light purple, 10-30 cm, 1.5-3 mm in diam., base densely covered with large scales similar to rhizome scales, mixed with brown small scales. Lamina deeply or shallowly bipinnatifid or bipinnatipartite, light green abaxially, darker adaxially when dry. Pinnae 25-40 pairs, alternate or nearly opposite, veins indistinct on both surfaces, pinnate on lobes, veinlets mostly free, often forked on auricles. Sori 1-4 per lobe, up to 6 per auricle; indusia brown when mature, small, caducous.

**Specimens examined:** Mizoram, Mamit district, DTR, 817 m alt., Dt. 20. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00077.

## 78. Polystichum luctuosum (Kunze) T. Moore.

Index Filic, 95. 1858.

**Description:** Plants evergreen. Rhizome erect, densely scaly; scales dark brown, narrowly lanceolate. Fronds 30-60 cm; stipe stramineous, 16-30 cm, 2-4 mm in diam. at base; rachis without proliferous bulbils, densely scaly abaxially; scales blackish brown or brown, linear with dilated base, margins ciliate. Pinnae 20-26 pairs, alternate, attached

at right angles to rachis or slightly ascendant, frond texture thinly leathery; venation pinnate on pinnules, lateral veins often forked, abaxially slightly concave or raised, adaxially indistinct. Sori in 1 row on each side of pinnule midrib, 3-9 per pinnule; indusia present, entire.

**Specimens examined:** Mizoram, Champhai district, MNP, 1447 m alt., Dt. 25. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00078.

79. *Polystichum polyblepharum* (Roem. ex Kunze) C. Presl.

Abh. Königl. Böhm. Ges. Wiss., ser. 5, 6: 416. 1851.

**Description:** Plants perennial, terrestrial, evergreen or summer-green. Rhizome short, erect or ascending, together with basal stipe often covered with scales; scales linear to ovate, rarely with hairs. Fronds caespitose, monomorphic or rarely nearly dimorphic; stipe stramineous to purplish brown, canaliculate adaxially. Lamina linear-lanceolate, lanceolate, ovate-lanceolate, lorate, or oblong, pinnate, bipinnatifid, or bipinnate, rarely finely divided and tripinnatifid or tetrapinnatifid; frond texture thinly papery, papery, or leathery, with lanceolate, subulate, linear, or ovate microscales abaxially and sometimes also adaxially; venation pinnate, free or rarely anastomosing to form 1 or 2 rows of areoles. Sori orbicular, terminal on veins of pinnae, sometimes abaxial or nearly terminal on veins, indusiate or rarely exindusiate; indusia orbicular, peltate, membranous, entire, erose, or irregularly toothed.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1237 m alt., Dt. 09. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00079.

## 80. Polystichum semifertile (Clarke) Ching.

Lingnan Sci. J., 15: 398. 1936; Tagawa and K. Iwats., Fl. Thailand, 3: 338, F. 29.6 and 29.7. 1988; Boonkerd and Pollawatn, Pterid. Thailand, 209. 2000.

**Description:** Rhizome massive, erect; scales oblong-subtriangular, up to 25 by 8 mm, bicoloured, central portion shining castaneous brown to nearly black, the edges light brown, ferrugineous. Stipes 40–60 cm long; scales at base of two kinds, one like those on rhizome, the other smaller, linear, up to 8 mm long, toothed at margin. Laminae oblong, acuminate at apex, truncate at base, 50–70 by 25–38 cm; rachis densely covered with downy linear scales; pinnae about 20 pairs; pinna-rachis densely scaly, grooved above; pinnules falcate, acute at apex, distinctly auricled at acroscopic and dimidiate at basiscopic bases. Sori scattered on basal pinnae, from near rachis towards posterior portion, in one row, medial to costular, fertile pinnules smaller than sterile ones.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1817 m alt., Dt. 30. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00080.

# 81. Polystichum squarrosum (D. Don) Fée.

Mém. Foug., 5: 278. 1852.

**Description:** Plants evergreen. Rhizome erect, densely covered with broadly lanceolate brown scales. Fronds 50-80 cm; stipe amber, 18-42 cm, up to 1 cm in diam. at base, densely scaly; scales brown or reddish brown, ovate and linear, basal stipe scales dark brown at middle. Lamina bipinnate, narrowly ovate or narrowly oblong,  $45-62 \times 9-16$  cm, rounded-cuneate, sometimes slightly contracted at base, acuminate; rachis without proliferous bulbils, densely scaly; scales reddish brown or brown, lanceolate with ciliate margins or linear, apex twisted. Pinnae 30-40 pairs, alternate, slightly ascendant, linear-lanceolate or broadly lanceolate. Pinnules 8-18 pairs, alternate, slightly ascendant, frond texture leathery; venation pinnate on pinnules, not concave or slightly raised abaxially, slightly concave adaxially. Sori in 1 row on each side of midrib; indusia present, entire.

**Specimens examined:** Mizoram, Mamit district, DTR, 617 m alt., Dt. 20. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00081.

#### 82. Polystichum yunnanense Christ.

Notul. Syst. Paris, 1(2): 34. 1909.

**Description:** Plants evergreen. Rhizome erect or ascending, short, densely covered with linear brown scales. Fronds up to 80 cm; stipe yellowish brown, 16-39 cm, 2-4 mm in diam. at base, densely covered with linear, lanceolate and larger scales; large scales bicolorous, blackish brown at middle, shiny, ovate, ovate-lanceolate, and broadly lanceolate; rachis without proliferous bulbils, abaxially densely covered with linear,

lanceolate and larger scales; large scales bicolorous, blackish brown at middle, shiny, ovate-lanceolate or broadly lanceolate. Pinnae 14-21 pairs, alternate, attached at right angles to rachis, frond texture papery; venation pinnate, lateral veins 5-7 pairs, dichotomous, distinct. Sori 4-6 pairs per pinnule, in 1 row on each side of midrib, slightly close to midrib, terminal on veinlets.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1327 m alt., Dt. 24. 07. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00082.

83. Pronephrium lakhimpurense (Rosenstock) Holttum.

Blumea, 20: 110. 1972.

**Description:** Plants ca. 1.5 m tall. Rhizomes long creeping. Fronds remote; stipes 80-90 cm, occasionally with 1 or 2 scales at bases, dark stramineous; laminae oblong-lanceolate or ovate-oblong, 60-85 cm, 1-imparipinnate, acuminate at apices; lateral pinnae 8-12 pairs, subobliquely spreading, pinnae on proximal to middle part broadly lanceolate,  $24-32 \times 4-6$  cm, stalk ca. 2 mm, somewhat rounded at bases, entire or undulate, shortly caudate-pointed at apices; terminal pinna of similar shape as proximal ones, stalk 1.5-2 cm. Veins slender, evident abaxially, veinlets subobliquely spreading and parallel to each other, veinlets 13-17 pairs. Laminae thinly papery or herbaceous, dark brown, glabrous on both surfaces, occasionally with 1 or 2 short setae abaxially, rachises, costae, and veins with sparse short hairs. Sori orbicular, attached on middle or

above middle part of veinlets and arranged in 2 rows, occasionally confluent when mature, exindusiate.

Specimens examined: Mizoram, Champhai district, MNP, 1317 m alt., Dt. 21. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00083.

84. *Pteridium aquilinum* (L.) Kuhn.

Bot. Ost-Afrika, 3(3): 11 1879; Cat. N. Amer. Pl., Ed. 3: 17. 1909.

**Description:** Plants up to 2 m tall. Rhizome long creeping, densely castaneous hairy. Fronds erect, 0.5-1.5 m (sometimes larger in shaded forms); stipe brown, up to 40 cm, woody, basally with short brown hairs, apically glabrous; lamina 3- or 4-pinnate-pinnatifid; rachis pale brown, thinly pubescent, becoming glabrous; lobes present between 2 adjacent segments; pinnae ascending or horizontal, ovate-triangular to oblong, up to  $40 \times 15$  cm, apex acute; pinnules or segments linear to oblong, glabrous except for pinnule margins and midvein, deeply pinnatifid in larger pinnae, apex shortly bluntly or long caudate. Sori elongate, submarginal on ultimate segments; outer indusium ca. 0.5 mm wide, membranous, ciliate, inner indusium vestigial and fimbriate.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1217 m alt., Dt. 04. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00084.

## 85. Pteridium revolutum (Blume) Nakai.

Bot. Mag. Tokyo, 39: 109. 1925.

**Description:** Plants up to 1 m tall. Rhizome long creeping. Fronds subleathery when dried, margins often revolute; stipe straw-colored or brown, 35-50 cm, 5-8 mm in diam. at base, adaxially grooved, densely clothed with pallid hairs when young, glabrous when old; lamina 3-pinnate-pinnatifid, broadly triangular or ovate-triangular in outline, 30-80 x 30-50 cm, apex acuminate; pinnae 4-6 pairs, opposite, decumbent, oblong, base subtruncate, stalked (2-3 cm), apex acuminate; basal pinnae 2-pinnate-pinnatifid,slightly triangular, 20-30 x 10-15 cm, stalked (2-3 cm); pinnules to 12-18 pairs per pinna, opposite or alternate; veins prominent abaxially, grooved adaxially; rachises, costae, and costules approximate, with pallid or light brown hairs or verrucose, glabrescent.

**Specimens examined:** Mizoram, Mamit district, DTR, 817 m alt., Dt. 20. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00085.

86. Pteris arisanensis Tagawa,

Acta Phytotax. Geobo., 5: 102. 1936.

**Description:** Plants 1-1.5 m tall. Rhizome erect, short, 1.5-2 cm in diam., apex with black-brown scales. Fronds clustered (6-8 per plant); stipe basally brown, upper part straw-colored, slightly lustrous, as long as fronds, 3-4 mm in diam., glabrous; lamina 2- or 3-pinnatipartite, oblong-ovate in outline, 50-70 x 20-30 cm; costae with 6-10 mm

wide wings, prominent abaxially, straw-colored, glabrous, grooved adaxially, with spines; veins conspicuous and convex on both sides, decumbent, 2-forked at base, basiscopic vein of segment base arising from rachis, and acroscopic vein from base of costa, 2 opposite veins of pinna base arriving at margin of incision and forming a fork or triangle, or sometimes interlinked into a continuous triangular mesh, and other veins outward from mesh separate and extending to base of incision; lamina green, yellowish green, or brown-green, subleathery when dried, glabrous.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1217 m alt., Dt. 27. 07. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00086.

87. Pteris aspericaulis Wallich ex J. Agardh,

Recens. Spec. Pter., 22. 1839.

**Description:** Plants 0.3-1.5 m tall. Rhizome ascending, short, 1.5-2 cm in diam., apex densely scaly; scales blackish brown with brown margins, lustrous, linear-lanceolate, 2-7  $\times$  0.1-0.4 mm. Fronds clustered; stipe often light purple, sometimes straw-colored or green, 10-42 cm, ca. 2 mm in diam., glabrous,  $\pm$  rough, with scattered pustules, scaly at base; lamina 2- or 3-pinnatipartite, oblong-ovate in outline, 18-80  $\times$  (3-)8-25 cm; lateral pinnae (1 or)2-14 pairs, opposite or subopposite, decumbent, lanceolate, 2-18(-23) cm, sessile or basiscopically shortly stalked, costae prominent abaxially, glabrous, grooved adaxially, with short and robust spines along groove; veins conspicuous on both sides,

decumbent, 2-forked from base, lateral veins of segment arising from rachis, 2 opposite veins of base reaching incision of pinnae; lamina gray-green, often mauve at margin, subleathery when dried, glabrous (abaxially glabrous, adaxially subglabrous when young.

Specimens examined: Mizoram, Champhai district, MNP, 1457 m alt., Dt. 20. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00087.

## 88. Pteris biaurita L.

Sp. Pl., 2: 1076. 1753; Baishya and Rao, Ferns and Fern-Allies Meghalaya, 91. 1982; Jamir and Rao, Ferns Nagaland, 211. 1988.

**Description:** Plants 70-110 cm tall. Rhizome erect, robust, 2-2.5 cm in diam., woody, apex densely clothed with brown scales. Fronds clustered; stipe light brown, apically straw-colored to pale green, slightly lustrous, 40-60 cm, 3-5 mm in diam., glabrous, scaly, rarely with a few scales, adaxially narrowly grooved; rachis straw-colored, glabrous, narrowly grooved adaxially; lamina 2- or 3-pinnatipartite, oblong-ovate in outline,  $40-55 \times 20-30$  cm; costae prominent abaxially, straw-colored, glabrous, slightly grooved adaxially, with short spines on both sides; veins slightly raised, conspicuous on both surfaces, acroscopic veinlet of lobe base and a basiscopic veinlet of upper lobe base combining to form an arcuate vein, anastomosing to form a series of narrow areoles along costules, with 5 or 6 free veinlets extending to margin at arcuate vein, and a

majority of veinlets outward from areole usually 2-forked; lamina gray-green, thickly papery when dried, glabrous. Indusia light brown, membranous, entire, and persistent.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1117 m alt., Dt. 18. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00088.

89. Pteris confusa T.G. Walker.

Kew Bulletin, 14: 329, F. 5, pl. 5(B). 1960.

**Description:** Rhizome erect, densely covered by linear lanceolate scales at the apex. Stipes upto 60 cm long, dark brown at the base, stramineous above, densely scaly at the very base, glabrous above. Lamina broadly ovate or deltoid, bipinnate; pinnae upto 15 pairs, ascending, opposite, sub-sessile; pinnules upto 25 pairs, linear oblong, margin entire; pinnae dark green, small pinnule present on the junction of costa and costule. Sori all along the margin except at the apex of the pinnule, dark brown; indusia rigid, pale brown; spores dark brown.

**Specimens examined:** Mizoram, Mamit district, DTR, 617 m alt., Dt. 21. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00089.

## 90. Pteris cretica L.

Mant. Pl., 130. 1767; Bedd., Handb. Ferns Brit. India, 106. 1883; Boonkerd and Pollawatn, Pterid. Thailand, 127. 2000.

**Description:** Rhizome short creeping or ascending, bearing closely spaced fronds, scaly at apex; scales brown, up to 5 mm long, entire. Stipes stramineous to castaneous or deep purple, nearly black and sparsely hairy at base, puberulous upwards, usually 10–30 cm or sometimes more than 50 cm long, those of fertile frond longer. Laminae more or less dimorphic, imparipinnate, 15–40 by 6–35 cm; lateral pinnae up to 7 pairs, narrowing towards base, caudately long-acuminate at apex, serrate at margin, sessile or shortly stalked at base, up to 23 by 2 cm in sterile and 1.2 cm in fertile ones, but commonly about 12 cm long, papyraceous to subcoriaceous, light green; veins ascending, forked, all free. Sori along the margin of pinnae; indusia firm, brown.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1917 m alt., Dt. 05. 08. 2015,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00090.

## 91. Pteris linearis Poir.

Encycl., 5(1): 723. 1804. Baishya and Rao, Ferns and Fern-allies Meghalaya, 91. 1982.

**Description:** Rizome erect; rhizome scales linear-lanceolate in outline,  $\pm$  5 mm long, with dark shiny centre stripe and pale margins. Fronds tufted, 1–2 m tall. Stipe straw-coloured, dull pale brown to reddish and with scattered scales near base, 50–80 cm long. Lamina  $\pm$  oblong in outline, 60–70 × 35 cm, pinnate with lower pinnae branched near base; upper pinnae in 6–15 pairs, narrowly oblong in outline, Sori up to 3 cm long,

occupying up to 5/6th of length of segments.Pinnate lamina with branched lower pinnae near base, anastomising veins and rounded entire apices of pinna lobes.

Specimens examined: Mizoram, Champhai district, MNP, 1437 m alt., Dt. 20. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00091.

#### 92. Pteris quadriaurita Retz.

Obs. Bot., 6. 38. 1791; Bedd. Ferns South. India, T. 31. 1864; Handb. Ferns Brit. India, 110. 1883; Baishya and Rao, Ferns and Fern-Allies Meghalaya, 111. 1982; Jamir and Rao, Ferns Nagaland, 152. 1988; Manickam and Irudayaraj, Pterid. Fl. West. Ghats-S. India, 79. T. 55. 1992.

**Description:** Rhizome erect or suberect, short, ca 2.5 cm thick, scaly at the apex; scales ca 4 x 0.5 mm, linear-lanceolate, apex acuminate, membranaceous at periphery, thick at the centre, dark-brown, margin hairy. Stipes ca  $30-50 \times 0.7$  cm, glabrous, glossy above, erect, ridged, green in living, pale-dark, to purplish when dry. Lamina ca 75 x 30 cm, deltoid or broadly ovate, bipinnatifid, with an apical pinna, basal pinnae simple or forked once at the base; pinnae ca  $20 - 35 \times 4 - 7$  cm, opposite or subopposite, lanceolate, with caudate, acuminate apex, base broadly cuneate; margin entire; texture subcoriaceous, pinnae dark-green; upper surface of costae and costules with spinules; veins distinct below, forked once, free. Sori blackish-brown, generally partial on the margins of the segments, rarely upto the apex.

Specimens examined: Mizoram, Aizawl district, TWS, 1327 m alt., Dt. 12. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00092.

#### 93. Pteris vittata L.

Sp. Pl., 1074. 1753; Tagawa and K. Iwats., Fl. Thailand, 3: 233. 1985; Boonkerd and Pollawatn, Pterid. Thailand, 132. 2000. Jamir and Rao, Ferns Nagaland, 152. 1988.

**Description:** Rhizome short, ascending, bearing a tuft of fronds, scaly; scales light brown, narrow, up to 5 mm long. Stipes up to 20 cm long, densely scaly on lower part, stramineous. Laminae imparipinnate, oblanceolate, widest at upper portion; pinnae simple, lower ones gradually becoming smaller downwards to mere auricles, middle or upper ones linear, nearly straight, up to 15 cm by 8–12 mm, sessile and cordate at base, caudately long-acuminate at apex, serrate at non-soriferous margin; terminal pinnae usually much longer, up to 20 cm or more long, about 1 cm broad; rachis grooved on upper surface, minutely scaly; veins forked, free except when connected by soral commissure. Sori marginal, continuous along margin of pinnae; indusia thin, pale.

Specimens examined: Mizoram, Mamit district, DTR, 1417 m alt., Dt. 05. 08. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00093.

## 94. *Pyrrosia lanceolata* (L.) Farw.

Amer. Midl. Natur., 12: 245. 1931; Hovenkamp, Leiden Bot. Ser. 9: 191, F. 25. 1986; Tagawa & K. Iwats., Fl. Thailand, 3: 500. 1989; Hovenkamp, Fl. Males., Ser. II, Ferns and Fern Allies 3: 161. 1998; Boonkerd and Pollawatn, Pterid. Thailand, 286. 2000; Jamir and Rao, Ferns Nagaland, 152. 1988.

**Description:** Rhizome long creeping, about 1–2 mm diam., bearing fronds 1–5 cm apart, dark brown, densely scaly throughout; scales oblong-subdeltoid to lanceolate, gradually narrowing towards apex, 1–8 by 0.5–1.2 mm, brown in central portion, paler towards margin, hairy at margin, appressed to spreading, imbricate. Fronds simple. Stipes 0-4(-9) cm long, scaly at base, sparsely to densely stellate hairy. Fronds slightly to distinctly dimorphic, when dimorphic fertile fronds longer. Laminae linear-lanceolate, gradually narrowing towards both apex and base, widest below, around or above the middle, 3.5-20(-31) by 0.5-2.5 cm, base attenuate to cuneate, apex rounded, obtuse or acute; midrib distinct on both surfaces, grooved on upper surface, veins visible or not, anastomosing, with free included veinlets; coriaceous, thick, both surfaces sparsely to densely covered with stellate hairs or becoming glabrescent above. Sporangia covering the whole lower surface of apical half, becoming narrow in soriferous portion; sori round, covered with dense stellate hairs when young, hairs monomorphic. It was epiphytic and found on *Bridelia squamosa* (Lam.) Gehrm.

Specimens examined: Mizoram, Champhai district, MNP, 1317 m alt., Dt. 26. 07. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00094.

## 95. Pyrrosia flocculosa (D. Don) Ching

Bull. Chin. Bot. Soc., 1: 70. 1935; Jamir and Rao, Ferns Nagaland, 152. 1988.

**Description:** Plants 10-20 cm tall. Rhizome long creeping. Scales peltate,  $3.9-7.8 \times 0.3$ -1.3 mm, base entire to ciliate; acumen light brown, often with a distinct hyaline margin, ciliate; short, orbicular to ovate scales usually present. Fronds subdimorphic. Stipes 1-4 cm; lamina widest below or at middle,  $10-25 \times 1-1.8$  cm, base attenuate, decurrent, apex long caudate. Fertile fronds narrower. Indument monomorphic, sparse, whitish to brown; hairs 0.2-1.2 mm with erect-spreading to appressed, boat-shaped to acicular rays. Sori sunken, with a distinct central bundle of stellate paraphyses. It was epiphytic and found on *Syzygium ramosissimum* (Blume)N.P. Balakr

Specimens examined: Mizoram, Aizawl district, TWS, 1218 m alt., Dt. 22. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00095.

## 96. Selaginella bisulcata Spring.

Mém. Acad. Roy. Sci. Belgique, 24: 259. 1850.

**Description:** Plants terrestrial, evergreen, creeping, 20-35 cm. Rhizophores at intervals throughout length of main stem, borne on ventral side in axils of branches. Main stems branched from near base upward, pinnately branched, stramineous, main stem 1.2-1.8 mm. Strobili solitary, terminal, compact, dorsiventrally complanate,  $6-10 \times 3.5-5.5$  mm; sporophylls unlike sterile leaves, strongly dimorphic (very basal sporophylls on ventral

side similar to lateral sterile leaves), resupinate, not obviously white-margined; dorsal sporophylls oblong-lanceolate, carinate, margin ciliolate, apex acuminate or aristate, with sporophyll-pteryx incomplete and ciliolate; ventral sporophylls ovate-lanceolate or oblong-ovate, carinate or not carinate, base dilated, margin ciliolate or lacerate-ciliolate; megasporophylls in basal portion on lower side of strobilus, or megasporophylls and microsporophylls at intervals.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1517 m alt., Dt. 30. 08. 2016,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00096.0

# 97. Selaginella involvens (Swartz) Spring.

Bull. Acad. Roy. Sci. Bruxelles, 10: 136. 1843; Jamir and Rao, Ferns Nagaland, 152. 1988.

**Description:** Green, erect, 15-35 (-65) cm, with creeping sybterranean rhizome and stolons; leaves on rhizome and stolons scalelike, pale yellow. Rhizophores restricted to creeping rhizomes and stolons. Main stems branched from middle upward, pinnately branched, stramineous, unbranched main stem 5 - 25 cm tall, 1 - 1.5. Dorsal leaves imbricate, ovate-triangular or ovate- elliptic,  $0.6 - 1.2 \ge 0.2 - 0.5$  mm, slightly carinate, base cuneate, margin denticulate, apex long acuminate to shortly aristate, parallel to axis. Ventral leaves contiguous or overlapping, slightly ascending, ovate to triangular,  $1.4 - 2.4 \ge 0.4 - 1.4$  mm, apex subacute or apiculate; basiscopic base rounded, margin

entire; acroscopic base enlarged, broader, overlapping stem and branches, margin hyaline, denticulate. Strobili solitary, terminal, compactmicrosporangia elliptic, rather thin, cells regular; microspores yellowish orange, megaspores whitish or brown, with equatorial flange.

Specimens examined: Mizoram, Champhai district, MNP, 1617 m alt., Dt. 10. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00097.

98. Sphaeropteris brunoniana (Wall ex Hooker) R. M. Tryon.

Contr. Gray Herb., 200: 21. 1970.

**Description:** Trunk erect, up to 10-20 m tall, up to 20 cm in diameter. Stipe and rachis yellowish to purplish, smooth, or finely warty at base; scales pale brown or brown, thin, with setiferous edges; lamina 2-pinnate-pinnatifid, 2-3  $\times$  to 1.6 m; pinnae 20-30 pairs, ascending, lanceolate; largest pinnae up to 90  $\times$  25 cm; pinnules narrowly lanceolate, 9-14  $\times$  2-3 cm, slightly narrowed at base, apex caudate, pinnatifid to pinnatisect; pinnule segments 16-25 pairs, falcate, 10-16  $\times$  3-5 mm, wider at base, entire or minutely crenate, rarely with small segments; veins 2- or 3-forked; abaxial side of pinnules glabrous, adaxial side glabrous or with sparse hairs; lamina glaucous abaxially; adaxial side of pinna rachis with pale antrorse hairs, a few pale hairs and scales along costules abaxially. Sori close to midveins of fertile pinnule segments, often throughout lower

lamina; paraphyses pale to brown, filamentous, longer than sporangia or equal in length; indusia absent.

**Specimens examined:** Mizoram, Mamiti district, DTR, 717 m alt., Dt. 10. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00098.

99. Tectaria coadunata (J. Smith) C. Chr.

Contr. U.S. Natl. Herb., 26: 331. 1931.

**Description:** Plants terrestrial, 30-100 cm tall. Rhizome shortly creeping or erect, thick, densely scaly at apex and stipe bases; scales stiff, dark brown with a light margin, lanceolate, 6-7 mm, entire. Fronds clustered; stipe stramineous to pale castaneous, glossy, 20-40 cm, glabrescent above. Lamina pinnatifid to quadripinnatifid, light green to brown when dried, secondary pinnules falcate-lanceolate, bases crenate or pinnatifid, apices rounded; segments falcate-lanceolate, entire, obtuse. Veins copiously anastomosing, with included free veinlets. Sori orbicular, at apex of included veinlets, in a single row on each side of midrib of ultimate lobes, medial indusiate; indusia rather large, brown, entire, clypeate, membranous, glabrous or hairy.

Specimens examined: Mizoram, Lawngtlai district, PNP, 1417 m alt., Dt. 11. 08. 2014,R. Vanlalpeka, Accession Number: MZU/Bot/Pt-00099.

100. Thelypteris clarkei (Bedd.) Ching.

Bull. Fan. Mem. Inst. Biol. Bot., 8: 178. 1938.; Fraser-Jenk., New Sp. Syndr. Indian Pteridol., 259. 1997; Tagawa and K. Iwats., Fl. Thailand 3: 423. 1988; Holttum, Kew Bull, 31: 308. 1976.

**Description:** Rhizome creeping, about 5 mm diam.; scales narrow, up to 7 by 1 mm, brown, hairy. Stipes up to 70 cm long, hairy throughout, scaly and dark at base. Laminae oblong-lanceolate, acute at apex, up to 48 by 28 cm, rachis and costa rather densely hairy throughout; segments oblong, oblique, moderately acute to rounded at apex, entire, up to 7 by 3 mm; venation pinnate, veinlets simple, up to 18 pairs; texture thinly papyraceous, pale green, densely hairy with setose unicellular hairs, glandular on lower surface; glands orange to red, round to clavate, sessile, rather dense.Sori medial; indusia small, persistent, shortly hairy.

Specimens examined: Mizoram, Aizawl district, TWS, 1417 m alt., Dt. 09. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000100.

101. Thelypteris nudata (Roxb.) C.V. Morton.

Contrib.U.S. Natl. Herb., 38(7):352. 1974; B.K. Nayar and S. Kaur, Companion Beddome's Handb. Ferns Brit. India, 66, 69. 1974; Holttum, Dansk Bot. Ark., 20: 23. 1961.

**Description:** Rhizome creeping, about 5 mm diam.; scales caducous, dark brown, hairy. Stipes 35-80 cm long, scaly at base. Laminae oblong, 50 – 100 by 25 – 50 cm; lateral pinnae lanceolate, sessile, ascending, gradually narrowing towards long-acuminate apex, terminal pinna like lateral ones, rounded to subtruncate at base; marginal lobes acute at apex, with cartilaginous margin; chartaceous, green, verrucose on lower surface; venation meniscioid. Sori rather close to excurrent veinlets or medial in two rows between costules; indusia setose; sporangia glabrous.

**Specimens examined:** Mizoram, Mamit district, DTR, 917 m alt., Dt. 20. 07. 2015, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000101

102. Thelypteris procera (D. Don) Fraser-Jenk.

Taxon. Revis. Indian Subcontinental Pteridophytes, 183: 2008.

**Description:** Plants 60-120 cm tall. Rhizomes long creeping, including stipe bases with brown lanceolate scales. Fronds distant; stipes 20-40 cm, stramineous; veinlets 10-15 pairs, basal pair anastomosing. Laminae herbaceous, yellowish green when dried, with sparse long pale acicular hairs along abaxial surface of rachises and both sides of veins, and minute yellow glands along veins abaxially, glabrous between veins on both surfaces. Sori orbicular, submarginal; indusia very small, glabrous or with several long acicular hairs. Sporangia bearing small orange glands on stalks. Spores variously winged, tuberculate, or echinate.

Specimens examined: Mizoram, Champhai district, MNP, 1317 m alt., Dt. 28. 08. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000102.

103. Thelypteris siamensis Tagawa and K. Iwats.

Acta Phytotax. Geobot., 22: 101, F. 5. 1967. 1967.

**Description:** Plants 70-100 cm tall. Rhizomes erect, apices along with stipe bases with dense linear-lanceolate brown scales. Fronds clustered; stipes 20-40 cm, stramineous, subglabrous or with sparse pale long acicular hairs distally; laminae  $50-70 \times 30-40$  cm, bases not narrowed or slightly so. Laminae herbaceous, yellowish green when dried, with pale long acicular hairs along rachises, costae, and veins on both surfaces. Sori orbicular, medial; indusia with dense pale acicular hairs. Sporangia bearing golden glands on stalks. Spores echinate or tuberculate.

**Specimens examined:** Mizoram, Aizawl district, TWS, 1517 m alt., Dt. 30. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000103.

104. Tricholepidium normale (D. Don) Ching.Acta Phytotax. Geobot., 29: 43. 1978.

**Description:** Rhizome thick, creeping; scales adpressed, brown or pale brown, orbicular, with a tuft of hairs at middle on adaxial side. Fronds close or distant; stipe thick, short or frond subsessile, scaly at base; lamina green or yellow-green, lanceolate to loriform,  $35-60 \times 2-4$  cm, widest at middle, herbaceous or thickly papery, glabrous,

margin entire or undulate; midrib prominent, veins forming 2 or 3 irregular areoles on each side of midrib, distinct, free included veinlets ending with hydathodes, veinlets at margin of laminae free. Sori orbicular, superficial, or slightly sunken, in 1-3 irregular rows on each side of midrib; paraphyses peltate, clathrate.

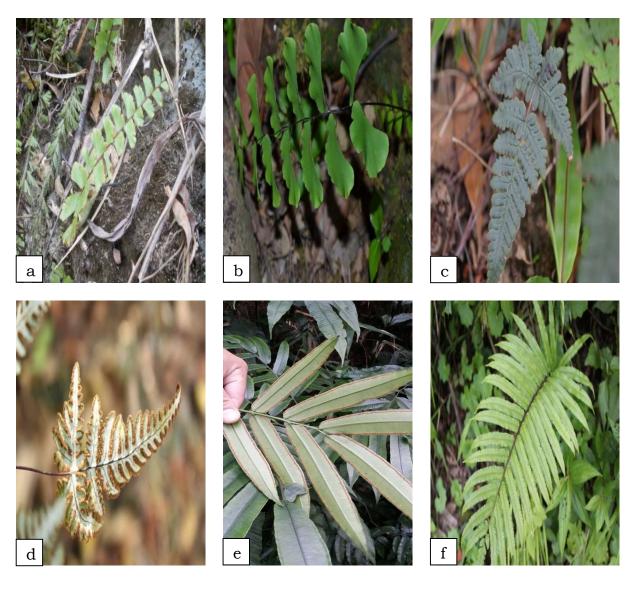
Specimens examined: Mizoram, Champhai district, MNP, 1117 m alt., Dt. 14. 08. 2014, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000104.

## 105. Vittaria fluexuosa Fee.

Mem. Fem. Foug., 3: 16. 1851; Hooker Sp. Fil., 5: 178.1864; Clarke, Trans. Linn. Soc. Bot., 1: 574. 1880.

**Description:** Rhizome short-creeping, dia. 0.3-0.4 cm scaly; scales brown, bicolorous, dark in the centre paler at the margin, narrow, linear- lanceolate, apex acuminate. Stipes short, up to 6 cm long, thick, dia. 0.1-0.2 cm, glabrous but scaly and fibrillose towards the base; rachis prominently raised, glabrous. Lamina simple, up to 30 cm or so long, 0.5 cm broad, linear, the basal part gradually narrowed and decurrent on the stipe, margin often narrowly recurved, texture thick, glabrous. Sori exindusiate, located in a deep intramarginal shallow furrow with the margin of frond extended beyond the furrow. Spores light-brown. It was epiphytic and found on *Artocarpus chama* Buch. Ham.

Specimens examined: Mizoram, Mamit district, DTR, 817 m alt., Dt. 11. 07. 2016, R. Vanlalpeka, Accession Number: MZU/Bot/Pt-000105.



# Photo Plate 4:

- a. Adiantum caudatum L.
- b. Adiantum phillipense L.
- c. Aleuritopteris formosana (Hayata) Tagawa
- d. Aleuritopteris dubia (C. Hope) Ching
- e. Angiopteris helferiana C. Presl
- f. Anisocampium cuspidatum (Bedd.) Y.C. Liu, W.L. Chiou & M. Kato.



Photo Plate 5:

- a. Athyrium attenuatum (Wall. ex C.B. Clarke) Tagawa
- b. *Blechnum orientale* L.
- c. Christella dentata (Forssk.) Brownsey & Jermy
- d. Colysis insignis (Blume) J. Sm.
- e. Coniogramme fraxinea (D. Don) Fée ex Diels
- f. Cyathea chinensis Copel.



Photo Plate 6:

- a. Cyclosorus aridus (D. Don) Tagawa
- b. Cyclosorus falcilobus Panigrahi
- c. Dicranopteris linearis (Brum.F) Underw
- d. Didymochlaena truncatula (Sw.) J. Sm.
- e. Dryopteris cochleata (D. Don) C. Chr.
- f. Diplazium latifolium T. Moore

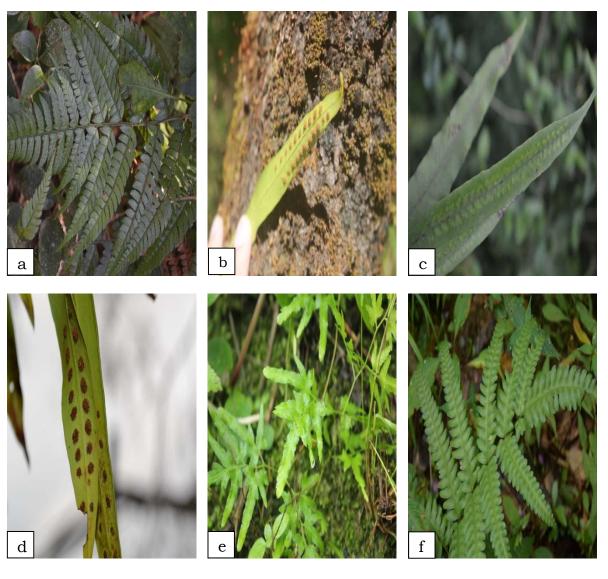


Photo Plate 7:

- a. Dryopteris stenolepis (Baker) C. Chr
- b. Lepisorus contortus (Christ) Ching
- c. Lepisorus excavatus (Bory ex Willd.) Ching
- d. Lepisorus nudus (Hook.) Ching
- e. Lygodium flexuosum (L.) Sw.
- f. Macrothelypteris torresiana (Gaudich.) Ching

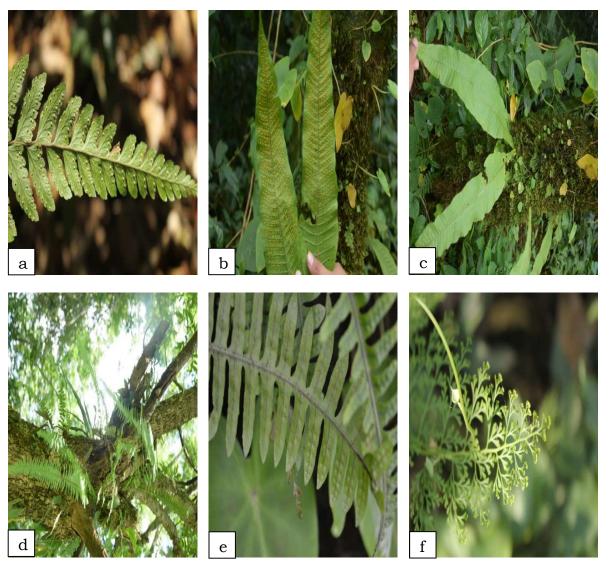


Photo Plate 8:

- a. Microlepia strigosa (Thunb.) C. Presl
- b. Microsorum membranaceum (D. Don) Ching
- c. Neocheiropteris zippelii (Blume) Bosman
- d. Nephrolepis auriculata (L.) Trimen
- e. Nephrolepis cordifolia (L.) C. Presl
- f. Odontosoria biflora (Kaulf) C. Chr



Photo Plate 9:

- a. Odontosoria chinensis (L.) J. Sm.
- b. Odontosoria krameri Freser Jenk
- c. Peranema aspidioides (Blume) Mett.
- d. Pronephrium lakhimpurense (Rosenst.) Holttum
- e. Polypodiodes amoena (Wall. ex Mett.) Ching
- f. Polystichum lentum (D. Don) T. Moore



Photo Plate 10:

- a. Thelypteris nudata (Roxb.) C.V. Morton
- b. Polystichum obliquum (D. Don) T. Moore
- c. Polystichum polyblepharum (Roem. ex Kunze) C. Presl
- d. Thelypteris clarkei C.F. Reed
- e. Polystichum semifertile (C.B. Clarke) Ching
- f. Polystichum luctuosum (Kunze) T. Moore



Photo Plate 11:

- a. Thelypteris siamensis Tagawa & K. Iwats.
- b. Polystichum squarrosum (D. Don) Fée
- c. Polystichum yunnanense Christ
- d. Pteris vittata L.
- e. Pteris arisanesis Tagawa
- f. *Pteris biaurita L.*

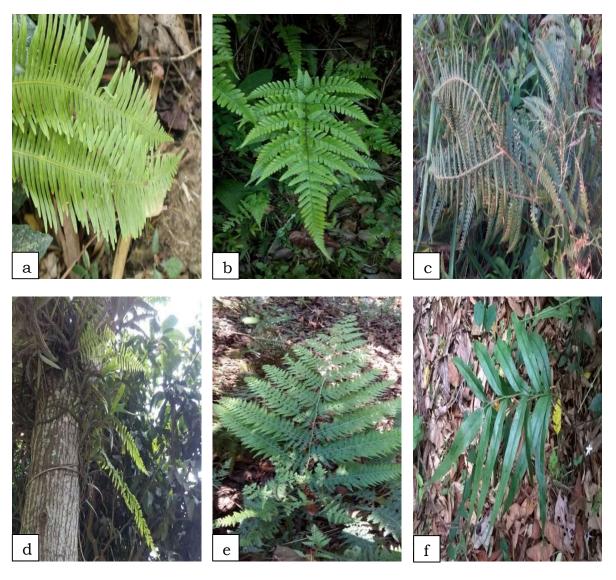


Photo Plate 12:

- a. Dicranopteris splendida (Hand. Mazz.) Ching
- b. Dryopteris sparsa (D. Don) Kuntze
- c. Pteridium revolutum (Blume) Nakai
- d. Drynaria propinqua (Wall. ex Mett.) Bedd.
- e. Microlepia speluncae (L.) T. Moore
- f. Lindsaea ensifolia Sw.



Photo Plate 13:

- a. Lygodium salicifolium C. Presl
- b. Pseudodrynaria coronans (Wall. ex Mett.) Ching
- c. Pteridium aquilinum (L.) Kuhn
- d. Lycopodiella cernua (L.) Pic. Serm.
- e. Pteris aspericaulis Wall. ex J. Agardh
- f. Pteris confusa T.G. Walker

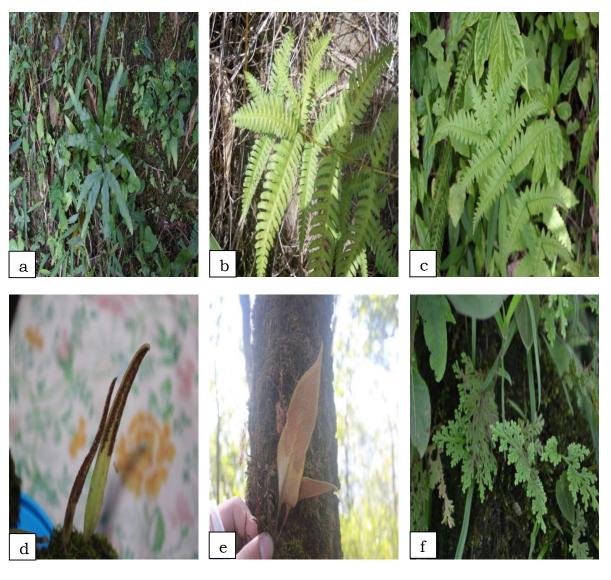


Photo Plate 14:

- a. *Pteris cretica L*.
- b. Pteris linearis Poir.
- c. Pteris quadriaurita Retz.
- d. Pyrrosia lanceolata (L.) Farw.
- e. Pyrrosia flocculosa (D. Don) Ching
- f. Selaginella involvens Swartz



Photo Plate 15:

- a. Sphaeropteris brunoniana (Wall ex Hook) R.M. Tryon
- b. Tectaria coadunata ((J. Smith) C. Chr
- c. Lepisorus oligolepidus (Baker) Ching
- d. Angiopteris evecta (G. Forst.) Hoffm
- e. Diplazium dilatatum Blume
- f. Asplenium yoshinagae Makino



Photo Plate 16:

- a. Asplenium nidus L.
- b. Cyclosorus parasiticus (L.) Farw
- c. Deparia boryana (Willd.) M. Kato
- d. Diplazium polypodioides Blume
- e. Egenolfia appendiculata (Willd.) J. Sm.
- f. Goniophlebium lachnopus (Wall ex Hook) Bedd



Photo Plate 17:

- a. Diplazium esculentum (Retz.) Sw.
- b. Leucostegia immersa Wall. ex C. Presl
- c. Loxogramme involuta (D. Don) C. Presl
- d. Microlepia rhomboidea C. Presl
- e. Selaginella bisulcata Spring
- f. Tricholepidium superficiale (Blume) Fraser-Jenk.



Photo Plate 18:

- a. Tricholepidium normale (D. Don) Ching
- b. Vittaria flexuosa Fée
- c. Oleandra undulata (Willd.) Ching
- d. Polypodiodes lachnopus (Wall. ex Hook.) Ch
- e. Athyrium falcatum Bedd.
- f. Lepisorus thunbergianus (Kaulf.) Ching

	Sl. No	Species	Family	Accession No.	Habit
	1	Adiantum phillipense L.	Adiantaceae	MZU/Bot/Pt-00001	Terrestrial
	2	Adiantum caudatum L.	Adiantaceae	MZU/Bot/Pt-00002	Terrestrial
	3	Aleuritopteris formosana (Hayata) Tagawa	Pteridaceae	MZU/Bot/Pt-00003	Lithophytic
117	4	Aleuritopteris dubia (C. Hope) Ching	Pteridaceae	MZU/Bot/Pt-00004	Terrestrial
	5	Angiopteris evecta (G. Forst.) Hoffm	Marratiaceae	MZU/Bot/Pt-00005	Terrestrial
	6	Angiopteris helferiana C. Presl	Marratiaceae	MZU/Bot/Pt-00006	Terrestrial
	7	Asplenium cheilosorum Kunze ex Mett	Aspleniaceae	MZU/Bot/Pt-00007	Lithophytic
	8	Asplenium unilaterale Lam	Aspleniaceae	MZU/Bot/Pt-00008	Terrestrial

# Table 1. List of pteridophytes collected along with their habits.

	9	Asplenium yoshinagae Makino	Aspleniaceae	MZU/Bot/Pt-00009	Lithophytic
	10	Asplenium laciniatum D. Don	Aspleniaceae	MZU/Bot/Pt-00010	Lithophytic
	11	Asplenium nidus L.	Aspleniaceae	MZU/Bot/Pt-00011	Epiphytic
	12	Athyrium attenuatum (Wall. ex C.B. Clarke) Tagawa	Athyriaceae	MZU/Bot/Pt-00012	Terrestrial
118	13	Anisocampium cuspidatum (Bedd.) Y.C. Liu, W.L. Chiou & M. Kato	Athyriaceae	MZU/Bot/Pt-00013	Terrestrial
	14	Athyrium falcatum Bedd.	Athyriaceae	MZU/Bot/Pt-00014	Terrestrial
	15	Blechnum orientale L.	Blechnaceae	MZU/Bot/Pt-00015	Terrestrial
	16	Christella dentata (Forssk.) Brownsey & Jermy	Thelypteridaceae	MZU/Bot/Pt-00016	Terrestrial
	17	Christella evoluta (Clarke & Baker) Holttum	Thelypteidaceae	MZU/Bot/Pt-00017	Terrestrial
	18	Coniogramme fraxinea (D. Don) Fée ex Diels	Pteridaceae	MZU/Bot/Pt-00018	Lithophytic

	19	Cyclosorus aridus (D. Don) Tagawa	Thelypteidaceae	MZU/Bot/Pt-00019	Terrestrial
	20	Cyclosorus parasiticus (L.) Farw	Thelypteridaceae	MZU/Bot/Pt-00020	Terrestrial
	21	Cyathea chinensis Copel.	Cyatheaceae	MZU/Bot/Pt-00021	Terrestrial
	22	Cyclosorus falcilobus Panigrahi	Pteridaceae	MZU/Bot/Pt-00022	Terrestrial
	23	Cystopteris fragilis (L.) Bernh.	Cystopteridaceae	MZU/Bot/Pt-00023	Terrestrial
119	24	Deparia boryana (Willd.) M. Kato	Athyriaceae	MZU/Bot/Pt-00024	Terrestrial
	25	Deparia petersenii (Kunze) M. Kato	Athyriaceae	MZU/Bot/Pt-00025	Terrestrial
	26	Dicranopteris linearis (Brum.F) Underw	Gleicheniaceae	MZU/Bot/Pt-00026	Terrestrial
	27	Dicranopteris splendida (HandMazz.) Ching	Gleicheniaceae	MZU/Bot/Pt-00027	Terrestrial
	28	Didymochlaena truncatula (Sw.) J. Sm.	Hypodematiaceae	MZU/Bot/Pt-00028	Terrestrial

	29	Asplenium bantamense (Blume) Baker	Aspleniaceae	MZU/Bot/Pt-00029	Terrestrial
	30	Diplazium dilatatum Blume	Athyriaceae	MZU/Bot/Pt-00030	Terrestrial
	31	Diplazium latifolium T. Moore	Athyriaceae	MZU/Bot/Pt-00031	Terrestrial
	32	Diplazium esculentum (Retz.) Sw.	Athyriaceae	MZU/Bot/Pt-00032	Terrestrial
	33	Diplazium polypodioides Blume	Athyriaceae	MZU/Bot/Pt-00033	Epiphytic
120	34	Pseudodrynaria coronans (Wall. ex Mett.) Ching	Polypodiaceae	MZU/Bot/Pt-00034	Epiphytic
	35	Drynaria propinqua (Wall. ex Mett.) Bedd.	Polypodiaceae	MZU/Bot/Pt-00035	Epiphytic
	36	Dryopteris cochleata (D. Don) C. Chr.	Dryopteridaceae	MZU/Bot/Pt-00036	Terrestrial
	37	Dryopteris sparsa (D. Don) Kuntze	Dryopteridaceae	MZU/Bot/Pt-00037	Terrestrial
	38	Dryopteris stenolepis (Baker) C. Chr	Dryopteridaceae	MZU/Bot/Pt-00038	Terrestrial

	39	Egenolfia appendiculata (Willd.) J. Sm.	Dryopteridaceae	MZU/Bot/Pt-00039	Terrestrial
	40	Goniophlebium lachnopus (Wall ex Hook) Bedd	Dipteridaceae	MZU/Bot/Pt-00040	Epiphytic
	41	Lepisorus contortus (Christ) Ching	Polypodiaceae	MZU/Bot/Pt-00041	Epiphytic
	42	Lepisorus excavatus (Bory ex Willd.) Ching	Polypodiaceae	MZU/Bot/Pt-00042	Epiphytic
	43	Lepisorus nudus (Hook.) Ching	Polypodiaceae	MZU/Bot/Pt-00043	Epiphytic
121	44	Lepisorus oligolepidus (Baker) Ching	Polypodiaceae	MZU/Bot/Pt-00044	Epiphytic
	45	Lepisorus thunbergianus (Kaulf.) Ching	Polypodiaceae	MZU/Bot/Pt-00045	Epiphytic
	46	Leucostegia immersa Wall. ex C. Presl	Hypodematiaceae	MZU/Bot/Pt-00046	Epiphytic
	47	Leucostegia truncata (D. Don) Fraser-Jenk.	Hypodematiaceae	MZU/Bot/Pt-00047	Terrestrial
	48	Lindsaea ensifolia Sw.	Lindsaeaceae	MZU/Bot/Pt-00048	Terrestrial

49	Lindsaea lobata Poir.	Lindsaeaceae	MZU/Bot/Pt-00049	Terrestrial
50	Loxogramme involuta (D. Don) C. Presl	Polypodiaceae	MZU/Bot/Pt-00050	Lithophytic
51	Loxogramme porcata M.G. Price	Polypodiaceae	MZU/Bot/Pt-00051	Lithophytic
52	Lycopodiella cernua (L.) Pic. Serm.	Lycopodaceae	MZU/Bot/Pt-00052	Lithophytic
53	Lygodium flexuosum (L.) Sw.	Schizaeaceae	MZU/Bot/Pt-00053	Lithophytic
54	Lygodium salicifolium C. Presl	Schizaeaceae	MZU/Bot/Pt-00054	Lithophytic
55	Macrothelypteris torresiana (Gaudich.) Ching	Thelypteidaceae	MZU/Bot/Pt-00055	Terrestrial
56	Microlepia hancei Prantl	Dennstaedtiaceae	MZU/Bot/Pt-00056	Terrestrial
57	Microlepia hallbergii C.Chr.	Dennstaedtiaceae	MZU/Bot/Pt-00057	Terrestrial
58	Microlepia calvescens (Wall ex Hook) Ching	Dennstaedtiaceae	MZU/Bot/Pt-00058	Terrestrial

	59	Microlepia rhomboidea C. Presl	Dennstaedtiaceae	MZU/Bot/Pt-00059	Terrestrial
	60	Microlepia speluncae (L.) T. Moore	Dennstaedtiaceae	MZU/Bot/Pt-00060	Terrestrial
	61	Microlepia strigosa (Thunb.) C. Presl	Dennstaedtiaceae	MZU/Bot/Pt-00061	Terrestrial
	62	Colysis insignis (Blume) J. Sm.	Polypodiaceae	MZU/Bot/Pt-00062	Lithophytic
	63	Microsorum membranaceum (D. Don) Ching	Polypodiaceae	MZU/Bot/Pt-00063	Terrestrial
123	64	Tricholepidium superficiale (Blume) Fraser-Jenk.	Polypodiaceae	MZU/Bot/Pt-00064	Epiphytic
	65	Neocheiropteris zippelii (Blume) Bosman	Polypodiaceae	MZU/Bot/Pt-00065	Terrestrial
	66	Nephrolepis auriculata (L.) Trimen	Nephrolepidaceae	MZU/Bot/Pt-00066	Epiphytic
	67	Nephrolepis cordifolia (L.) C. Presl	Nephrolepidaceae	MZU/Bot/Pt-00067	Lithophytic
	68	Odontosoria chinensis (L.) J. Sm.	Lindsaeaceae	MZU/Bot/Pt-00068	Lithophytic

	69	Odontosoria biflora (Kaulf) C. Chr	Lindsaeaceae	MZU/Bot/Pt-00069	Lithophytic
	70	Odontosoria krameri Freser Jenk	Lindsaeaceae	MZU/Bot/Pt-00070	Lithophytic
	71	Oleandra undulata (Willd.) Ching	Oleandraceae	MZU/Bot/Pt-00071	Epiphytic
	72	Peranema aspidioides (Blume) Mett.	Dryopteridaceae	MZU/Bot/Pt-00072	Terrestrial
	73	Plagiogyria pycnophylla (Kunze) Mett.	Plagiogyriaceae	MZU/Bot/Pt-00073	Terrestrial
124	74	Polypodiodes amoena (Wall. ex Mett.) Ching	Polypodiaceae	MZU/Bot/Pt-00074	Terrestrial
	75	Polypodiodes lachnopus (Wall. ex Hook.) Ch	Polypodiaceae	MZU/Bot/Pt-00075	Terrestrial
	76	Polystichum obliquum (D. Don) T. Moore	Dryopteridaceae	MZU/Bot/Pt-00076	Terrestrial
	77	Polystichum lentum (D. Don) T. Moore	Dryopteridaceae	MZU/Bot/Pt-00077	Terrestrial
	78	Polystichum luctuosum (Kunze) T. Moore	Dryopteridaceae	MZU/Bot/Pt-00078	Terrestrial
		•			

	79	Polystichum polyblepharum (Roem. ex Kunze) C. Presl	Dryopteridaceae	MZU/Bot/Pt-00079	Terrestrial
	80	Polystichum semifertile (C.B. Clarke) Ching	Dryopteridaceae	MZU/Bot/Pt-00080	Terrestrial
	81	Polystichum squarrosum (D. Don) Fée	Dryopteridaceae	MZU/Bot/Pt-00081	Terrestrial
	82	Polystichum yunnanense Christ	Dryopteridaceae	MZU/Bot/Pt-00082	Terrestrial
	83	Pronephrium lakhimpurense (Rosenst.) Holttum	Thelypteidaceae	MZU/Bot/Pt-00083	Terrestrial
125	84	Pteridium aquilinum (L.) Kuhn	Dennstaedtiaceae	MZU/Bot/Pt-00084	Terrestrial
	85	Pteridium revolutum (Blume) Nakai	Dennstaedtiaceae	MZU/Bot/Pt-00085	Terrestrial
	86	Pteris arisanesis Tagawa	Pteridaceae	MZU/Bot/Pt-00086	Terrestrial
	87	Pteris aspericaulis Wall. ex J. Agardh	Pteridaceae	MZU/Bot/Pt-00087	Terrestrial
	88	Pteris biaurita L.	Pteridaceae	MZU/Bot/Pt-00088	Terrestrial

	89	Pteris confusa T.G. Walker	Pteridaceae	MZU/Bot/Pt-00089	Terrestrial
	90	Pteris cretica L.	Pteridaceae	MZU/Bot/Pt-00090	Terrestrial
	91	Pteris linearis Poir.	Pteridaceae	MZU/Bot/Pt-00091	Terrestrial
	92	Pteris quadriaurita Retz.	Pteridaceae	MZU/Bot/Pt-00092	Terrestrial
	93	Pteris vittata L.	Pteridaceae	MZU/Bot/Pt-00093	Terrestrial
126	94	Pyrrosia lanceolata (L.) Farw.	Polypodiaceae	MZU/Bot/Pt-00094	Epiphytic
	95	Pyrrosia flocculosa (D. Don) Ching	Polypodiaceae	MZU/Bot/Pt-00095	Epiphytic
	96	Selaginella bisulcata Spring	Selaginelaceae	MZU/Bot/Pt-00096	Lithophytic
	97	Selaginella involvens Swartz	Selaginelaceae	MZU/Bot/Pt-00097	Lithophytic
	98	Sphaeropteris brunoniana (Wall ex Hook) R.M. Tryon	Cyatheaceae	MZU/Bot/Pt-00098	Terrestrial

	99	Tectaria coadunata ((J. Smith) C. Chr	Tectariaceae	MZU/Bot/Pt-00099	Terrestrial
	100	Thelypteris clarkei C.F. Reed	Thelypteidaceae	MZU/Bot/Pt-00100	Terrestrial
	101	Thelypteris nudata (Roxb.) C.V. Morton	Thelypteidaceae	MZU/Bot/Pt-00101	Terrestrial
	102	Thelypteris procera (D. Don) Fraser-Jenk.	Thelypteidaceae	MZU/Bot/Pt-00102	Terrestrial
	103	Thelypteris siamensis Tagawa & K. Iwats.	Thelypteidaceae	MZU/Bot/Pt-00103	Terrestrial
127	104	Tricholepidium normale (D. Don) Ching	Polypodiaceae	MZU/Bot/Pt-00104	Terrestrial
	105	Vittaria flexuosa Fée	Vittariaceae	MZU/Bot/Pt-00105	Epiphytic

Sl. No	Name of the Species	PNP	MNP	TWS	DTR
1	Adiantum phillipense L	+	+	+	+
2	Adiantum caudatum L	+	+	+	+
3	Aleuritopteris formosana (Hayata) Tagawa	-	+	+	-
4	Aleuritopteris dubia (C. Hope) Ching	+	-	+	-
5	Angiopteris evecta (G. Forst.) Hoffm	-	+	+	+
6	Angiopteris helferiana C. Presl	-	+	+	+
7	Asplenium cheilosorum Kunze ex Mett	+	+	+	+
8	Asplenium unilaterale Lam	+	+	+	-

# Table 2. Comparative distribution of Pteridophyte Flora of PNP, MNP, TWS, DTR of Mizoram.

9	Asplenium yoshinagae Makino	-	+	+	+
10	Asplenium laciniatum D. Don	+	+	+	-
11	Asplenium nidus L.	+	+	+	+
12	Athyrium attenuatum (Wall. ex C.B. Clarke) Tagawa	-	+	+	-
13	Anisocampium cuspidatum (Bedd.) Y.C. Liu, W.L. Chiou & M. Kato	-	+	+	-
14	Athyrium falcatum Bedd.	+	+	+	+
15	Blechnum orientale L.	+	+	+	+
16	Christella dentata (Forssk.) Brownsey & Jermy	+	+	+	-
17	<i>Christella evoluta</i> (Clarke & Baker) Holttum	+	+	-	+
18	Coniogramme fraxinea (D. Don) Fée ex Diels	-	-	-	+

19	Cyclosorus aridus (D. Don) Tagawa	-	-	-	+
20	Cyclosorus parasiticus (L.) Farw	+	-	+	+
21	Cyathea chinensis Copel.	-	+	+	-
22	Cyclosorus falcilobus Panigrahi	+	+	+	+
23	Cystopteris fragilis (L.) Bernh.	+	+	+	+
24	Deparia boryana (Willd.) M. Kato	-	+	+	-
25	Deparia petersenii (Kunze) M. Kato	-	+	+	+
26	Dicranopteris linearis (Burm.F) Underw	+	+	+	+
27	Dicranopteris splendida (Hand Mazz.) Ching	+	+	+	-
28	Didymochlaena truncatula (Sw.) J. Sm.	-	-	+	-

29	Asplenium bantamense (Blume) Baker	-	+	+	+
30	Diplazium dilatatum Blume	+	+	+	+
31	Diplazium latifolium T. Moore	+	+	-	-
32	Diplazium esculentum (Retz.) Sw.	+	+	+	+
33	Diplazium polypodioides Blume	+	+	+	+
34	Pseudodrynaria coronans (Wall. ex Mett.) Ching	+	+	+	+
35	Drynaria propinqua (Wall. ex Mett.) Bedd.	+	+	+	+
36	Dryopteris cochleata (D. Don) C. Chr.	+	+	+	-
37	Dryopteris sparsa (D. Don) Kuntze	+	+	+	-
38	Dryopteris stenolepis (Baker) C. Chr	-	+	-	-

39	Egenolfia appendiculata (Willd.) J. Sm.	+	-	-	-
40	Goniophlebium lachnopus (Wall ex Hook) Bedd.	-	-	-	+
41	Lepisorus contortus (Christ) Ching	-	+	+	+
42	Lepisorus excavatus (Bory ex Willd.) Ching	-	+	+	+
43	Lepisorus nudus (Hook.) Ching	+	+	+	+
44	Lepisorus oligolepidus (Baker) Ching	-	+	-	+
45	Lepisorus thunbergianus (Kaulf.) Ching	-	+	-	+
46	Leucostegia immersa Wall. ex C. Presl	+	+	+	+
47	Leucostegia truncata (D. Don) Fraser-Jenk.	-	+	+	-
48	Lindsaea ensifolia Sw.	+	+	+	+

49	Lindsaea lobata Poir.	+	+	+	+
50	Loxogramme involuta (D. Don) C. Presl	-	-	+	+
51	Loxogramme porcata M.G. Price	-	+	+	-
52	Lycopodiella cernua (L.) Pic. Serm.	+	+	+	+
53	Lygodium flexuosum (L.) Sw.	+	+	+	+
54	Lygodium salicifolium C. Presl	-	+	-	-
55	Macrothelypteris torresiana (Gaudich.) Ching	+	+	+	+
56	Microlepia hancei Prantl	+	-	-	+
57	Microlepia hallbergii C.Chr.	+	+	-	-
58	Microlepia calvesens (Wall ex Hook) Ching	+	+	+	+

59	Microlepia rhomboidea C. Presl	+	+	+	-
60	Microlepia speluncae (L.) T. Moore	+	+	+	+
61	Microlepia strigosa (Thunb.) C. Presl	+	+	+	+
62	Colysis insignis (Blume) J. Sm.	-	+	+	-
63	Microsorum membranaceum (D. Don) Ching	+	+	+	+
64	Tricholepidium superficiale (Blume) Fraser-Jenk.	-	+	-	-
65	Neocheiropteris zippelii (Blume) Bosman	-	-	+	-
66	Nephrolepis auriculata (L.) Trimen	-	-	+	-
67	Nephrolepis cordifolia (L.) C. Presl	-	+	+	-
68	Odontosoria chinensis (L.) J. Sm.	-	+	+	-

69	Odontosoria biflora (Kaulf) C.Chr	-	+	+	+
70	Odontosoria krameri Fraser Jenk	-	+	+	+
71	Oleandra undulata (Willd.) Ching	-	-	+	-
72	Peranema aspidioides (Blume) Mett.	+	+	-	-
73	Plagiogyria pycnophylla (Kunze) Mett.	-	+	-	-
74	Polypodiodes amoena (Wall. ex Mett.) Ching	+	+	+	+
75	Polypodiodes lachnopus (Wall. ex Hook.) Ch	+	+	+	+
76	Polystichum obliquum (D. Don) T. Moore	+	+	+	+
77	Polystichum lentum (D. Don) T. Moore	-	-	-	+
78	Polystichum luctuosum (Kunze) T. Moore	+	+	+	+

79	Polystichum polyblepharum (Roem. ex Kunze) C. Presl	+	+	+	+
80	Polystichum semifertile (C.B. Clarke) Ching	+	+	+	+
81	Polystichum squarrosum (D. Don) Fée	+	+	+	+
82	Polystichum yunnanense Christ	+	-	-	+
83	Pronephrium lakhimpurense (Rosenst.) Holttum	+	+	+	+
84	Pteridium aquilinum (L.) Kuhn	+	+	+	+
85	Pteridium revolutum (Blume) Nakai	+	+	+	+
86	Pteris arisanesis Tagawa	+	+	-	+
87	Pteris aspericaulis Wall. ex J. Agardh	+	+	+	+
88	Pteris biaurita L.	+	+	+	+

89	Pteris confusa T.G. Walker	+	+	+	+
90	Pteris cretica L.	+	+	+	+
91	Pteris linearis Poir.	+	+	+	+
92	Pteris quadriaurita Retz.	+	+	+	+
93	Pteris vittata L.	+	+	+	+
94	Pyrrosia lanceolata (L.) Farw.	-	+	+	-
95	Pyrrosia flocculosa (D. Don) Ching	-	+	+	-
96	Selaginella bisulcata Spring	+	-	+	+
97	Selaginella involvens Swartz	-	+	+	-
98	Sphaeropteris brunoniana (Wall ex Hoo) R.M. Tryon	-	+	+	+

99	Tectaria coadnata (J. Smith) C. Chr	+	-	-	-
100	Thelypteris clarkei (Bedd) C.F. Reed	+	+	+	+
101	Thelypteris nudata (Roxb.) C.V. Morton	+	+	+	+
102	Thelypteris procera (D. Don) Fraser-Jenk.	+	+	+	+
103	Thelypteris siamensis Tagawa & K. Iwats.	-	+	+	-
104	Tricholepidium normale (D. Don) Ching	-	+	-	-
105	Vittaria flexuosa Fée	+	+	+	+
	Total	65	89	85	69

### Table 3. Floristic analysis of Pteridophyte flora Phawngpui National Park.

Families	Genera	Species
18	32	65

### Table 4. Floristic analysis of Pteridophyte flora of Murlen National Park.

Families	Genera	Species
21	44	89

## Table 5. Floristic analysis of Pteridophyte flora Tawi Wildlife Sanctuary.

Families	Genera	Species
21	43	85

## Table 6. Floristic analysis of Pteridophyte flora of Dampa Tiger Reserve.

Families	Genera	Species
20	34	69

#### 4.4. DISCUSSION

In the present study, a total of 105 species belonging to 51 genera and 24 families of fern have been recorded from Murlen National Park, Phawngpui National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve.

The percent of occurrence of 105 Pteridophytes species under 24 different families during the study shows highest value for Polypodiaceae 17.14 percent (18 species), followed by Pteridaceae and Dryopteridaceae with 11.43 percent (12 species) each while Thelypteridaceae has 9.5 percent (10 species) occupying the third largest families recorded during the study. The rest of the families like, Athyriaceae exhibit 8.57 percent (9 species), Dennstaedtiaceae 7.62 percent (8 species), Aspleniaceae 5.71 percent (6 species), Lindsaeaceae 4.76 percent (5 species) and families like Adiantaceae, Cyatheaceae, Gleicheniaceae, Marratiaceae, Nephrolepidaceae, Selaginelaceae, Schizaeaceae exhibit 1.90 percent (2 species) each adding to their family while Blechnaceae, Cystopteridaceae, Dipteridaceae, Hypodematiaceae, Lycopodiaceae, Oleandraceae, Plagiogyriaceae, Tectariaceae and Vittariaceae accounts only for 0.95 percent having 1 species each (Figure 5). In the present study it has been notice that the terrestrial species constitute more than 68 percent (71 species) out of 105 species recorded, while epiphytes and lithophytes communities constitute approximately 16 percent (17 species) each of the total pteridophytes species collected (Figure 6).

The availability of number of pteridophytes species the collected from four reserve forest sites are different in which a total no of 89 species have been collected

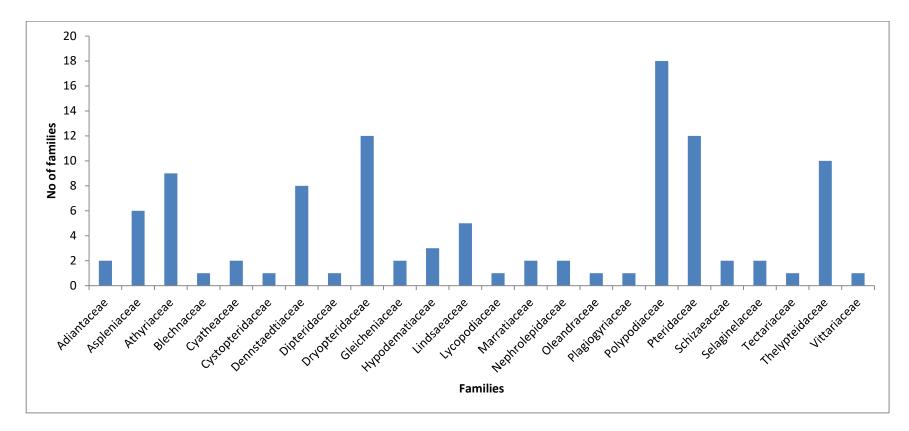


Figure 5. Family distribution of Pteridophytes in PNP, MNP, TWS and DTR.

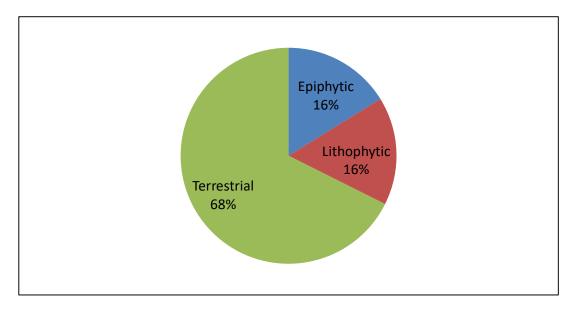


Figure 6. Percentage showing their state of habit.

from Murlen National Park, 85 species from Tawi Wildlife sanctuary, 69 species from Dampa Tiger Reserve and 65 species from Phawngpui National Park.

Out of 105 species collected species like Adiantum phillipense L, Adiantum caudatum L, Asplenium cheilosorum Kunze ex Mett, Asplenium nidus L., Macrothelypteris torresiana (Gaudich.) Ching, Athyrium falcatum Bedd., Blechnum orientale L., Cyclosorus falcilobus Panigrahi, Cystopteris fragilis (L.) Bernh., Dicranopteris lanigera Fraser-Jenk, Diplazium dilatatum Blume, Diplazium esculentum (Retz.) Sw., Diplazium polypodioides Blume, Pseudodrynaria coronans (Wall. ex Mett.) Ching, Drynaria propingua (Wall. ex Mett.) Bedd., Lepisorus nudus (Hook.) Ching, Leucostegia immersa Wall. ex C. Presl, Lindsaea ensifolia Sw., Lindsaea lobata Poir., Lindsaea lobata Poir., Lycopodiella cernua (L.) Pic. Serm. Lygodium flexuosum (L.) Sw., Microlepia polypodioides (Sw.) C. Presl, Microlepia strigosa (Thunb.) C. Presl, Microsorum membranaceum (D. Don) Ching, Polypodiodes lachnopus (Wall. ex Hook.) Ch, Polystichum obliquum (D. Don) T. Moore, Polystichum luctuosum (Kunze) T. Moore, Polystichum polyblepharum (Roem. ex Kunze) C. Presl, Polystichum semifertile (C.B. Clarke) Ching, Polystichum squarrosum (D. Don) Fée, Pronephrium lakhimpurense (Rosenst.) Holttum, Pteridium aquilinum (L.) Kuhn, Pteridium revolutum (Blume) Nakai, Pteris aspericaulis Wall. ex J. Agardh, Pteris biaurita L., Pteris confusa T.G. Walker, Pteris cretica L., Pteris linearis Poir., Pteris quadriaurita Retz., Pteris vittata L., Thelypteris clarkei C.F. Reed, Thelypteris nudata (Roxb.) C.V. Morton and Thelypteris procera (D. Don) Fraser-Jenk. are found to have occur in all the four forests reserve sites selected. Also, there are some species which are specific to a

particular place, means they are found only in one place amongst the four sites selected like *Coniogramme fraxinea* (D. Don) Fée ex Diels, Polystichum lentum (D. Don) T. Moore. and *Cyclosorus aridus* (D. Don) Tagawa found only in Dampa Tiger Reserve. *Didymochlaena truncatula* (*Sw.*) *J. Sm. and Oleandra undulata* (*Willd.*) *Ching are found in Tawi Wildlife sanctuary while Dryopteris stenolepis (Baker) C. Chr, Lygodium salicifolium C. Presl, Tricholepidium superficiale (Blume) Fraser-Jenk, Tricholepidium normale (D. Don) Ching and Plagiogyria pycnophylla (Kunze) Mett.* are found only in Murlen National Park and *Egenolfia appendiculata (Willd.) J. Sm. is found only in Phawngpui National Park.* Besides these, 5 species viz. *Angiopteris evecta, Asplenium nidus, Asplenium unilaterale, Drynaria propinqua* and *Pseudodrynaria coronans* recorded in the present study are known to be rare and endangered species for Northeastern India (Bir 1987).

In PNP a total no of 65 species belonging to 32 genera and 18 families has been recorded (Table 3). The highest no. of family in PNP is exhibited by Dryopteridaceae and Pteridaceae having 10 species each which is followed by Dennstaedtiaceae having 8 species and Polypodiaceae having 7 species accounting to it. The rest of the families have lesser no. of species as compared to the four families mentioned (Figure 7) and out of the 65 species recorded from PNP 53 species belongs to the terrestrial community, 7 belongs to epiphytic and 5 belongs to lithophytes community (Figure 8).

In MNP a total no of 89 species belonging to 44 genera and 21 families has been recorded (Table 4). The highest no. of family in MNP is exhibited by

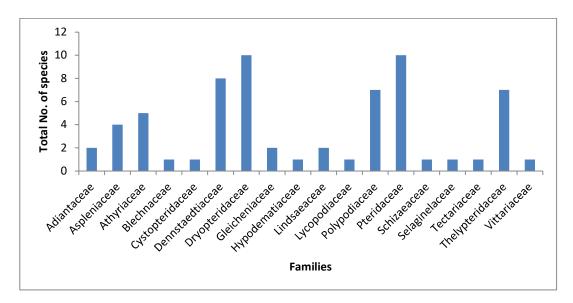


Figure 7. Family distribution in Phawngpui National Park.

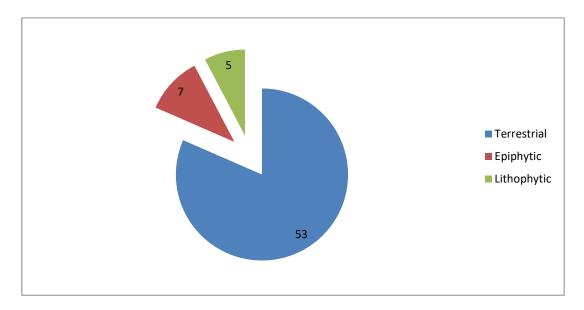


Figure 8. Percentage showing their state of habit from Phawngpui National Park.

Polypodiaceae with 16 species, the second highest is and Pteridaceae having 11 species each which is followed by Dryopteridaceae having 10 species, Dennstaedtiaceae having 7 species, Athyriaceae 9 species, Aspleniaceae and Thelypteridaceae having 6 species listed to the families. The rest of the families have lesser no. of species as compared to the families mentioned above (Figure 9) and out of the 89 species recorded from MNP 61 species belongs to the terrestrial community, 14 belongs to both epiphytic and lithophytes community (Figure 10).

In TWS a total no of 85 species belonging to 43 genera and 21 families has been recorded (Table 5) which has one genus less as compared to MNP. The highest no. of family in TWS is exhibited by Polypodiaceae with 13 species, the second highest is and Pteridaceae having 10 species each which is followed by Thelypteridaceae having 8 species, Dryopteridaceae having 7 species, Dennstaedtiaceae having 6 species Athyriaceae and Aspleniaceae both having 8 species and Lindsaeaceae having 5 species listed to the families. (Figure 11) and out of the 85 species recorded from TWS 57 species belongs to the terrestrial community, 13 belongs to epiphytic and 15 belongs to lithophytic community (Figure 12).

In DTR a total no of 69 species belonging to 34 genera and 20 families has been recorded (Table 6) which has a recorded genera, a margin higher as compared to PNP. The highest no. of family in DTR is exhibited by Polypodiaceae with 11 species, the second highest is and Pteridaceae having 10 species each which is followed by Thelypteridaceae having 8 species, Dryopteridaceae having 7 species,

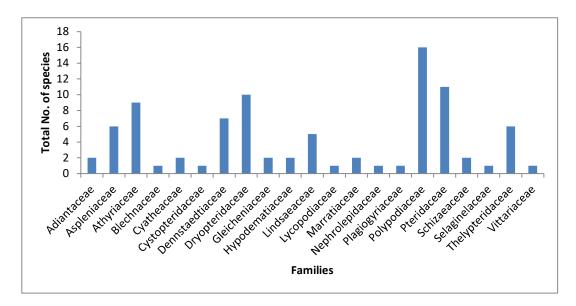


Figure 9. Family distribution in Murlen National Park.

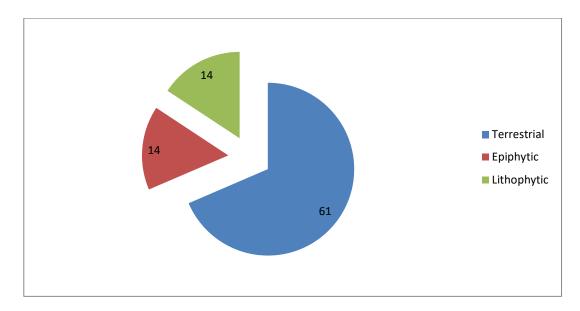


Figure 10. Percentage showing their state of habit from Murlen National Park.

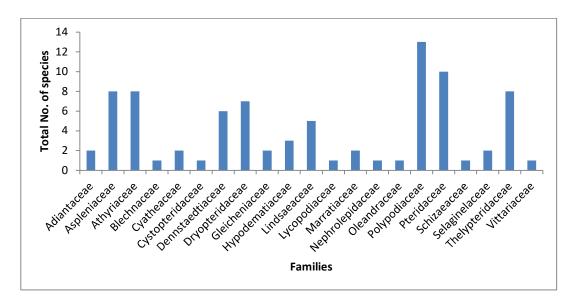


Figure 11. Family distribution in Murlen National Park.

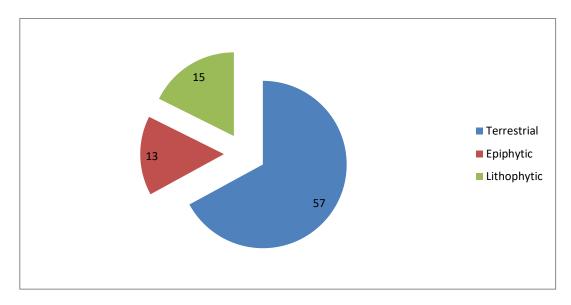


Figure 12. Percentage showing their state of habit from Murlen National Park.

Dennstaedtiaceae having 6 species Athyriaceae 5 species and Aspleniaceae having 4 species listed to the families. (Figure 13) and out of the 69 species recorded from DTR 48 species belongs to the terrestrial community, 12 belongs to epiphytic and 9 belongs to lithophytic community (Figure 14). Amongst the four sites selected Murlen National Park has the greatest number of species, genera and families recorded followed by Tawi Wildlife Sanctuary, Dampa Tiger reserve and Phawngpui National Park.

Owing to various anthropogenic activities like shifting cultivation, expansion of agriculture, burning of forest and other human activities near the reserve forest there is less diversity and less luxuriant growth of pteridophytes in Phawngpui National park as compared to Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve.

The numerous and intensive programme for development in the state have also largely contribute to the loss of many natural forests. These have resulted in loss of biodiversity in the state, which is accelarated during the last few decades. It is therefore, become essential to take proper steps for conservation of rare species in the state. As pointed out by Bir (1987) ex-situ conservation of fern species in Botanic gardens pose special problems and for which he suggested the establishment of forest conservatories as the best way to conserve fern species.

The present study reaffirmed that Mizoram is a huge pteridophyte reservoir, however it is depleting at a very fast rate, epiphytic and lithophyte pteridophytes in particular due various anthropogenic activities. Recently ethnomedicinal discipline

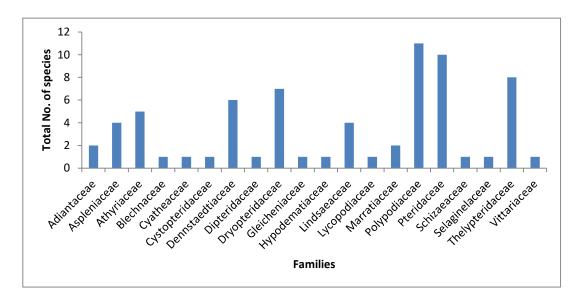


Figure 13. Family distribution in Dampa Tiger Reserve.

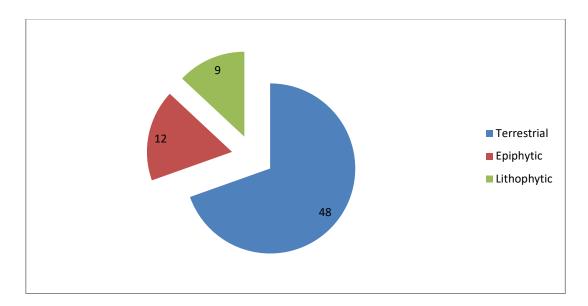


Figure 14. Percentage showing their state of habit from Dampa Tiger Reserve.

drew the attention of many researchers to discover of new drug, its conservation and utilization for socio-economic sustainability within the tribal tracts. The northeastern region including Mizoram being within biodiversity hot spot is the best custodian for much valuable plants. Despite the discovery of many drugs, it is of the view that many are yet to be explored, identified for pharmaceutical, cosmetic and ecological studies. The conservation is of great concern as the local inhabitants are unable to explore with such a rapid anthropogenic pressure. The study of cyptogamic plants would be insufficient if we happen to ignore the pteridophytic flora. Although there had been a study on pteridophyte diversity on Murlen National Park, it was the first and foremost systematic pioneer work in Tawi Wildlife Sanctuary, Phawngpui National park and Dampa Tiger reserve. There is a great scope on the study for further investigation in the state of Mizoram.

#### **CHAPTER-V**

#### PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY

### **5.1. INTRODUCTION**

Plants are a valuable resource of novel bioactive molecules to combat microbial diseases. Plants have been used since ancient times as a natural product for traditionally maintaining human health. Plant products in their natural state do not cause side effects. The medical value of plants emanates from ranges of active chemicals that produce physiological effects in the human body. The flavonoids, tannins, alkaloids and phenolics are the most beneficial bioactive compounds obtained from plants.

On an empirical basis, medicinal plants are the reliable source of traditional treatment of various diseases. The invention of antimicrobials from higher plants might undoubtedly lead to phytomedicines against microbes. The extracts of plants possess enormous therapeutic potential with lesser side effects. Based on random sampling there are several surveys on phytochemicals. The alkaloids, steroids and saponins were the major chemical substances of interest but other phytocomponents documented are flavonoids, tannins, unsaturated steroids, triterpenoids and essential oils. Davvamani *et al.*, (2005) mentioned as "the use of antibiotics and synthesized medicines cure microbial infections very fast but they may equally disturb the innate immunity of the body and cause a variety of side effects. This has keenly aroused

considerable interest in plant products that can support or substitute synthetic drugs. Due to antimicrobial activity, the enormous diversity of medicinal plants is beneficial for mankind." Pteridophytes are less explored plant group with reverence to economic and medicinal uses. Medicinal use of pteridophytes has been tethered as Folk medicine (Ethnobotany). There are many reports on medicinal uses of ferns and fern-allies Caius (1935-36); Chopra (1933); Chopra et al., (1958); Nayar (1959); Puri (1970); May (1978); Sharma and Vyas (1985); Kaushik and Dhiman (1995); Manandhar (1996); Dhiman (1998); Vasudeva (1999); Singh et al., (2001); Gogoi (2002); Jha et al., (2003); Baltrushes (2006); Sher and Khan (2006); Mannan et al., (2008). The medicinal uses of ferns have been mentioned by Greek botanist Theophrastus (ca. 327-287 B.C.); Dioscorides (c. 50 A.D.); Sushruta (ca. 100 A.D.); Charak (ca. 100 A.D.); Unani physicians and Chinese doctors (cf. Manickam et al., 2005). There are several reports on the antibacterial property of Pteridophytes (Maruzzella 1961; Banerjee and Sen 1980; Basile 1997; Kumar and Kausik 1999; Reddy et al., (2001); Parihar and Bohra 2002a, b, 2003a, b, 2004). Alcoholic extract of Adiantum capillus-veneris exerts an antibacterial effect (Kumar and Kaushik 1999). Parihar et al., (2003) demonstrated a profound inhibitory effect of alcoholic extract of roots and stem of Marsilea minuta L. against Staphylococcus aureus. Aqueous and alcoholic extracts of *Adiantum capillus-veneris*, A. incisum, and A. lunulatum inhibit the growth of Salmonella typhi (Parihar and Bohra, 2004).

Antibacterial effect of *Marsilea minuta* L. against human and plant pathogenic bacteria has been reported by Parihar *et al.*, (2007a, 2008). As the isolation of antimicrobial compounds from Indian ferns is concerned, less work has

been carried out. Previously, Banerjee and Sen (1980) screened antibiotic activity of 114 species of Pteridophyta including some Thelypteroid ferns. In the prior study, the plants were extracted in water, methanol, 70% ethanol, acetone and ether and assayed against three gram-positive bacteria, one acid-fast bacterium, five gramnegative bacteria and three fungal plant pathogens. Further, the above study reported that Ampelopteris prolifera (Retz.) Copel., Christella contiguum (Rosent.) Holttm., Cyclosorus gongylodes (Schk.) Link, Sphaerostephanos subtruncatus (Berg.) Holttm. and S. unitus (L.) Holttum possess significant antibacterial activities against test organisms. Spinelli et al., (2000) confined three new coumarin derivatives, three new furanocoumarins and a novel dioxocane derivative from the fern Cyclosorus interruptus (Willd.) with excellent antibacterial activity. The antibacterial activity of leaf glands of Christella parasitica (L.) Lev. against nine human pathogens was reported by Manickam et al., (2005). Also, the antibacterial property of the alcoholic and chloroform extracts of Christella dentate against Bacillus subtilis, Escherichia *coli*, *Salmonella typhi* and *Staphylococcus aureus* is reported by Kumar and Kaushik (2011).

The present Chapter 5 deals with profiling of three abundant traditionally used ferns (*Lycopodiella cernua* (L.) Pic. Serm, *Diplazium esculentum* (Retz.) Sw. *and Selaginella bisulcate Spring*.) for the presence and diversity of vital phytochemicals like alkaloid, carbohydrates, proteins, tannins, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids. Further, the present Chapter describes the antibacterial activities in methanolic and chloroform extracts of above three ferns *L. cernua*, *D. esculentum* and *S. bisulcata*. Results showed that all three ferns studied showed excellent antibacterial activities.

#### **5.2. MATERIALS AND METHODS**

#### 5.2.1. PHYTOCHEMICAL SCREENING

The methods and tests as described by Kokate *et al.*, (2013) and given in Chapter 3 were used in common for the extensive screening of phytochemicals in the selected species of Pteridophytes. Selected Pteridophytes were screened for the following compounds: alkaloid, carbohydrates, proteins, tannins, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids.

### 5.2.2. TEST FOR ANTIBACTERIAL ACTIVITY

Agar well-diffusion method described by Murray *et al.*, (1995) and modified by Olurinola (1996) was followed to reliably determine the antimicrobial activity. Nutrient agar (NA) plates were carefully prepared as per the method described in Chapter 3. Briefly, nutrient agar plates were swabbed (sterile cotton swabs) with 8 hours old-broth culture of respective bacteria. Wells (7mm diameter and about 2 cm apart) were made in each of these plates using a sterile cork borer. The stock solution of each plant extract was prepared at a concentration of 100 mg ml<sup>-1</sup> and was diluted to different concentration viz 20, 40, 60 and 80 mg ml<sup>-1</sup> using a serial dilution method of methanol extract of diverse kind of plants. About 70  $\mu$ l of plant extracts were added into the well by using a sterile syringe and allowed to diffuse at the room temperature. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured, and the activity index was calculated. 1.5 gm of Ceftriaxone and Sulbactam antibiotics were used as control. The test was conducted on three bacteria strains namely *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* 

#### **5.3. RESULTS**

### 5.3.1. STUDIES ON PHYTOCHEMICAL SCREENING OF EXTRACTS

The results of phytochemical group tests for chloroform, methanol, aqueous and petroleum ether crude extracts of *Lycopodiella cernua* (L.) Pic. Serm., *Diplazium esculentum* (Retz.) Sw. *and Selaginella bisulcata* Spring. are given in Tables 7, 8 and 9, respectively. The results evidenced the existence of alkaloids, carbohydrates, proteins, tannins, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids in all three studied species of Pteridophytes. The chloroform extract revealed the most significant phytochemical diversity. Simultaneously, least phytochemical diversity was noted in petroleum ether extracts of plants. Furthermore, no phytochemical was detected in petroleum ether extract of *S. bisulcata*.

 Table 7. Phytochemical analysis of the methanolic, aqueous and petroleum ether

 extracts of Lycopodiella cernua (L.) Pic. Serm.

Phytochemical compounds	Chloroform extract	Methanol extract	Aqueous extract	Petroleum- ether extract
Alkaloids	-	+	+	-
Carbrohydrates	+	-	-	-
Proteins	+	-	-	-

Tannins	+	-	+	-
Terpenoids	+	-	+	-
Phenols	+	+	+	-
Glycosides	+	+	+	+
Saponins	+	-	+	-
Phytosterols	+	+	-	-
Flavanoids	+	+	+	-

+ = present; - = absent

## Table 8. Phytochemical analysis of the methanolic, aqueous and petroleum ether

extracts of Diplazium esculentum (Retz.) Sw.

Phytochemical compounds	Chloroform extract	Methanol extract	Aqueous extract	Petroleum- ether extract
Alkaloids	+	+	+	-
Carbrohydrates	+	-	-	-
Proteins	+	+	-	-
Tannins	+	-	+	-
Terpenoids	+	+	-	-
Phenols	+	+	+	-
Glycosides	+	+	+	+
Saponins	+	-	+	-
Phytosterols	+	+	-	-
Flavanoids	+	+	+	-

+ = present; - = absent

 Table 9: Phytochemical analysis for the methanolic, aqueous and petroleum

ether extracts of Selaginella bisulcata Spring.

Phytochemicals	Chloroform	Methanol	Aqueous	Petroleum
compounds	extract	extract	extract	ether extract
Alkaloids	+	+	-	-
Carbrohydrates	+	+	-	-
Proteins	+	+	-	-
Tannins	+	-	-	-
Terpenoids	+	+	+	-
Phenols	+	+	-	-
Glycosides	+	-	+	-
Saponins	+	+	+	-
Phytosterols	+	+	-	-
Flavanoids	+	+	-	-

+ = present; - = absent

# 5.3.2. Antibacterial activity of *Lycopodiella cernua* (L.) Pic. Serm. extract against *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtili*

The antibacterial activities of the extracts obtained from *L. cernua* were examined by the agar-well-diffusion method. The assessment of the antibacterial activity was based on determination of the diameter of the zone of inhibition (mm) that formed around the well filled with the extract. The antibacterial activity of methanolic extracts of *L. cernua* was determined at various concentrations viz. 20, 40, 60, and 80 mg ml<sup>-1</sup>. Control makes up  $25\pm0.00$ ,  $24\pm0.00$  and  $22.0\pm0.00$  mm

inhibition zone in agar plate of *Escherichia coli* (ATCC-10536), *Klebsiella pneumoniae* (ATCC - 10031) and *Bacillus subtilis* (ATCC - 11774), respectively.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *L. cernua* caused 7.00 $\pm$  0.0, 7.5 $\pm$ 0.0, 7.83 $\pm$ 0.29 and 14.33 $\pm$ 0.57 mm inhibition zone in agar plate of *E. coli*, respectively (Figure 15). The known antibiotic treatment produced an inhibition zone of 25 mm. It is suggested that methanolic extract of *L. cernua* at 80 mg ml<sup>-1</sup> concentration was the most potent in inhibition of growth of *E. coli*. The antimicrobial proficiency of 80 mg L<sup>-1</sup> methanolic extract of *L. cernua* against *E. coli* was 57.3% when equated with a commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *L. cernua* caused 7.83±0.23, 9.2±0.57, 10.33±0.67 and 12.00±0.57 mm inhibition zone in agar plate of *K. pneumoniae*, respectively (Figure 16). The known antibiotic treatment caused an inhibition zone of 24.0±0.00 mm. The inhibition potential of methanolic extract of *L. cernua* increased with a rise in the concentration of extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *L. cernua* against *K. pneumoniae* was 50% when compared with a commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *L. cernua* caused 7.0 $\pm$ 0.00, 7.5 $\pm$ 0.03, 8.0 $\pm$ 0.00 and 9.0 $\pm$ 0.00 mm inhibition zone in agar plate of *B. subtilis*, respectively (Figure 17). The known antibiotic treatment resulted in an inhibition zone of 22 mm. The inhibition potential of methanolic extract of *L. cernua* increased with an increase in the concentration of extract. The antimicrobial

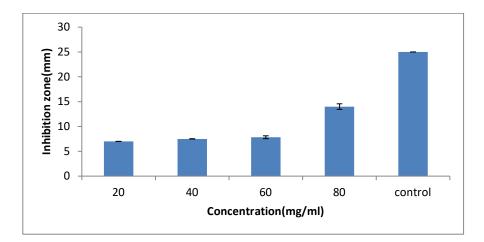


Figure 15. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. against *Escherichia coli*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

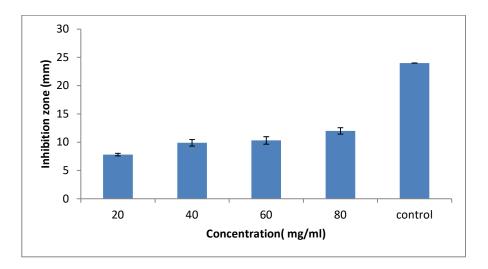


Figure 16. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. against *Klebsiella pneumoniae*. The values are mean of three replicates. Vertical bars show  $\pm$ SE.

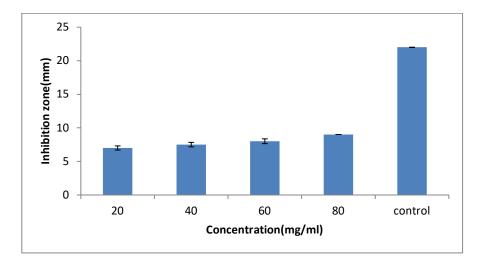


Figure 17. The antibacterial activity in the methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

efficiency of 80 mg L<sup>-1</sup> methanolic extract of *L. cernua* against *B. subtilis was* 41% when compared with a commercial antibiotic.

Above results suggested that methanolic extract of *L. cernua* has strongest antibacterial activity against *E. coli* followed by *K. pneumoniae and B. subtilis.* Results also indicated that gram (-) bacteria are more sensitive towards the methanolic extract of *L. cernua*.

The results of one-way analysis of variance (ANOVA) significant variation  $(p \le 0.05)$  between different concentrations of the extracts tested on three bacterial strains are given in Table 10,11 and 12.

Table 10. F- and p-values of One-Way ANOVA for the inhibition zone in *E. Coli* subjected to various concentrations of methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. The marked effects are significant at p≤ 0.05.

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	2620.03	0.00*
2	CrtlXC1	2620.03	0.00*
3	CrtlXC2	2547.21	0.00*
4	CrtlXC3	2450.00	0.00*
5	CrtlXC4	899.04	0.00*
6	C1XC2	73.22	0.00*
7	C1XC3	181.79	0.00*
8	C1XC4	1210.00	0.00*

9	C2XC3	1920.80	0.00*
10	C2XC4	2800.12	0.00*
11	C3XC4	800.12	0.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>

Table 11. F- and p-values of One-Way ANOVA for the inhibition zone in *Klebsiella pneumoniae* subjected to various concentrations of methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. The marked effects are significant at p≤ 0.05.

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	2451.24	0.00*
2	CrtlXC1	2451.24	0.00*
3	CrtlXC2	2016.96	0.00*
4	CrtlXC3	1616.62	0.00*
5	CrtlXC4	1362.78	0.00*
6	C1XC2	344.10	0.00*
7	C1XC3	472.07	0.00*
8	C1XC4	1400.81	0.00*
9	C2XC3	80.40	0.00*
10	C2XC4	1513.80	0.00*
11	C3XC4	950.12	0.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>.

Table 12. F- and p-values of One-Way ANOVA for the inhibition zone in *Bacillus subtilis* subjected to various concentrations of methanolic extract of *Lycopodiella cernua* (L.) Pic. Serm. The marked effects are significant at p≤ 0.05.

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	1237.54	.00*
2	CrtlXC1	1237.54	.00*
3	CrtlXC2	2202.89	.00*
4	CrtlXC3	2386.94	.00*
5	CrtlXC4	2020.79	.00*
6	C1XC2	.08	.93
7	C1XC3	3.57	.13
8	C1XC4	17.93	.01*
9	C2XC3	22.05	.09
10	C2XC4	84.96	.01*
11	C3XC4	650.10	.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml.

# 5.3.3. Effect of *Diplazium esculentum* (Retz.) Sw. extract against *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis*

The antibacterial activities of the concentrated extracts obtained from *D*. *esculentum* were investigated by agar-well-diffusion approach. The inhibition zone

was measured in nutrient agar plates of *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtili* after treatment with methanolic extract of *D. esculentum* at 20, 40, 60 and 100 mg ml<sup>-1</sup> concentrations.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *D. esculentum* caused  $9.33\pm0.57$ ,  $11.33\pm0.57$ ,  $12.33\pm0.58$  and  $16.67\pm0.75$  mm inhibition zones in agar plate of *E. coli* (ATCC - 10536), respectively (Figure 18). The treatment with known antibiotic led to an inhibition zone of 25 mm. Results subtly indicated that antibacterial activity of extract increased with an elevation in concentration of extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *D. esculentum* against *E. coli* (ATCC-10536) was 66.7% when set against with the antibacterial efficiency of the commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *D. esculentum* caused 7.66±0.57, 10.0±0.75, 11.33±0.57 and 12.33±0.57 mm inhibition zones in agar plate of *K. pneumoniae* (ATCC-10031), respectively (Figure 19). The treatment of known antibiotic resulted in an inhibition zone of 24.0±0.00 mm. The inhibitory potentiality of methanolic extract of *D. esculentum* increased with an increment in the concentration of extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *D. esculentum* against *K. pneumoniae* (ATCC-10031) was 51.4% when equated with the inhibitory capability of a tested commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extract of *D. esculentum* caused  $8.66\pm0.57$ ,  $9.66\pm0.53$ ,  $10.00\pm0.57$  and  $11.16\pm0.76$  mm inhibition zones in agar plate of *B. subtilis (ATCC - 11774)*, respectively (Figure 20). The known antibiotic

treatment has caused an inhibition zone of 22 mm. The antibacterial potential of methanolic extract of *D. esculentum* increased with an increase in the concentration of extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *D. esculentum* against *B. subtilis (ATCC-11774)* was 50.7% when compared with the antibacterial potential of tested commercial antibiotic.

Above results suggested that methanolic extract of *D. esculentum* has the highest antibacterial activity against *E. coli* followed by *K. pneumoniae and B. subtilis.* Comparable results also marked that gram (-) bacteria are more sensitive towards the methanolic extract of *D. esculentum*.

The results of one-way analysis of variance (ANOVA) significant variation  $(p \le 0.05)$  between different concentrations of the extracts tested on three bacterial strains are given in (Table 13,14 and 15).

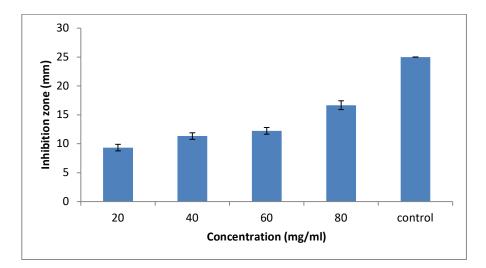


Figure 18. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *E.coli*. The values are mean of three replicates. The vertical bars show  $\pm$  SE.

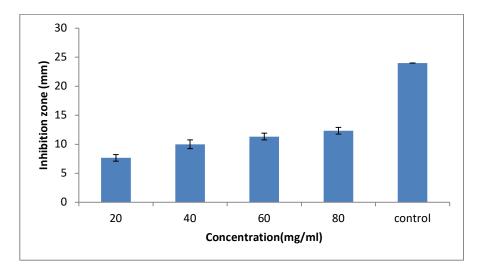


Figure 19. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *Klebsiella pneumoniae*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

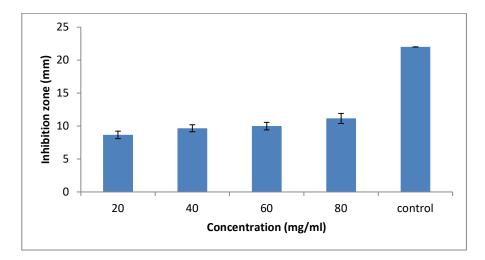


Figure 20. The antibacterial activity in the methanolic extract of *Diplazium* esculentum (Retz.) Sw. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

Table 13. F- and p-values of One-Way ANOVA for the inhibition zone in *E. Coli* subjected to various concentrations of methanolic extract of *Diplazium esculentum* (Retz.) Sw. The marked effects are significant at  $p \le 0.05$ 

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	1909.24	0.00*
2	CrtlXC1	1909.24	0.00*
3	CrtlXC2	1419.16	0.00*
4	CrtlXC3	1232.26	0.00*
5	CrtlXC4	391.13	0.00*
6	C1XC2	175.39	0.00*
7	C1XC3	473.67	0.00*
8	C1XC4	727.13	0.00*
9	C2XC3	49.95	0.02*
10	C2XC4	365.87	0.00*
11	C3XC4	700.12	0.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>

Table 14. F- and p-values of One-Way ANOVA for the inhibition zone in *Klebsiella pneumoniae* subjected to various concentrations of methanolic extract of *Diplazium esculentum* (Retz.) Sw. The marked effects are significant at p≤ 0.05.

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	1906.68	0.00*
2	CrtlXC1	1906.68	0.00*
3	CrtlXC2	1760.33	0.00*
4	CrtlXC3	1407.60	0.00*
5	CrtlXC4	1231.57	0.00*
6	C1XC2	130.06	0.00*
7	C1XC3	261.09	0.00*
8	C1XC4	461.23	0.00*
9	C2XC3	72.16	0.01*
10	C2XC4	299.22	0.00*
11	C3XC4	780.10	0.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-</sup>

Table 15. F- and p-values of One-Way ANOVA for inhibition zone in *Bacillus* subtilis subjected to various concentrations of methanolic extract of *Diplazium* esculentum (Retz.) Sw. The marked effects are significant at  $p \le 0.05$ .

Sl. No	Source of variance	F- Value	P- value
1	CrtlXC1XC2XC3XC4	2346.50	0.00*
2	CrtlXC1	2346.50	0.00*
3	CrtlXC2	2136.14	0.00*

4	CrtlXC3	1665.62	0.00*
5	CrtlXC4	1140.17	0.00*
6	C1XC2	3.57	0.13
7	C1XC3	390.76	0.00*
8	C1XC4	274.42	0.01*
9	C2XC3	200.41	0.03*
10	C2XC4	200.70	0.00*
11	C3XC4	180.14	0.05*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>.

# 5.3.4: Effect of *Selaginella bisulcata* Spring. extract against *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus subtilis*

The antibacterial activities in methanolic extract of *S. bisulcata* were evaluated by the agar-well-diffusion technique. The antibacterial activity of the extract was quantified at concentrations 20, 40, 60 and 80 mg ml<sup>-1</sup> against three strains of bacteria. The activity was measured in terms of inhibition zone and compared with the inhibition zone caused by a commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extracts of *S. bisulcata* caused  $8.33\pm0.57$ ,  $9.33\pm0.57$ ,  $11.00\pm0.90$  and  $13.33\pm0.57$  mm inhibition zone in agar plate of *E. coli* (ATCC-10536), respectively (Figure 21). The known antibiotic treatment has caused an inhibition zone of 25 mm. Results suggested that antibacterial activity of extract increased with an increase in the concentration of extract. The

antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *S. bisulcata* against *E. coli* (ATCC-10536) was 53.32% when weighed with the antibacterial efficiency of a commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extracts of *S. bisulcata* caused 8.66±0.57, 10.33±0.33, 11.66±0.57 and 12.66±0.57 mm inhibition zones in the agar-nutrient plate of *K. pneumoniae* (ATCC - 10031), respectively (Figure 22). The treatment with known antibiotic caused an inhibition zone of 24.0±0.00 mm. The inhibitory potential of methanolic extract of *S. bisulcata* increased with an increase in the concentration of the extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *S. bisulcata* against *K. pneumoniae* (ATCC-10031) was 52.75% when compared with the inhibitory potential of a commercial antibiotic.

The 20, 40, 60 and 80 mg ml<sup>-1</sup> methanolic extracts of *S. bisulcata* caused  $8.00\pm0.57$ ,  $8.33\pm0.53$ ,  $10.33\pm0.57$  and  $11.66\pm0.76$  mm inhibition zones in agar plate of *B. subtilis (ATCC-11774)*, respectively (Figure 23). The known antibiotic treatment has caused an inhibition zone of 22 mm. The antibacterial potential of methanolic extract of *S. bisulcata* increased with a gradual increase in the concentration of the extract. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *S. bisulcata* against *B. subtilis (ATCC-11774)* was 53.0% when compared with the antibacterial potential of a tested commercial antibiotic.

Above results suggested that methanolic extract of *S. bisulcata* possess on a par antibacterial activity against all the three strains of bacteria. Weighed up to the

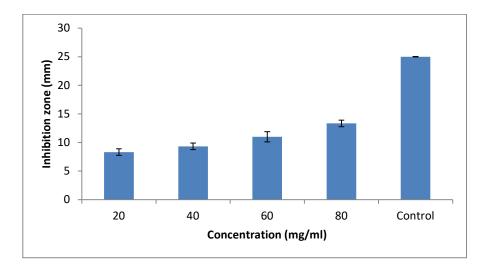


Figure 21. The antibacterial activity in the methanolic extract of *Selaginella bisulcata* Spring. against *E. coli*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

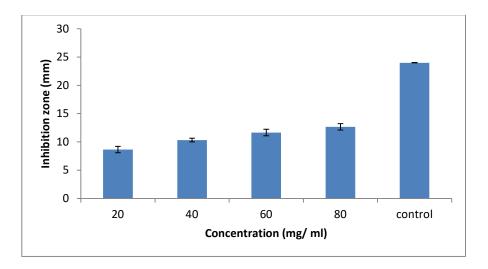


Figure 22. The antibacterial activity in the methanolic extract of *Selaginella bisulcata* Spring. against *Klebsiella pneumoniae*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

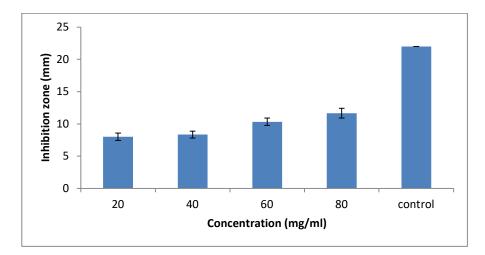


Figure 23. The antibacterial activity in the methanolic extract of *Selaginella bisulcata* Spring. against *Bacillus subtilis*. The values are mean of three replicates. Vertical bars show  $\pm$  SE.

antibacterial potential of a commercial antibiotic, the extract manifested 53% efficiency in inhibition of bacterial growth.

The results of F- and p-values of one-way ANOVA for the inhibition zone in three selected strains of bacteria is given in Tables 10, 11, 12, 13, 14, 15, 16, 17 and 18)

Table 16. F- and p-values of One-Way ANOVA for inhibitiion zone in *E. Coli* subjected to various concentrations of methanolic extract of *Selaginella bisulcata* Spring. The marked effects are significant at  $p \le 0.05$ .

Sl. No	Source of variation	F- Value	P- value
1	CrtlXC1XC2XC3XC4	2136.14	0.00*
2	CrtlXC1	2136.14	0.00*
3	CrtlXC2	1912.35	0.00*
4	CrtlXC3	1392.00	0.00*
5	CrtlXC4	990.02	0.00*
6	C1XC2	49.33	0.02*
7	C1XC3	212.51	0.00*
8	C1XC4	851.26	0.00*
9	C2XC3	85.96	0.01*
10	C2XC4	585.53	0.00*
11	C3XC4	481.52	0.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>

Table 17. F- and p-values of One-Way ANOVA for inhibition zone in *Klebsiella* pneumoniae subjected to various concentrations of methanolic extract of Selaginella bisulcata Spring. The marked effects are significant at  $p \le 0.05$ 

Sl. No	Source of variation	F- Value	P- value
1	CrtlXC1XC2XC3XC4	1737.36	0.00*
2	CrtlXC1	1737.36	0.00*
3	CrtlXC2	537.89	0.00*
4	CrtlXC3	1112.82	0.00*
5	CrtlXC4	970.02	0.00*
6	C1XC2	20.74	0.01*
7	C1XC3	123.24	0.00*
8	C1XC4	221.30	0.00*
9	C2XC3	16.72	0.60
10	C2XC4	10.07	0.03*
11	C3XC4	189.48	0.03*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>

Table 18. F- and p-values of One-Way ANOVA for inhibiton zone in *Bacillus* subtilis subjected to various concentrations of methanolic extract of *Selaginella* bisulcata Spring. The marked effects are significant at  $p \le 0.05$ 

Sl. No	Source of variation	F- Value	P- value
1	CrtlXC1XC2XC3XC4	1737.36	.00*
2	CrtlXC1	1737.36	.00*
3	CrtlXC2	1516.67	.00*
4	CrtlXC3	963.98	.00*
5	CrtlXC4	763.13	.00*
6	C1XC2	14.31	.01*
7	C1XC3	5.89	.07
8	C1XC4	33.99	.04*
9	C2XC3	.01	.90
10	C2XC4	11.08	.02*
11	C3XC4	290.14	.00*

Crtl: Control, C1: 20 mg ml<sup>-1</sup>, C2: 40 mg ml<sup>-1</sup>, C3: 60 mg ml<sup>-1</sup>, C4: 80 mg ml<sup>-1</sup>

# **5.4. DISCUSSION**

In the present study, phytochemical screening of chloroform extract of *Lycopodiella cernua* (L.) Pic. Serm. *shows the presence of* carbohydrates, protein, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids whereas alkaloid and tannins were lacking in chloroform extract. In methanol extract, all the phytochemicals tested were present apart from carbohydrates, tannins and terpenoids. In the aqueous extract, the phytochemical tested were present except carbohydrates, tannins and terpenoids whereas in petroleum ether extract all the phytochemical compounds tested were absent (Table 7).

Results showed that the chloroform extract of *Diplazium esculentum* (Retz.) Sw. possesses alkaloid, carbohydrates, protein, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids whereas tannins were absent. In methanol extract, all the phytochemicals tested were present except saponins, tannins and terpenoids. In the aqueous extract, the phytochemical tested were present except carbohydrates, proteins, tannins and phytosterols. In petroleum ether extract all the phytochemical compounds tested in this study were absent (Table 8).

Alkaloid, carbohydrates, protein, tannins, terpenoids, phenols, glycosides, saponins, phytosterols and flavonoids were detected in chloroform extract of *Selaginella bisulcata* Spring. In methanol extract, all the phytochemical tested were present except tannins and glycosides. In the aqueous extract, the all phytochemicals tested were detected except carbohydrates, proteins, tannins, phenol, phytosterols and flavanoid whereas in petroleum ether extract all the phytochemical compounds tested were absent. (Table 9).

It can be concluded that the selection of the solvent has a profound influence in phytochemial profile of plants. In the present study, methanolic extract *of L. cernua*, *D. esculentum and S. bisulcata.* were further evaluated for antibacterial activity because it demonstrated an intermediate diversity of phytochemicals compared with methanol and aqueous extracts.

The remarkable antibacterial activity may be associated with phenolic compounds present in methanolic extracts of all three species of Pteridophytes. The

phenolic compounds have been of considerable interest to humans because of their pronounced physiological, medicinal and antibacterial properties (Herrero, 2013)

In the present study, the inhibitory effect of methanol extract of *L. cernua*, *D. esculentum*. and *S. bisulcata*. were also evaluated against three bacterial strains viz *Escherichia coli (ATCC - 10536), Klebsiella pneumoniae (ATCC - 10031), Bacillus subtilis (ATCC- 11774)*. The results revealed that methanol extracts were potent antibacterial against all the microorganisms studied.

Similar to the present study there are several reports on antibacterial activity of Pteridophytes. An extract of *Athyrium filix-femina*, *Dryopteris affinis* and *Dryopteris filix-mas* shows an inhibition zone of  $8.50\pm 0.70$ ,  $9.00\pm 1.41$  and  $8.0\pm 0.0$ respectively; tested on five pathogenic bacteria (Soare *et al.*, 2012). The leaf extract of *Angiopteris helferiana*, *Cyathea brunoniana* and *Pronephrium nudatum* has also been tested effective against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Kathakali Nath *et al.*, 2017). Also, pteridophyte like *Botrychium lanuginosum*, *Dryopteris cochleate*, *Polystichum molluscens*, *Polystichum squarrosum*, *Salvinia radicata*, *Sphaerostephanos unitus*, *Pteris cretica* and *Pteris vittata* are documented to have anti-bacterial activity (Singh, 1999).

The presence of phytoconstituents like flavonoid, phenols, tannins and other phytoconstituents in methanol extracts are known to have anti-microbial agents, that is why the difference in susceptibility of various test bacteria towards the extracts as observed in the study could be due to the nature of anti-microbial agents present in the extracts and their mode of action on the different test bacteria. (Rizvi *et al.*, 2011).

Although the attempt has not been made in the present study, other researchers have isolated several active compounds from Pteridophytes. Two glycosides, 6'-0-(3,4-dihydroxy benzoyl)  $\beta$ -d-glucopyranosyl ester and 4-O- $\beta$ -d-glucopyranoside-3-hydroxy methyl benzoate along with five other compounds namely methyl benzoate, hypogallic acid, caffeic acid, paeoniflorin and pikuroside are isolated from a fresh water fern *Salvinia molesta* (Choudhary *et al.*, 2008). Also from the whole plant of *Pteris multifida*, which is used for treating antipyretic, detoxification, antibiotic, anti-inflammatory, and antimutagenic activity (Lee and Lin, 1988) several bioactive compounds have been isolated like luteolin-7-*O*-glucoside (Murakami and Machashi, 1985; Lu *et al.*, 1999), 16-hydroxy-kaurane-2- $\beta$ -D-glucoside (Liu and Qin, 2002), luteolin, palmitic acid, apigenin 4-*O*- $\alpha$ -L-rhamnoside (Lu *et al.*, 1999; Qin *et al.*, 2006), quercetin, hyperin, isoquercitrin, kaempferol, rutin (Lu *et al.*, 1999; Wang *et al.*, 2010; Hoang and Tran, 2014).

Results suggested that the extracts of all the three pteridophytes examined in the present study possess an antibacterial activity which can be used as a potent drug for inhibiting the growth of pathogenic gram (-) as well as gram (+) bacteria. Further studies are required to isolate bioactive compounds from three fern species.



Photo plate 19:

- a. Soxhlet apparatus used for plant extraction
- b. Rotary evaporator used for concentration of active components
- c. Crude extract stored in a container for further used
- d. Phytochemical screening of selected plants extract

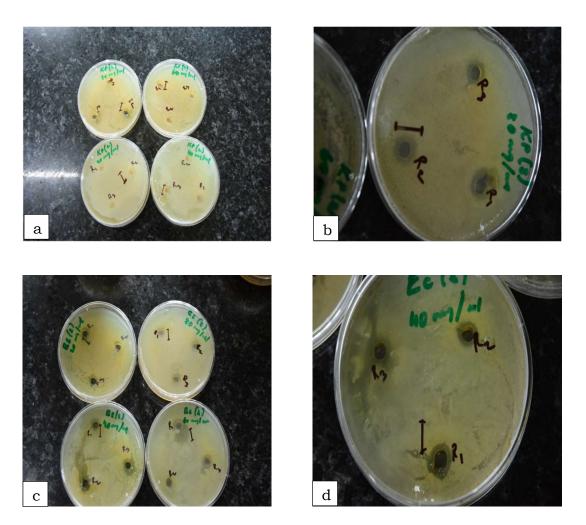


Photo plate 20:

- a. Extract of *Selaginella bisulcata* Spring. tested on *Klebsiella pneumoniae* at various concentration
- Extract of Selaginella bisulcata Spring. showing clear inhibition zone on Klebsiella pneumoniae at 80 mg ml<sup>-1</sup> concentration.
- c. Extract of Lycopodiella cernua L. tested on Bacillus subtilis at various concentration
- d. Extract of *Lycopodiella cernua* L. showing clear inhibition zone on *Bacillus* subtilis at 40 mg ml<sup>-1</sup> concentration.

#### **CHAPTER- VI**

# DETERMINATION OF TOTAL PHENOLICS, FLAVONOIDS CONTENTS AND ANTI-OXIDANT ACTIVITIES

## **6.1. INTRODUCTION**

Plant phenolics constitute the primary source for the majority of FDAapproved agents and are continued to be one of the key sources of inspiration for subsequent drug discovery (Bhuwan *et al.*, 2011). Plant phenolics possess tremendous free-radical scavenging and antioxidant activities. Phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins, stilbenes) are one of the main free radical scavenging molecules in plants (Cai *et al.*, 2003; Zheng and Wang 2001). Epidemiological studies have shown that many of these compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Owen *et al.*, 2000; Sala *et al.*, 2002; Cushnie and Lamb 2005; El-Hela *et al.*, 2011).

Phenolic or phenol carboxylic acids are main plant phenolic compounds having one carboxylic group. In plants, phenolic compounds are found mainly in a bound form such as amide, ester, or glycosides (Pereira *et al.*, 2009). Phenolic compounds are further classified in to two groups: droxybenzoic and hydroxycinnamic acids. Ferulic, caffeic, *p*-coumaric, and sinapic acids are the most abundant hydroxycinnamic acids in plants and they are derived from cinnamic acid. Hydroxybenzoic acids are found in soluble form and possess standard structure as C6-C1. The *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids are the most commonly found hydroxybenzoic acids in some plants at low concentrations.

Polyphenolics compounds are secondary metabolites synthesized through the shikimate pathway from L-phenylalanine and L-tyrosine. The shikimate or shikimic acid pathway involves the conversion of simple carbohydrate into phenylalanine and tryptophan. The shikimate pathway is the main source of the phenolics in bacteria, fungi and plants. Although plants have abundant phenolics, the role of phenolic compounds in plants is not completely known. Some known functions of phenolics in plants are nutrient uptake, as a structural component, enzyme activity, protein synthesis, photosynthesis, and allelopathy (Lyu *et al.*, 1990). Plant phenolic acids serve as signaling and play a key role in plant defense.

Phenolic acids receive broad therapeutic applications such as: antioxidant, anticancer, anti-inflammatory, anti-diabetic, neuro-protective, and antimicrobials. Plant phenolics are antioxidants or free radical scavengers. Plant extracts with significant phenolic contents also show high flavonoid content as reported for other plant species (Makepeace *et al.*, 1985). ROS (reactive oxygen species) have been considered to cause harm to live organisms and therefore occupy a significant role in many human diseases such as arthritis, myocardial infarction, atherosclerosis, diabetes mellitus and cancer (Elzaawely and Tawata, 2012). The phenolic acids have very high market demand as they are precursors of other bioactive compounds commonly used in therapeutics, cosmetics, and food industries.

Flavonoids are important secondary metabolites and are found ubiquitously in plants. Flavonoids are hydroxylated polyphenolic substances with a benzo- $\gamma$ -pyrone structure. Flavonoids exhibit antioxidant, anti-inflammatory and antiallergic properties. Due to the presence of hydroxyl groups, the flavonoids are good antioxidant and showed high free radicals scavenging properties as well as metal chelating capacity (Mishra and Pandey 2013). Other reported pharmaceuticals activities of flavonoids are hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral (Kumar and Pandey 2013). The synthesis of flavonoid in plants is initiated by the microbial attack and flavonoid levels in plants is greatly influenced by harvest time, shade netting, planting time, development stage etc. Many types of medicinally important flavonoids have been extracted from ferns (Kumar and Pandey 2013). Ferns have some unusual flavon-4-ol glycosides and flavones (Kumar and Pandey 2013). Wang et al., (2017) analyzed flavonoid contents in extracts of 49 ferns from Tianmu Mountain, China and showed the total flavonoid contents of 49 ferns ranged from 3.3 to 191.7 mg g<sup>-1</sup> (w/w). Cao et al. (2012) reported total flavonoid content as 14 per cent in a full plant of fern Dryopteris erythrosora.

Several studies have reported antioxidant activities of ferns (Shin 2010; Soare *et al.*, 2012; Gupta *et al.*, 2014) have evaluated eight species of ferns from Meghalaya, India and showed that *Aleuritopteris flava* and *Lindsaea odorata* possess the best antioxidant activity compared to other ferns like *Pteris scabristipes, Microlepia rhomboidea, Diplazium esculentum, Asplenium khasianum, Microlepia hallbergii and Adiantum edgeworthii.* Due to the high content of antioxidant

compounds ferns can be used as a therapeutic substance in preventing oxidative stress-related diseases.

North-East India, including Mizoram, enjoys a tremendous diversity of Pteridophytes, ferns represent an important food supplement in various ethnic communities in North East India. Limited studies have been carried out on determination of phytochemical contents and antioxidant activities of ferns of Mizoram, India. Work described in this Chapter aimed to quantitatively determine the total polyphenols and flavonoids contents in the methanolic and chloroform extracts obtained from the three abundant species of Ferns in Mizoram. *Lycopodiella cernua, Diplazium esculentum* and *Selaginella bisulcata*. The antioxidant activities in three above fern species were equally determined.

#### **6.2. MATERIALS AND METHODS:**

#### 6.2.1. PREPARATION OF PLANT EXTRACTS

The dried plants of *L. cernua, D. esculentum and S. bisulcata* were macerated to powder form using a hand grinder. The plant powder was subjected to a sequential continuous hot extraction in a Soxhlet apparatus using chloroform or methanol as a solvent. Extraction was run for 72 h. The methanolic and chloroform extracts were concentrated separately in a vacuum rotary evaporator (Buch iRotavapor® R-215). The plant extracts produced in the form of semi-solid mass were refrigerated at 4°C until further use.

#### **6.2.2. DETERMINATION OF TOTAL POLYPHENOLS CONTENT**

Total phenolic content of extracts of *L. cernua*, *D. esculentum and S. bisulcata* were determined according to the method of Mc Donald *et al.* (2001) with Folin Ciocalteu reagent. The extract of the pteridophytes was prepared by taking 10 mg of semi-solid extract in a test tube and 10 ml of methanol (90%, v/v) was added. It was next diluted with 10- fold from the stock solution. After dilution 1 ml of plant extract (50  $\mu$ g ml<sup>-1</sup>) was mix with 5 ml of FCR. After three minutes, 4.0 ml of sodium carbonate solution (0.7 M) was added, and the mixture was allowed to stand for 1 hour at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. 1 ml extract (50  $\mu$ g ml<sup>-1</sup>) was also mixed with the reagents above and after 1 hour the absorbance was measured to determine total plant phenolic content. With the help of the calibration curve, the phenolic concentrations of extracts were determined and expressed as Gallic acid equivalents (GAE) per g of dried extract. Preparation on standard gallic acid calibration curve is described in chapter 2 (Material and Methods).

# 6.2.4. QUANTITATIVE DETERMINATION OF TOTAL FLAVONOID CONTENT

Total flavonoid contents of the extracts were determined by the aluminium chloride method (Chang *et. al.*, 2002). The extract of the plants was prepared by taking 10.0 mg of extract in a test tube and 10 ml of methanol was added. It was then diluted with 10-fold from the stock solution. After dilution 1.0 ml of the extract (50  $\mu$ g ml<sup>-1</sup>) was mixed with 2 ml of distilled water. After five minutes, 3.0 ml of 5% sodium

nitrite (NaNO<sub>2</sub>) and 0.3 ml of 10% aluminum chloride (AlCl<sub>3</sub>) were added. After six minutes, 2.0 ml of NaOH (1.0 M) was added, and the volume was made up to 10 ml with distilled water. It was subsequently incubated at room temperature for 1.0 hour and absorbance was taken at 510 nm in a UV-Vis spectrophotometer (Thermo Scientific Evolution TM-200). A standard calibration curve at different concentrations (10, 20, 40, 60, 80, and 100  $\mu$ g ml<sup>-1</sup>) of Quercetin was prepared and used for quantification of total flavonoid content (Chapter 2: Material and Methods). From the calibration curve, the total flavonoid content was determined and expressed as milligrams of quercetin equivalents (QE) per g of extract.

## 6.2.6. DETERMINATION OF ANTIOXIDANT CAPACITY

DPPH (2, 2-diphenyl-1-picrylhydrazil) radical scavenging was carried out according to the method described by Kim *et al.* (2017). A standard stock solution of the plant extracts was made by dissolving a 10 mg of plant extract in 10 ml of distilled water to get 0.0005, 0.001, 0.005, 0.01, 0.025 and 0.05 mg ml<sup>-1</sup> concentrations of each plant extracts. The 3.0 ml of plant extract was subsequently mixed with 1.0 ml of 0.1 mM DPPH solution (in MeOH) and the volume was made six ml with distilled water in a test tube. The reaction mixture was furthermore vortex and incubated at 37<sup>o</sup>C for 30 minutes. The absorbance of the solution was then measured at 517 nm using Thermo Scientific EVOLUTION 200 UV-Visible spectrophotometer. The percentage of DPPH scavenging was calculated by comparing the absorbance values of the test samples with those of the controls (not treated with plant extract). The Free radical scavenging percentage (I%) was calculated as radical scavenging activity as follows:

$$I(\%) = \left(\frac{Absorbance \ of \ control - Absorbance \ of \ sample}{Absorbance \ of \ Control}\right) x100$$

The free radical scavenging capacity is expressed as an  $IC_{50}$  value defined as the concentration (µg ml<sup>-1</sup>) of extract that inhibits the formation of DPPH radicals by 50%. Lower the  $IC_{50}$  values the better the radical scavenging capacity.

## 6.3. RESULTS

## **6.3.1. TOTAL POLYPHENOL CONTENT**

The total phenolic contents in the chloroform and methanolic extracts of three fern species were determined from the calibration curve (Figure 24) and results are shown in Table 19 and Table 20, respectively.

Table 19. Polyphenol contents (mean± SE) in mg GAE per g of chloroformextract. Values are mean of three replicates ± SE.

S.No.	Fern species	Total phenols
1.	Lycopodiella cernua	134.60±0.03
2.	Diplazium esculentum	71.30±0.02
3.	Selaginella bisulcata	127.20±0.02

 Table 20. Polyphenol contents (mean± SE) in mg GAE per g of methanolic

 extract. Values are mean of three replicates ± SE.

Sl.No.	Fern species	Total phenols
1.	Lycopodiella cernua	137.50±0.01
2.	Diplazium esculentum	91.90±0.01
3.	Selaginella bisulcata	131.60±0.03

The polyphenol content in chloroform extracts of three fern species varies between 71.3 mg GAE g<sup>-1</sup> to 134.0 mg GAE g<sup>-1</sup>. The highest polyphenol content was observed in *L. cernua* followed by *S. bisulcata* and *D. esculentum*. Similarly, methanolic extract of *L. cernua* showed the highest (137.5 mg GAE g<sup>-1</sup>) phenolic content. The lowest polyphenolic content was found in the methanolic extract of *D. esculentum*. Results also showed that methanolic extraction gives higher polyphenol yield compared to chloroform extraction in all three examined fern species.

#### **6.3.2. TOTAL FLAVONOID CONTENT**

The total flavonoids content in chloroform and methanolic extracts of the three fern species was determined rom the calibration curve (Figure 25). The results are shown in Table 21 and Table 22.

Table 21. Total flavonoid contents (mean± SE) in mg QE per g of chlorofor	m
extract. Values are mean of three replicates $\pm$ SE.	

Sl. No.	Fern species	Total flavonoids
1.	Lycopodiella cernua	116.90±0.02
2.	Diplazium esculentum	72.79±0.01
3.	Selaginella bisulcata	72.79±0.02

 Table 22. Total flavonoid contents (mean± SE) in mg QE per g of methanol

 extracts of three fern species. Values are mean of three replicates ± SE

Sl.No.	Fern species	Total flavonoids
1.	Lycopodiella cernua	131.60±0.02
2.	Diplazium esculentum	87.50±0.04
3.	Selaginella bisulcata	102.20±0.03

*L. cernua* showed highest flavonoids content in chloroform as well as methanolic extracts compared to the other two fern species. However, polyphenol content was higher (131.6 mg QE g<sup>-1</sup>) in methanol extract of *L. cernua* compared to chloroform extract (116.90 mg QE g<sup>-1</sup>). Contrary to the above results, methanolic extract of *S. bisculata* showed higher total flavonoid content (102.2 mg QE g<sup>-1</sup>) compared to

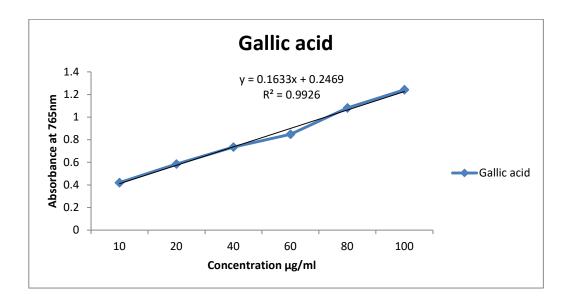


Figure 24. Calibration curve for gallic acid.

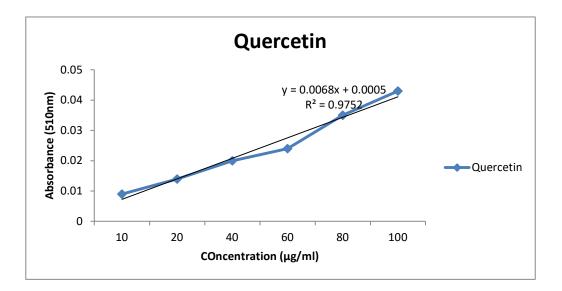


Figure 25. Calibration curve for quercetin.

chloroform extract (72.79 QE g<sup>-1</sup>). *D. esculentum* showed the least flavonoid content compared to *L. cernua* and *S. bisulcata*. The above results suggest that the selection of solvent for extraction profoundly affect the yield and quantification of flavonoids in plants.

# 6.3.3. DPPH RADICAL SCAVENGING ACTIVITY AND IC50

The antioxidant activities of chloroform and methanolic extract of *L. cernua*, *D. esculentum and S. bisulcata* were determined using a methanol solution of DPPH reagent.

The antioxidant activities of methanol extract from *L. cernua*, *D. esculentum* and *S. bisulcata* were expressed in terms of percentage of DPPH scavenging activity and IC<sub>50</sub> values in  $\mu$ g ml<sup>-1</sup> (Table 23, 24 and 25). BHT was used as the standard compound, parallel to an examination of the antioxidant activity of plant extracts; the values for the standard compound were obtained and compared to the values of the antioxidant activity.

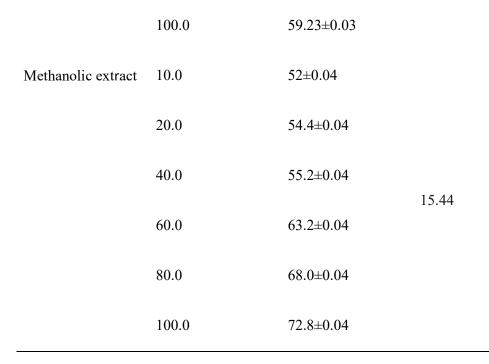
Table 23. Results of DPPH radical scavenging assay of BHT. Values are mean ±SE of three replicates.

Extract	Concentration	DPPH Scavenging activity, I (%)	
	(µg ml <sup>-1</sup> )		(µg ml <sup>-1</sup> )
BHT	10.0	52.73±0.01	2.62

20.0	55.38±0.01
40.0	65.20±0.02
60.0	72.10±0.01
80.0	81.05±0.03
100.0	85.35±0.03

Table 24. Results of DPPH radical scavenging assay of chloroform and methanolic extract of *Lycopodiella cernua*. Values are mean  $\pm$  SE of three replicates.

Extract	Concentration (µg ml <sup>-1</sup> )	DPPH Scavenging activity, I (%)	IC50 (μg ml <sup>-1</sup> )
Chloroform Extract	10.0	45.38±0.01	
	20.0	47.69±0.07	
	40.0	50.0±0.01	
	60.0	53.07±0.00	39.07
	80.0	56.15±0.03	



Results of DPPH scavenging assay showed that methanolic extract of *L*. *cernua* showed 7.2-folds higher antioxidant activity compared to chloroform extract as indicated by IC<sub>50</sub> values obtained for both extracts. IC<sub>50</sub> values obtained for both types of extracts of *D. esculentum* were higher than values obtained for the extracts of *L. cernua* and suggested that *L. cernua* has higher free radical scavenging potential than *D. esculentum*. For, the IC<sub>50</sub> value was higher for the methanolic extract compared to chloroform extract. The aforementioned results suggested that methanolic extract has better antioxidant activity compared to chloroform extract. The similar results were obtained for the radical scavenging potential of methanolic and chloroform extracts of *S. bisulcata*. The methanolic extract of *S. bisulcata* showed 2.23 folds higher radical scavenging capacity compared to chloroform extract (Table 26) The three fern species evaluated in the present study can be arranged based on decreasing order of free radical scavenging potential as *L. cernua* > *S. bisulcata* > *D. esculentum*.

Table 25. Results of DPPH radical scavenging assay of chloroform and methanolic extract of *Diplazium esculentum*. Values are mean  $\pm$  SE of three replicates.

Extract	Concentration (µg ml <sup>-1</sup> )	DPPH Scavenging activity, I (%)	IC50 (μg ml <sup>-1</sup> )
Chloroform Extract	10.0	42.50±0.01	
	20.0	43.33±0.01	
	40.0	45.33±0.01	110.24
	60.0	46.67±0.01	118.34
	80.0	47.50±0.02	
	100.0	48.33±0.02	
Methanolic extact	10.0	37.27±0.01	
	20.0	40.0±0.002	105.43
	40.0	41.81±0.01	

60.0	46.36±0.03
80.0	47.27±0.01
100.0	48.18±0.00

Table 26. Results of DPPH radical scavenging assay of chloroform and methanolic extract of *Selaginella bisulcata*. Values are mean  $\pm$  SE of three replicates.

Extract	Concentration (µg ml <sup>-1</sup> )	DPPH Scavenging activity, I (%)	IC50 (μg ml <sup>-1</sup> )
Chloroform Extract	10.0	42.48±0.01	
	20.0	43.36±0.01	
	40.0	43.36±0.01	100.05
	60.0	44.25±0.01	133.37
	80.0	46.02±0.01	
	100.0	46.90±0.01	
Methanolic extact	10.0	43.69±0.01	59.76

20.0	45.38±0.02
40.0	47.06±0.02
60.0	50.42±0.02
80.0	52.10±0.02
100.0	55.46±0.01

### **6.4. DISCUSSION**

Since ancient time ferns are being used as food and medicine. Ferns are well known for their antioxidant properties (Shin and Lee 2010; Chai *et al.*, 2012). In Mizoram and other North East region of India Ferns are sold in local markets. It is reported that the nutritional values of ferns are superior to other leafy vegetables (Voon and Kueh 1999). Along with food values, the ferns are vital part of traditional medicine in many countries to treat fever, skin diseases, ulcers and stomach-ache (Benjamin and Manickam 2007). Phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins) are one of the main free radical scavenging molecules in ferns (Cai *et al.*, 2003; Zheng and Wang 2001). Many of these compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities (Owen *et al.*, 2000; Sala *et al.*, 2002; Cushnie and Lamb 2005; El-Hela *et al.*, 2011).

Plant phenolics comprise an extensive group of compounds acting as primary antioxidants or free radical scavengers. Plant extracts with significant phenolic contents also show high flavonoid content as reported for other plant species (Makepeace et al., 1985). ROS (reactive oxygen species) have been considered to cause harm to live organisms and therefore play a significant role in many human diseases like arthritis, myocardial infarction, atherosclerosis, diabetes mellitus and cancer (Elzaawely and Tawata 2012). In addition, flavonoids comprise a class of secondary plant metabolites endowed with significant antioxidant and chelating attributes (Heim et al., 2002). Chloroform and methanolic extracts from L. cernua, D. esculentum and, S. bisulcata possess a good amount of total flavonoids, which is in correlation with intense antioxidant activity of these extracts. The antioxidant activity may be because of the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, quinones and other structural motifs (Patt and Hudson 1990, Demiray et al., 2009). The commercially available synthetic antioxidants have been suspected of causing or instigating negative health effects, so harsh restrictions imposed over their application and there is an urgent trend to substitute them with naturally occurring antioxidants (Hosny and Rosazza 2002; Molyneux 2004) while the intake of natural antioxidants has been associated with the concomitant reduced risks of cancer, cardiovascular disease, diabetes, and other diseases related with age as they have the advantage of being virtually devoid of side effects (Yang et al., 2001; Sun et al., 2002).

In the present study, the total phenolic contents in the examined chloroform extracts ranged from 1.323 to 10.15 mg GAE g<sup>-1</sup>, whereas the extract from methanol

compound ranges from 0.772 to 8.564 mg GAE g<sup>-1</sup>. The highest amount of total phenolics in chloroform extract was detected in the extract of *L. cernua*, followed by an extract of *S. bisculata* while the extract of *D. esculentum* has the lowest phenolic content (Table 19). Also, in methanol extract the highest amount of phenolic content was found in *L. cernua* followed by *S. bisulcata* and the lowest phenolic content was found in *D. esculentum* (Table 20).

Although limited studies have been carried out on determination of phenolic contents in pteridophyte, comparison of results is critical because of various units used in conflicting studies. Edible fern *Stenochlaena palustris* contains 51.69 mg g<sup>-1</sup> dry matter total polyphenol, 58.05 mg g<sup>-1</sup> dry matter flavonoids, and 48.80 mg g<sup>-1</sup> dry matter hydroxycinnamic acids (Chai *et al.*, 2012). Soare *et al.*, (2012) have reported polyphenols content as 2340 mg GAE  $100^{-1}$  g FW in *Dryopteris filix-mas* and 887.0 mg GAE  $100^{-1}$  g FW in *D. affinis*. A study carried out in Tunisia has reported phenolic contents of ethyl acetate extracts in a range of 49.3 to 55.4 mg GAE g<sup>-1</sup> into two ferns *Asplenium adiantum-nigrum and Asplenium trichomanes* (Hammami *et al.*, 2016).

The total phenolic contents in the extracts of the three plant species under study depend on the type of solvent, i.e. the polarity of solvent used in extraction. Therefore, the elevated concentration of these compounds may be because of high solubility of phenols in solvents like CHCl<sub>3</sub>, MeOH (Mohsen and Ammar 2008; Zhou and Yu 2004). The concentration of flavonoids in chloroform extracts ranged from 6.617 to 11.02 mg QE g<sup>-1</sup> (Table 21), and in methanol extract, the concentration ranges from 8.09 to 12.50 mg QE g<sup>-1</sup> (Table 22). The intensest amount of total flavonoids in chloroform extract was found in *L. cernua*, followed by *S. bisculata* and *D. esculentum* whereas the highest concentration of flavonoid in methanol extract was obtained in *L. cernua* followed by *S. bisulcata* and the lowest concentration was recorded in *D. esculentum*. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao 2005). Several reports have conclusively provided the correlation between antioxidant activity and the amount of total phenolics/total flavonoids (Negro *et al.*, 2003; Ramadeep and Geoffrey 2005; Anna *et al.*, 2003).

The more significant contents of phenolics and flavonoids prompt us to determine the antioxidant activities of three fern species. The free radical scavenging capacity of chloroform and methanolic extracts of all three ferns was studied. Similar to phenolics and flavonoids contents, the free radical scavenging also varied in three fern species viz. *L. cernua., D. esculentum* and *S. bisulcata*. The DPPH radical scavenging capacity was determined at several concentrations of extract ranging from 10-100  $\mu$ g ml<sup>-1</sup> of the plant extracts. It was observed that the DPPH free radicals scavenging capacity of the extracts increased with increasing the concentrations of the extract. The above results suggested that free radical scavenging capacity of all three tested ferns depends on the concentration of extract. In CHCl<sub>3</sub> extract of *L. cernua* the DPPH radical scavenging ranges from 45.38 to 59.23 per cent inhibition whereas in MeOH extract the inhibition percentage ranges

from 52 to 72.8 with a concentration range from 10-100µl mg<sup>-1</sup>. In CHCl<sub>3</sub> extract of *S. bisulcata* the inhibition ranges from 42.48 to 47.79 per cent whereas in MeOH extract the inhibition percentage ranges from 43.69- 48.18 per cent with a concentration range from 10-100 µl mg<sup>-1</sup>. In CHCl<sub>3</sub> extract of *D. esculentum* the obtained values range from 42.5 to 48.33 per cent inhibition whereas in MeOH extract the inhibition percentage ranges from 37.27 to 48.18 with a concentration range from 10 to100 µg ml<sup>-1</sup>. Amongst the three Pteridophytes studied, the most substantial capacity to neutralize DPPH radicals was found in methanolic extract of *L.cernua* which neutralized 50% off free radicals at the extract concentration of 5.44 µg ml<sup>-1</sup> whereas the most diminished capacity to inhibit DPPH radicals scavenging was discovered in CHCl<sub>3</sub> extract of *S. bisulcata* for which the calculated IC<sub>50</sub> was 133.37µg ml<sup>-1</sup>. In comparison to IC<sub>50</sub> values of BHT, MeOH extract of *L. cernua* manifested the strongest capacity of DPPH neutralization.

Similar to results obtained in the present study, many studies have confirmed the radical scavenging and antioxidant properties in Pteridophytes. *Polystichum aculeatum* showed significant antioxidant properties with an IC<sub>50</sub> value of  $0.45\pm0.02$ µg ml<sup>-1</sup> (Valizadeh *et al.*, 2015). Fern *Asplenium trichomanes* ethyl acetate extract exhibited the antioxidant activity, DPPH IC<sub>50</sub> as  $0.59\pm0.05$  mg ml<sup>-1</sup> (Hammami *et al.*, 2016). Therefore, it is remarkable that ferns evaluated in the present study showed much higher radical scavenging capacity compared to reported capacity in previous studies. The methanolic as well as chloroform extracts of all three species of ferns examined in the present study exhibited antioxidant activities. The antioxidant activity may be attributed to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, quinones and other structural motifs (Patt and Hudson 1990, Demiray *et al.*, 2009). Similar to the present study, several reports have conclusively shown a correlation between the antioxidant activity and the amount of total phenolics or total flavonoids (Negro *et al.* 2003; Ramadeep and Geoffrey 2005). Plant extracts with high phenolic contents equally possess high flavonoid content as reported for other plant species (Sun *et al.*, 2002; Elzaawely and Tawata 2012). In our findings, the extracts that exhibit the highest antioxidant activity maintain the highest concentration of phenols too. Therefore, the phenolic content of plants may contribute directly to their antioxidant property (Tosun *et al.*, 2009). Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Borneo *et al.*, 2008; Katalinic *et al.*, 2004).

Based on the results, it can be concluded that methanol extracts of the three plants had a powerful *in vitro* antioxidant capacity against the various antioxidant systems in a dose-dependent manner.

#### CHAPTER-VII

#### **GENERAL DISCUSSION**

Pteridophytes are widely distributed throughout the world. About 250 million years ago Pteridophytes remained dominant vegetation on earth. Later on, the group Pteridophyta was gradually followed by seed-bearing plants (i.e. Gymnosperms and Angiosperms). Pteridophytes are considered as link between non-flowering and flowering plants. The world Pteridophytic flora consists of 12,717 species (Checklist of Ferns and Lycophytes of the World" by Michael Hassler and Bernd Schmitt 2020). According to an updated (January 2020) "Checklist of Ferns and Lycophytes of the World" by Michael Hassler and Evophytes of the World" by Michael Hassler and Lycophytes of the World" by Michael Hassler and Schmitt (2020), Indian Subcontinent and Indochina region possess 1642 species of Pteridophytes. As per the above checklist, the highest number of species are reported from India (1036 spp.) followed by Vietnam (855 spp.), Thailand (731 spp.), Nepal (550 spp.), Sri Lanka (338 spp.) and Pakistan (142 spp.).

In the present investigation, a total number of 105 Pteridophytes species from Phawngpui National Park, Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve has been collected. The present study showed that Polypodiaceae family possesses the most number of species (18 species) followed by Pteridaceae and Dryopteridaceae 12 species each while Thelypteridaceae has 10 species occupying the third vastest families recorded during the study. According to the study conducted all through India, the family Polypodiaceae accounts the highest number of species with 150 (Dixit, 2000) which correlate with our finding as Polypodiaceae being the family with the most number of species. Amongst the Pteridophytes that have been collected, 71 species out of 105 species recorded are terrestrial, while epiphytes and lithophytes have 17 species each. A total number of 45 species are present in all the four sites and 11 species a habit specific i.e. they are found barely in one place from the study site. Further, 5 species are rare and endangered species for Northeastern India (Bir, 1987). Among the four selected sites, Murlen National Park typically holds the highest number of Pteridophyte species (89 species, 44 genera and 21 families), followed by Tawi Wildlife sanctuary (85 species, 43 genera and 21 families) Dampa Tiger Reserve (69 species, 34 genera and 20 families) and, Phawngpui National Park (65 species, 32 genera and 18 families).

Beddome (1892) reported a total of 25 species of Pteridophytes from the then Cachar District and Sylhet District of Assam. In Manipur, there is a report of a total 20 ethnobotanical Pteridophytic plants belonging to 15 families (Yumkhan and Singh, 2011), In Meghalaya, a total of 113 species Pteridophytes are reported in the Nokrek Biosphere Reserve (Singh *et al.*, 2012). Benniamin (2011) conducted a study of medicinally valuable pteridophytes and had mentioned 51 pteridophytes belonging to 28 families. He had enumerated 76 species under 41 genera and 28 families in Arunachal Pradesh. From Mizoram, 36 species are recorded form Thorang Wildlife Sanctuary by Barbhuiya and Singh (2014), 19 species from Aizawl District and 27 species from Champhai District by Vanlalpeka and Laha (2014, 2015), 33 species from Ngengpui Wildlife Sanctuary by Verma *et al.*, (2013) and 35 species from Murlen by Sharma *et al.* (2017). Previous studies have reported 215 species from Assam (Borthakur *et al.*, 2000), 224 species from Meghalaya (Baishya and Rao, 1982), 280 species from Nagaland (Jamir and Rao, 1988), 338 species from Darjeeling and Sikkim (Mehra and Bir, 1964), 264 species from N. W. Himalayas (Dhir 1980), 239 species from Western Ghats (Manickam and Irudayaraj, 1992).

Compared to the present study the studies conducted in other States and regions showed higher species diversity. It is worthwhile to mention that the present study was confined to a specific location and not the whole state of Mizoram. Also, many factors like challenging terrain and anthropogenic activities like shifting cultivation, expansion of agriculture, the introduction of plantation crops, destruction of natural forest, collection of wood, infrastructure development and road construction play a role in the loss of the valuable pteridophytes resources. So. an urgent measure is demanded to prevent the anthropogenic activities, restored and to conserve the pteridophytic flora of these poorly explored regions.

The present study also aims at tapping out the valuable bioactive properties from overlooked plants so they can act as a substitute for other plants resources and with this in mind three species of Pteridophytes *Lycopodiella cernua* (L.) Pic. Serm., *Diplazium esculentum* Retz. and *Selaginella bisulcata* Spring. have been selected and were subjected for phytochemical screening, anti-bacterial activities test and antioxidant potential.

Extraction of secondary metabolites was the major objective of the present study. The results show the presence of alkaloid, carbohydrates, protein, terpenoids, phenols, glycosides, saponins, phytosterols, flavonoids and tannins in all the three species evaluated. A broad range of extraction solvents like water, methanol, acetone, chloroform, ethanol and their mixture with different polarity are frequently tested for the extraction of secondary metabolites from plants. In the present study water, methanol and chloroform were examined for the extraction of phytochemicals. We found that the selection of the extracting solvent has a profound influence in phytochemical profile of plants. A prior study has shown that polar solvent exhibits higher amount of bioactive compounds (Thouri *et al.*, 2017). In contrast to the above study, we observed the highest phytochemical diversity was noted in the methanolic extract followed by chloroform and aqueous extract of all species. Remarkably, there is no particular solvent which may be considered as standard.

In the present study, the three fern species *L. cernua*, *D. esculentum and S. bisulcata* were evaluated for antibacterial activities against three pathogenic bacterial strains namely *E. coli* (ATCC-10536), *K. pneumoniae* (ATCC-10031) and *B. subtilis* (ATCC-11774). *K. pneumoniae* belongs to Enterobacteriaceae family and *is* Gramnegative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It is one among the bacteria manifesting antibiotic resistance because of modification in the core genome. *B. subtilis* is spore-forming, motile, rod-

shaped, Gram-positive and facultative aerobe. It is utilized as pro-biotic and is commonly considered as non-pathogenic. However, several time the non-virulent *B. subtilis* causes illness in human (Weinstein and Colburn, 1950). *E. coli* is known for causing cholecystitis, cholangitis, urinary tract infection (UTI), and traveller's diarrhoea.

The methanolic extract of *L. cernua* showed 7.00±0.0, 7.5±0.0, 7.83±0.29 and 14.33±0.57 mm inhibition zone in agar plate of *E. coli* (ATCC-10536), 7.83±0.23, 9.2±0.57, 10.33±0.67 and 12.00±0.57 mm inhibition zone in agar plate of *K. pneumoniae* (ATCC-10031) and 7.00±0.00, 7.5±0.03, 8.00±0.00 and 9.00±0.00 mm inhibition zone in agar plate of *B. subtilis* (ATCC-11774), respectively. The antimicrobial efficiency of 80 mg L<sup>-1</sup> methanolic extract of *L. cernua* against *E. coli* (ATCC - 10536) was 57.3%, 50% against *K. pneumoniae* (ATCC - 10031) and 41 % against *B. subtilis* (ATCC-11774) when compared with a commercial antibiotic.

The study results revealed that methanol extracts were potent antibacterial against all the microorganisms studied. Besides the three pteridophytes subjected for test many other species can be tested to detect a potent antibacterial property like an extract of *Athyrium filix-femina*, *Dryopteris affinis* and *Dryopteris filix-mas* which shows an inhibition zone against five pathogenic bacteria (Soare *et al.*, 2012). The leaf extracts of *Angiopteris helferiana*, *Cyathea brunoniana* and *Pronephrium nudatum*, which are also found in Mizoram, have also been tested positive against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Kathakali Nath *et al.*, 2017). Also, Pteridophyte like *Botrychium lanuginosum*, *Dryopteris cochleate*,

Polystichum molluscens, Polystichum squarrosum, Salvinia radicata, Sphaero stephanosunitus, Pteris cretica and Pteris vittata are documented to retain antibacterial activity (Singh 1999). The presence of phytoconstituents like flavonoid, phenols, tannins in methanol extracts is known to have anti-microbial agents. The difference in susceptibility of various test bacteria towards extracts as observed in the study could be due to the nature of anti-microbial agents present in the extracts and their mode of action on the particular strains of bacteria (Rizvi *et al.*, 2011).

In the present study, the total phenolic contents in chloroform extracts and methanol extract which ranges from 1.323 to 10.15 mg GAE g<sup>-1</sup> in chloroform extract, whereas the extract from methanol compound ranges from 0.772 to 8.564 mg GAE g<sup>-1</sup>. The highest amount of total phenolics in chloroform extract and methanol was detected in the extract of *L. cernua*, followed by an extract of *S. bisculata* while the extract *of D. esculentum* has the lowest phenolic content. Although limited studies have been carried out on determination of phenolic contents in pteridophyte, comparison of results is impossible because of various units used in various studies. For example, *Stenochlaena palustris* contains 51.69 mg g<sup>-1</sup> dry matter total polyphenol, 58.05 mg g<sup>-1</sup> dry matter flavonoids, and 48.80 mg g<sup>-1</sup> dry matter hydroxycinnamic acids (Chai *et al.*, 2012). Soare *et al.* (2012) have reported polyphenols content as 2340 mg GAE 100<sup>-1</sup> g FW in *Dryopteris filix-mas* and 887 mg GAE 100<sup>-1</sup>g FW in *D. affinis*. A study carried out in Tunisia has reported phenolic contents of ethyl acetate extracts in a range of 49.3–55.4 mg GAEg<sup>-1</sup> in *Asplenium adiantum-nigrum and Asplenium trichomanes* (Hammami *et al.*, 2016).

The present study showed that the total phenolic content in the extracts of the three plant species depends on the type of extracting solvent, i.e. the polarity of solvent used in extraction. Therefore, the elevated concentration of these compounds may be because of high solubility of phenols in solvents like CHCl<sub>3</sub>, MeOH (Mohsen and Ammar 2008; Zhou and Yu 2004).

It was observed that flavonoids content in chloroform extracts ranged from 6.617 to 11.02 mg QE  $g^{-1}$  and in methanol extract from 8.09 to 12.50 mg QE  $g^{-1}$ . The intensest amount of total flavonoids in chloroform extract and methanol extract was found in L. cernua, followed by S. bisculata and D. esculentum. The more significant contents of phenolics and flavonoids inspired us to determine the antioxidant activities of three fern species. We determined the free radical scavenging capacity of chloroform and methanolic extracts of all three ferns. Similar to phenolics and flavonoids contents, the free radical scavenging ability also varied in three fern species L. cernua., D. esculentum and S. bisulcata. The DPPH radical scavenging capacity was determined at several concentrations of extract ranging from 10-100  $\mu$ g ml<sup>-1</sup>of the plant extracts. It was noted that the DPPH free radicals scavenging capacity of the extracts increased with gradual increment in the concentrations of the extract in the solvent. In CHCl<sub>3</sub> extract with a concentration range from 10-100  $\mu$ l mg<sup>-1</sup> of L. cernua the DPPH radical scavenging shows 45.38 to 59.23 per cent inhibition whereas in MeOH extract the inhibition percentage ranges from 52 to 72.8. In CHCl<sub>3</sub> extract of S. bisulcata, the inhibition ranges from 42.48 to 47.79 per cent whereas in MeOH extract the inhibition percentage ranges from 43.69 to 48.18. In CHCl<sub>3</sub> extract of *D. esculentum* the obtained values range from 42.5 to 48.33 per cent inhibition whereas in MeOH extract the inhibition percentage ranges from 37.27 to 48.18 with a concentration range from 10-100  $\mu$ l mg<sup>-1</sup>. Amongst the three pteridophytes tested, the highest capacity to neutralize DPPH radicals was found in methanolic extract of *L. cernua* (IC<sub>50</sub>= 5.44  $\mu$ g ml<sup>-1</sup>) whereas the most diminished capacity to inhibit DPPH radicals scavenging was discovered in CHCl<sub>3</sub> extract of *S. bisulcate* for (IC50= 133.37 $\mu$ g ml<sup>-1</sup>). Further, in comparison to IC<sub>50</sub> values of BHT, MeOH extract of *L. cernua* manifested the strongest capacity for neutralization of DPPH.

Similar to results obtained in the present study, many studies have confirmed the radical scavenging and antioxidant properties in Pteridophytes. *Polystichum aculeatum* showed significant antioxidant properties with IC<sub>50</sub> value of  $0.45\pm0.02 \ \mu g$ ml<sup>-1</sup>(Valizadeh *et al.*, 2015). Ethyl acetate extract of *Asplenium trichomanes* exhibited DPPH IC<sub>50</sub> as  $0.59 \pm 0.05 \ mg \ mL^{-1}$  (Hammami *et al.*, 2016). Therefore, it is remarkable that ferns evaluated in the present study showed much higher radical scavenging capacity compared to reported capacity in previous studies.

The methanolic, as well as chloroform extracts of all three species of ferns examined in the present study exhibited antioxidant activities. The antioxidant activity may be attributed to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, quinones and other structural motifs (Patt and Hudson, 1990, Demiray *et al.*, 2009). Plant extracts with high phenolic contents equally possess high flavonoid content as reported for other plant species (Sun *et al.*, 2002; Elzaawely and Tawata, 2012). In our findings, the extracts that exhibit the higher antioxidant activity showed the elevated concentration of phenols *per se*. Thus, the finding of the present study is in line with the results of an earlier study that showed phenolic content of plants contributes directly to their antioxidant property (Tosun *et al.*, 2009). Numerous investigations have further confirmed a direct linear correlation between the values of phenol concentration and antioxidant activity (Borneo *et al.*, 2008; Katalinic *et al.*, 2004). Based on the results, it can be concluded that methanol extracts of the two plants had a powerful *in vitro* antioxidant capacity in a dose-dependent manner.

Mizoram lies between  $21^{\circ}$  56'N – 24° 31'N latitudes and 92° 16'E – 93° 26'E longitudes sandwiched between two developing countries and it is of strategic significance geographically and because of its unique terrain and specific location the areas is rich in flora and fauna and naturally has a rich biodiversity with 10 ten protected sites for conserving its rich biodiversity. Out of the ten protected sites four sites viz Phawngpui National Park, Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve have been selected for the study sites. Based on three years (2013-2016) of field collections from the four selected sites a total number of 105 species of pteridophytes with 51 genera belonging to 24 families have been collected and identified. The identification of the plant specimen collected was made based on field observation, their distinctive characteristics, habit and their types, consulting various literature. Further, the plant specimen collected was mounted on to a standard herbarium sheet and was carefully brought to Botanical Survey of India (BSI), Shillong and CSIR- National Botanical Research Institute (NBRI), Lucknow for comparison and authentication.

In the present investigation which deals with the Pteridophytic flora of Mizoram which account 105 Pteridophytes species from Phawngpui National Park, Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve out of which Polypodiaceae family has the most number of species (18 species) which account for 17.14 per cent from the total pteridophytes collected, followed by

Pteridaceae and Dryopteridaceae with 11.43 per cent (12 species) each while Thelypteridaceae has 9.5 per cent(10 species) occupying the third largest families recorded during the study. The rest of the families like, Athyriaceae exhibit 8.57 per cent (nine species), Dennstaedtiaceae 7.62 per cent (eight species), Aspleniaceae 5.71 per cent (six species), Lindsaeaceae 4.76 per cent (five species) while the rest has lesser number of species with only 1 and 2 species accounting to their families. Also, in the present study it has been noticed that the terrestrial species constitute more than 68 per cent (71 species) out of 105 species recorded, while epiphytes and lithophytes communities represent approximately 16 per cent (17 species) each of the total pteridophytes species collected. A total no of 45 species are present in all the four sites and 11 species a habit specific i.e. they are found only in one place from the study site. Also, 5 species are rare and endangered species for Northeastern India (Bir 1987). Among the four selected sites, Murlen National Park typically holds higher number of pteridophyte species having a total no. of 89 species, 44 genera and 21 families, followed by Tawi Wildlife sanctuary 85 species, to 43 genera and 21 families from, 69 species, 34 genera and 20 families from Dampa Tiger Reserve and 65 species, 32 genera and 18 families from Phawngpui National Park. Most of the studied area exhibits a variety of macrohabitat which harbour good growth and colonization of several pteridophytes on different substrata. Because of difficult terrain and fewer anthropogenic activities, the studied area invariably showed luxuriant growth of pteridophytes like Adiantum phillipense L., Macrothelypteris torresiana (Gaudich.) Ching, Athyrium falcatum Bedd., Blechnum orientale L., Cyclosorus falcilobus Panigrahi, etc. But some part of the study region experienced anthropogenic problems like shifting cultivation, expansion of agriculture, the introduction of plantation crops, destruction of natural forest, collection of wood, infrastructure development and road construction, as a result, the valuable resources of pteridophytes are lost. The urgent need is to prevent the anthropogenic activities, restored and to conserve the pteridophytic flora of these poorly explored regions.

Since many plants have been used as a valuable resources since ancient times for the isolation of novel bioactive molecules to combat microbial diseases as well as maintaining human health, the present study aims at tapping out valuable bioactive properties from an overlooked plant so they can act as an excellent substitute for other plants resources and with this in mind three pteridophytes plant viz Lycopodiella cernua (L.) Pic. Serm., Diplazium esculentum Retz. and Selaginella bisulcata Spring. have been selected and were subjected to sequential extraction using pet ether, methanol, chloroform and water as the suitable solvent for phytochemical screening, anti-bacterial activity test and anti-oxidant test. In the present study Alkaloid, Carbohydrates, Protein, terpenoids, phenols, glycosides, saponins, phytosterols, flavonoids and tannins are invariably found to be present in all the three species selected. Then, the inhibitory effect of methanol extract and chloroform extract of Lycopodiella cernua (L.) Pic. Serm., Diplazium esculentum (Retz) Sw. and Selaginella bisulcata Spring. were evaluated against three bacterial strains viz Escherichia coli (ATCC-10536), Klebsiella pneumoniae (ATCC - 10031), Bacillus subtilis (ATCC - 11774). The antibacterial activity was determined using agar well-diffusion method and micro dilution method. Among the three pteridophytes selected and tested, D. esculentum has the largest inhibition zone against *E. Coli* at 80 mg ml<sup>-1</sup> concentration while the extract of *L. cernua* has the least inhibition zone at 80 mg ml<sup>-1</sup> concentration. From the experiment conducted the extracts of all the three pteridophytes tested shows antibacterial activity and the Analysis of Variance (ANOVA) also shows there was a significant variation between all the concentrations of *L. cernua*, *D. esculentum and S. bisulcata* on the test bacteria. Since, the presence of phytoconstituents like flavonoid, phenols, tannins and other phytoconstituents are known to have anti-microbial agents, this may be the reason why the the methanolic extract and chloroform extract of *L. cernua*, *D. esculentum and S. bisulcata* showed antibacterial activity and the difference in susceptibility of various test bacteria towards the extracts as observed in the study could be because of the nature of anti-microbial agents present in the extracts and their mode of action on the different test bacteria. (Rizvi, *et al.*, 2011).

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers and for this total flavonoid, total phenolic and antioxidant activity test has been carried out on the three selected plants. In the present study the total amount of phenolics content in CHCl<sub>3</sub> and MeOH extracts of *L. cernua* was determined to be 10.15 mg g<sup>-1</sup> and 8.564 mg g<sup>-1</sup>, *D. esculentum* 1.323 mg g<sup>-1</sup> and 0.722 mg g<sup>-1</sup> and *S. bisculata* 2.562 mg ml<sup>-1</sup> and 6.179 mg ml<sup>-1</sup> were determined to be equivalents of gallic acid respectively out of which the chloroform extract of *L. cernua* has the highest amount of phenolic content and the total amount of flavonoids content present in CHCl<sub>3</sub> and MeOH extracts of *L. cernua is* 11.02 mg g<sup>-1</sup> and 12.50 mg g<sup>-1</sup>, *D. esculentum* 6.617 mg g<sup>-1</sup> and 9.558 mg g<sup>-1</sup> and *S. bisculata* 

6.617 mg g<sup>-1</sup> and 8.09 mg g<sup>-1</sup> quercetin equivalents respectively out of which MeOH extracts of *L. cernua* exhibit the highest total flavonoid content.

The independent examination of antioxidant activities of methanol extracts and chloroform extract from *Lycopodiella cernua* (L.) Pic. Serm., *Diplazium esculentum* Retz. *and Selaginella bisulcata* Spring. showed different values and amongst the three pteridophytes tested the largest to inhibit antioxidant activities was found in MeOH extract of *L. cernua*. with an inhibition per centage ranges from 52-72.8 % followed by the MeOH extract of *S. bisculata* with an inhibition per centage ranges from 43.69- 48.18 % and CHCl<sub>3</sub> extract of *D. esculentum* with an inhibition per centage ranges from 42.5% to 48.33%. Also, IC<sub>50</sub> was calculated for each plant extract and the largest capacity to neutralize DPPH radicals was found in methanolic extract of *L. cernua* which neutralized 50% of free radicals at the concentration of 5.44 µg ml<sup>-1</sup> whereas the lowest capacity to inhibit DPPH radicals was found in CHCl<sub>3</sub> extract of *S. bisulcata* for which the IC<sub>50</sub> was calculated to be 133.37µg ml<sup>-1</sup>. In comparison to IC<sub>50</sub> values of BHA, MeOH extract of *L. cernua* manifested the strongest capacity for neutralization of DPPH radicals.

The notable presence of antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, quinones and other structural motifs (Patt and Hudson, 1990, Demiray *et al.*, 2009) and it also has been observed that many pharmacological effects of phenolics and flavonoids are linked together which act as a strong antioxidants (Saija *et al.*, 1995).

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2009	Graduation	Botany	MZU	First	61
2011	Post graduation	Botany	MZU	First	61.83
2017	National eligibility test		UGC-NET		

#### **Paper Published:**

- R. Vanlalpeka. and R.C. Laha. Ferns of Aizawl District, Mizoram, India. *Indian Forester*, 140 (11): 1114-1117, 2014.
- Zothanmawia, R. Vanlalpeka, Vanlalhruaii Ralte, H. Lalruatsanga, H.S. Thapa, John Zothanzama and PC Vanlalhluna. Diversity of wood decaying fungi in Pachhunga University College campus, Aizawl, Mizoram. *Issues and Trends of Wildlife conservation in Northeast India*, ISBN 978-81-924321-7-5, 2014
- R. Vanlalpeka and R.C. Laha. Forest pteridophytes of Champhai District, Mizoram, India. *International Journal of Recent Scientific Research*, Volume 6, Issue, 4: 3280-3283, April, 2015.
- R.C. Laha, Lalhriatpui, P.C.Lalremruata and R. Vanlalpeka. Forest Wild vegetable used by the Lai tribe in Lawngtlai district of Mizoram, India. *International Journal of Life Sciences Research.*, 6 (3): 212-217, 2017
- 5. R.C. Laha, Lalhriatpui, P.C.Lalremruata and **R. Vanlalpeka**. Ethnomedicinal plants used by Mara tribe in Siaha District of Mizoram, India *International Journal of Interdisciplinary Research and Innovations*, 6 (3): 365-368, 2017
- R.C. Laha, Lalhriatpui, P.C. Lalremruata and R. Vanlalpeka. Ethnomedicinal plants used by Lai tribe in Lawngtlai District of Mizoram, India. *International Journal of basic and applied research*, 8 (11): 371-376, 2017
- Lalnunmawia, Vanlalhruaii Ralte, H. Lalruatsanga, Zothanmawia, P.C. Vanlalhluna, H.S. Thapa and R. Vanlalpeka. Recovery of *Globba wengeri* (C.E.C. Fisch.) K. J. Williams, critically endangered plant species from Serchhip District in Mizoram, Northeast India. *Plant Science Today*, 7(1):1-4, 2020.

#### **Book published:**

 Thapa. H.S., Lalruatsanga. H., Ralte Vanlalhruaii, Vanlalhluna. P.C., Zothanmawia, Vanlalpeka, R. Crytogamic Diversity of Pachhunga University College. Mizo Academy of Sciences: ISBN 978-81-924321-9-9, 2014.

### **Conference papers presented:**

- R. Vanlalpeka. Pteridopytes diversity in Phawngpui National Park. Proceedings of National Conference on Biodiversity, Conservation and Utilization of Natural Resources with reference to North East India Areas in Chemistry (BCUNRNEI), 30th - 31st March, 2017, Department of Botany, Mizoram University, Aizawl.
- R. Vanlalpeka. Pteridopytes diversity in Tawi Wildlife Sanctuary, Mizoram, India. Proceedings of the & International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018), 12th -14th November, 2018, the 12th Annual Convention of Association of Biotechnology and Pharmacy (ABAP).

#### **Conference/Workshop and Seminar attended:**

- State Level Workshop on "Bamboo for Self Sufficiency" organized by Government of Mizoram on 6<sup>th</sup> March, 2014.
- National Seminar on "Issues of Wildlife Conservation in India with special reference to Mizoram" organized by Department of Environmental Science, Mizoram University and Department of Zoology, Pachhunga University College on 24<sup>th</sup> – 25<sup>th</sup> April, 2014 at Mizoram University, Aizawl, Mizoram.
- Participated on Training Programme on data entry, Data compilation and Basic queries in connection with DBT/GOI funded coordinated project entitled "Developing a digital database on bio-resources of N.E. India – Through network approach among North Eastern State" from 4<sup>th</sup> July – 5<sup>th</sup> July, 2014 at Department of Life Sciences, Manipur University, Canchipur.
- Participated on Short term Course on GIS and Remote Sensing organized by Academic Staff College, Mizoram University, UGC Sponsored Short Term Course on 26<sup>th</sup> August – 1<sup>st</sup> September, 2014.
- 5. National Workshop on "Capacity Development for Forests Management &

Personnel training" having Theme of "Training Improvement Plan" organized by Consultants State Project Management Unit, E&F Department Government of Mizoram, under the Japan International Cooperation Agency (JICA) Assisted project, held on 5<sup>th</sup> March, 2015 at ARCBR.

- Participated in the Workshop titled "Pathways in drug Discovery: from Forest to Drugstore" organized by DBT's Institutional Level Biotech Hub, Department of Pharmacy, RIPANS on 7-11<sup>th</sup> March, 2016.
- Technical Session/ Exhibition in Mizoram Science Congress held at Mizoram University during 13<sup>th</sup> – 14<sup>th</sup> October, 2016 organized by Mizoram Science Congress 2016 Organizing Committee.
- National Seminar on "Make in India: Science & Technology Driven Innovations" organized by Mizo Academy of Sciences in Collaboration with the Mizoram Science, Technology & Innovation Council (MISTIC), Government of Mizoram, catalyzed and supported by the National Council for Science & Technology Communication, Department of Science and Technology, New Delhi on 4<sup>th</sup> November, 2016.
- State Level Workshop on "Research Methodology and Monitoring of research projects" held at PUC Seminar hall, Aizawl on 11<sup>th</sup> August, 2017 organized by Project Committee, Pachhunga University College, Aizawl, Mizoram
- 10. State Level Workshop on "Challenges in Biosystematics in Mizoram" held at PUC Seminar hall, Aizawl on 29<sup>th</sup> Sept ember, 2017 jointly organized by Directorate of Science and Technology, Mizoram, Mizo Academy of Sciences, Aizawl and Project Committee, Pachhunga University College, Aizawl, Mizoram.
- Workshop on "Statistical and Computing Methods for Life-Science Data Analysis" jointly organized by Biological Anthropology Unit, Indian Statistical Institute, Kolkata and Department of Botany, Mizoram University, Aizawl on 5<sup>th</sup> – 10<sup>th</sup> March, 2018.
- 12. National Seminar on "Shifting Cultivation and its Environmental Impact in North-East India" organized by Department of Geography, Pachhunga University

College, on  $15^{\text{th}} - 16^{\text{th}}$  March, 2018.

- National Seminar on "Science and Technology for a Sustainable Future" organized by Mizo Academy of Sciences, Mizoram Science, Technology & Innovation Council (MISTIC), Government of Mizoram on 30<sup>th</sup> April, 2018.
- Participated in the Mizoram Science Congress, 2018, a National Conference, held at Pachhunga University College during 4<sup>th</sup> – 5<sup>th</sup> October, 2018 which was organized by Mizoram Science Congress 2018 Organising Committee.
- 15. One day Awareness Programme cum Workshop on Invasive Alien plants in Himalayas: Status, Ecological Impact and Management (Mizoram and Tripura chapter), organized by botanical Suvey of India in collaboration with Department of Botany, Mizoram University on 26<sup>th</sup> April, 2019.
- National Workshop on "Sensor Networks, Internet of things and Internet of Everything" funded by Department of Science and Technology, Government of India on 11-13<sup>th</sup> September, 2019.
- One Day National Workshop on "IPR and Plant Protection with special reference to NE India" organized by Department of Botany, Mizoram University and Department of Horticulture, Government of Mizoram on 18<sup>th</sup> December, 2019.
- Webinar on "The Importance of Symbiotic Microorganisms in drug Discovery" organized by Department of Forestry and Department of Environmental Sciences, Mizoram University on 6<sup>th</sup> June, 2020.
- Webinar on "Recent advances in science: Mankind and Change" organized by Government Serchhip College, Mizoram, India on 6<sup>th</sup> -7<sup>th</sup> July, 2020.

## PARTICULARS OF THE CANDIDATE

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DEPARTMENT	: Department of Botany
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DATE OF ADMISSION	: 20 <sup>th</sup> August 2013
APPROVAL OF RESEARCH PROPOSAL	
<ol> <li>BOS</li> <li>SCHOOL BOARD</li> </ol>	: 29 <sup>th</sup> April 2014 : 23 <sup>rd</sup> May 2014
REGISTRATION NO. & DATE	: MZU /Ph.D./671 of 23.05.2014
EXTENSION DATE	: Letter No. 16-2/MZU(Acad)/15/321
	Dated 17 <sup>th</sup> January 2019
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Head

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## ABSTRACT

# DIVERSITY AND PHYTOCHEMISTRY OF PTERIDOPHYTES FROM DIFFERENT RESERVE FORESTS IN MIZORAM

R. VANLALPEKA

DEPARTMENT OF BOTANY

MIZORAM UNIVERSITY

### ABSTRACT

# DIVERSITY AND PHYTOCHEMISTRY OF PTERIDOPHYTES FROM DIFFERENT RESERVE FORESTS IN MIZORAM

By

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Submitted

In partial fulfillment of the requirement for the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl

#### **ABSTRACT**

Pteridophytes, also known as 'vascular cryptogams' and 'ferns and fern allies', comprise about 12,000 species of vascular plants that do not produce flowers or seeds, reproducing instead via the production of spores. Pteridophytes occur in most terrestrial habitats on earth and are also present in some aquatic communities. They are an important part of the ground vegetation in many forest communities and, with about one-third of the species growing on the trunks and branches of trees; they are also an important component of many epiphytic plant communities. Certain territories of India fall under different biodiversity hot spots of the world. A good number of comprehensive works have also appeared for different parts of the country during the last three decades with the revival of interest in floristics of Pteridophytes in India.

The Eastern Himalaya with Mizoram which lies between  $21^{\circ} 56'N - 24^{\circ} 31'N$ latitudes and  $92^{\circ} 16'E - 93^{\circ} 26'E$  longitudes sandwiched between two countries is a biodiversity center of Pteridophytes and because of its unique terrain and location the areas is rich in flora and fauna and has a rich biodiversity with 10 ten protected sites for conserving its rich biodiversity. The combination of different factor like climate, temperature, edaphic, precipitation, humidity, altitude, forest type favors the growth of rich and luxuriant vegetation of Pteridophytes in Mizoram. The Pteridophytes are unique group of plants as they are strictly habitat specific, shade and moisture loving and any disturbances to the micro climate causes treat to their existence. At present anthropogenic activities like population growth, destruction of habitat particularly forest, urbanization, agriculture, slash and burn agricultural practice, clear felling practice together with mono-culture infrastructure development result in the alteration of the micro climate leading to habitat lost, destabilization of traditional management system cause tremendous threat to the existence of Pteridophyte in the state.

Also, considering the rich diversity of Indian plants including Pteridophytes which has been endowed with medicinal properties and various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases. The Greek botanist Theophrastus (ca. 372–287 BC) wrote about the medicinal value of pteridophytes. Dioscorides (ca.50 AD) in his de Materia Medica, including P. aquilinum and Dryopteris filix-mas, as having medicinal values. In ancient Indian medicine systems, several ferns were used to cure a number of human ailments. Sushruta (ca. 100 AD) and Charaka (ca. 100 AD) recommended the medicinal use of some ferns in their samhitas. Several ferns have been used by Unani physicians in India and Western Asia (Banerjee and Sen, 1980). So, it is of utmost importance that, the screening and testing of plant chemicals is needed to know their potential is much needed which will be beneficial for treating humans and plants diseases. So, knowing their importance and gap, the works have been carried out with the following objectives:1) To collect and identify the Pteridophytes diversity in selected sites of Mizoram. 2) To study the phytochemical analysis and antioxidant activity of selected species. 3) To study anti-bacterial activities of selected species.

The study sites have been confined to four reserve forest areas in Mizoram viz. Phawngpui National Park, Murlen National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve.

In identification of the plants species are presented under families as per the system of classification proposed by Pichi-Sermolli (1977) and literatures (Hovenkamp *et al.* 1998, Freser-Jenkins 2008) have been followed and for correct citation of the author of the species, Rodolfo E.G. Pichi Sermolli (1996) has been followed. The specimens collected were deposited in the Department of Botany, Mizoram University.

Three plants viz *Lycopodiella cernua* L. *Retz., Diplazium esculentum and Selaginella bisculata* Spring. were selected and subjected for phyto-chemical screening, anti-bacterial test and anti-oxidant activity test.

A test described by Kokate *et al.*, 2013 has been used for the screening of phytochemicals for the plants selected for anti-bacterial test agar well diffusion method (Murray *et al.*, 1995 later modified by Olurinola, 1996). Total phenolic content was determined by using the method of Mc Donald *et al.*, 2001 with slight modifications and total flavonoid contents of the extracts was determined by the aluminum chloride method (Chang *et.al.*, 2002). DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging was carried out according to the method describe by Kim.*et al.*, (2017).

In the present investigation which deals with the Pteridophytic flora of Mizoram which account 105 Pteridophytes species from Phawngpui National Park, Murlen

National Park, Tawi Wildlife Sanctuary and Dampa Tiger Reserve out of which Polypodiaceae family has the most number of species (18 species) which account for 17.14 percent from the total pteridophytes collected, followed by Pteridaceae and Dryopteridaceae with 11.43 percent (12 species) each while Thelypteridaceae has 9.5 percent (10 species) occupying the third largest families recorded during the study. The rest of the families like, Athyriaceae exhibit 8.57 percent (9 species), Dennstaedtiaceae 7.62 percent (8 species), Aspleniaceae 5.71 percent (6 species), Lindsaeaceae 4.76 percent (5 species) while the rest has lesser no of species with only 1 and 2 species accounting to their families. Also, in the present study it has been noticed that the terrestrial species constitute more than 68 percent (71 species) out of 105 species recorded, while epiphytes and lithophytes communities constitute approximately 16 percent (17 species) each of the total Pteridophytes species collected. A total no of 45 species have been found to be present in all the four sites and 11 species a habit specific i.e. they are found only in one place from the study site. Also, 5 species have been found to be rare and endangered species for North eastern India (Bir 1987).

Among the four selected sites, Murlen National Park holds higher number of pteridophyte species having a total no. of 89 species, 44 genera and 21 families, followed by Tawi Wildlife sanctuary 85 species, to 43 genera and 21 families from, 69 species, 34 genera and 20 families from Dampa Tiger Reserve and 65 species, 32 genera and 18 families from Phawngpui National Park. Most of the studied area exhibit variety of macrohabitat which harbor good growth and colonization of a number of pteridophytes on different substrata. Due to the difficult terrain and less anthropogenic

activities, the studied area showed luxuriant growth of pteridophytes like such as like *Adiantum phillipense* L., *Macrothelypteris torresiana (Gaudich.) Ching, Athyrium falcatum* Bedd., *Blechnum orientale* L., *Cyclosorus falcilobus Panigrahi*, etc. But some part of the study region experienced anthropogenic problems like shifting cultivation, expansion of agriculture, introduction of plantation crops, destruction of natural forest, collection of wood, infrastructure development and road construction as a result the valuable resources of lichens are lost. The urgent need is to prevent the anthropogenic activities, restored and to conserve the pteridophytic flora of these poorly explored regions.

Since many plants have been used as a valuable resources since ancient times for the isolation of novel bioactive molecules to combat microbial diseases as well as maintaining human health, the present study aims at tapping out valuable bioactive properties from an overlooked plant so they can act as an excellent substitute for other plants resources and with this in mind, three pteridophytes plant viz *Lycopodiella cernua L., Diplazium esculentum* Retz. *and Selaginella bisulcata* Spring. have been selected and were subjected to sequential extraction using pet ether, methanol, chloroform and water as the suitable solvent for phytochemical screening, anti-bacterial activity test and antioxidant test. In the present study alkaloid, carbohydrates, protein, terpenoids, phenols, glycosides, saponins, phytosterols, flavonoids and tannins are invariably found to be present in all the three species selected. Then, the inhibitory effect of methanol extract and chloroform extract of *L. cernua* L., *D. esculentum* (Retz) Sw. and *S. bisulcata* Spring, were evaluated against three bacterial strains viz *Escherichia coli (ATCC*-

10536), Klebsiella pneumoniae (ATCC - 10031), Bacillus subtilis (ATCC - 11774). The anti-bacterial activity was determined using agar well-diffusion method and micro dilution method. Among the three pteridophytes selected and tested, D. esculentum has the largest inhibition zone against *E*. *Coli* at 80 mg ml<sup>-1</sup> concentration while the extract of L. cernua has the least inhibition zone at 80 mg ml<sup>-1</sup> concentration. From the experiment conducted the extracts of all the three pteridophytes tested shows antibacterial activity and the Analysis of Variance (ANOVA) also shows there was a significant variation between all the concentrations of L. cernua, D. esculentum and S. bisulcata on the test bacteria. Since, the presence of phytoconstituents like flavonoid, phenols, tannins and other phytoconstituents are known to have anti-microbial agents, this may be the reason why the methanolic extract and chloroform extract of L. cernua, D. esculentum and S. bisulcata showed anti-bacterial activity and the difference in susceptibility of various test bacteria towards the extracts as observed in the study could be because of the nature of anti-microbial agents present in the extracts and their mode of action on the different test bacteria. (Rizvi, et al., 2011).

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers and for this total flavonoid, total phenolic and antioxidant activity test has been carried out on the three selected plants. In the present study the total amount of phenolics content in CHCl<sub>3</sub> and MeOH extracts of *L. cernua* was determined to be 10.15 mg g<sup>-1</sup> and 8.564 mg g<sup>-1</sup>, *D. esculentum* 1.323 mg g<sup>-1</sup> and 0.722 mg g<sup>-1</sup> and *S. bisculata* 2.562 mg ml<sup>-1</sup> and 6.179 mg ml<sup>-1</sup> were determined to be equivalents of gallic acid respectively out of which the chloroform extract of *L. cernua*  has the highest amount of phenolic content and the total amount of flavonoids content present in CHCl<sub>3</sub> and MeOH extracts of *L. cernua is* 11.02 mg g<sup>-1</sup> and 12.50 mg g<sup>-1</sup>, *D. esculentum* 6.617 mg g<sup>-1</sup> and 9.558 mg g<sup>-1</sup> and *S. bisculata* 6.617 mg g<sup>-1</sup> and 8.09 mg g<sup>-1</sup> quercetin equivalents respectively out of which MeOH extracts of *L. cernua* exhibit the highest total flavonoid content.

The independent examination of antioxidant activities of methanol extracts and chloroform extract from *Lycopodiella cernua* L., *Diplazium esculentum* Retz. *and Selaginella bisculata* Spring. showed different values and amongst the three pteridophytes tested the largest to inhibit antioxidant activities was found in MeOH extract of *L. cernua*. with an inhibition percentage ranges from 52- 72.8 % followed by the MeOH extract of *S. bisculata* with an inhibition percentage ranges from 43.69- 48.18 % and CHCl<sub>3</sub> extract of *D. esculentum* with an inhibition percentage ranges from 42.5% to 48.33%. Also, IC<sub>50</sub> was calculated for each plant extract and the largest capacity to neutralize DPPH radicals was found in methanolic extract of *L. cernua* which neutralized 50% of free radicals at the concentration of 5.44  $\mu$ g ml<sup>-1</sup> whereas the lowest capacity to inhibit DPPH radicals was found in CHCl<sub>3</sub> extract of *S. bisculata* for which the IC<sub>50</sub> was calculated to be 133.37 $\mu$ g ml<sup>-1</sup>. In comparison to IC<sub>50</sub> values of BHA, MeOH extract of *L. cernua* manifested the strongest capacity for neutralization of DPPH radicals.

The notable presence of antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic

groups, quinones and other structural motifs (Patt and Hudson, 1990, Demiray *et al.*, 2009) and it also has been observed that many pharmacological effects of phenolics and flavonoids are linked together which act as a strong antioxidants (Saija *et al.*, 1995).