Chapter 2
Nepalese Pteridophytes Used as Antimicrobials: Challenges and Opportunities

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Abstract Pteridophytes constitute the primitive vascular plant group, which are found scattered all over the world. There are 580 taxa of Nepalese pteridophytes, which are most diverse and adapted in different climatic zones forming an attractive component of the vegetation showing different ecological habits such as epiphytic, lithophytic, terrestrial, tree ferns, hanging club mosses, climbers, and hydrophytic. Human beings have been using pteridophytes because of their several useful properties, including food and medicine. Some important bioactive compounds have been identified from the Nepalese pteridophytes and the chemical compounds isolated have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. Finally, the challenges and opportunities of pteridophyte research are discussed.

Keywords Bioactivity · Central Nepal · Ethnomedicine · Fern · Natural antimicrobial

Abbreviations

ATCC	American Type Culture Collection;
CITES	International Trade in Endangered Species of Wild Fauna and Flora
IUCN	The International Union for Conservation of Nature
MBC	Minimum Bactericidal Concentration
mg	Milligram
MIC	Minimum Inhibitory Concentration
mL	Milliliter

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mm	Millimeter
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MTCC	Microbial Type Culture Collection
μg	Microgram

1 Introduction

Pteridophytes have a long geological history on our planet (Pandey et al. 1996). They are known from as far back as 380 million years. Fossils of pteridophytes have been obtained from rock strata belonging to Silurian and Devonian periods of the Paleozoic era (Pandey et al. 1996).

According to one school of thought, the pteridophytes like bryophytes have evolved from algae and both the groups are parallel to each other in their origin and they are not linked phylogenetically (Pandey et al. 1996). But, some researchers do not trace their origin from any particular group of algae (Eames 1936). Some believe that pteridophytes originated from a particular group of marine algae, that is, Phaeophyceae (Church 1919; Arnold 1947). Pteridophytes originated from erect and parenchymatous green algae with an isomorphic life cycle (Pandey et al. 1996). The pteridophytes resemble the bryophytes in many respects, and some researchers assumed that the pteridophytes have originated from bryophytes. The two groups (bryophytes and pteridophytes) are phylogenetically connected, but they are divergent evolutionary lines evolved from hypothetical terrestrial plants of primitive archegonia type (Zimmermann 1938; Pandey et al. 1996). They have arisen from an anthocerotean type of bryophytes.

Pteridophytes are nonflowering plants with well-developed vascular system. The most characteristic feature of pteridophytes is the presence of independent gametophytes and sporophytes at maturity. They have leaves (known as fronds), roots and sometimes true stems, and tree ferns have full trunks, constitute an essential and major group of plant kingdom possessing extinct and extant members (Pandey et al. 1996; www.plantlist.org). The root system is always adventitious. The stem is either underground or aerial. The leaves may be microphylls or megaphylls. Pteridophytes do not have seeds or flowers either; instead, they also reproduce via spores. They are divided into four classes: Psilopsida, Lycopsida, Sphenopsida, and Pteropsida (Sinnot 1935; Pandey et al. 1996).

Human beings have been using pteridophytes because of their several useful properties, including food and medicine. Some important bioactive compounds have been identified from the Nepalese pteridophytes and the chemical compounds isolated have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. The principal aim of this chapter is to collect the dispersed data about the Nepalese pteridophytes used as antimicrobials. Finally, the challenges and opportunities of pteridophyte research are discussed.

2 Diversity and Distribution

There are around 13,271 species of living pteridophytes in the world (Hassler 2018; www.plantlist.org), which accounts for nearly 3% of the world flora. The pteridophytes constitute an important component of the Nepalese flora. Fraser-Jenkins et al. (2015) listed 580 taxa of Nepalese pteridophytes (550 species and an additional 30 subspecies), based only on material seen and verified. Within Nepal, the region with the greatest number of species recorded is Central Nepal followed by Eastern Nepal. Western Nepal has the lowest number of species recorded (Fraser-Jenkins et al. 2015). Some fern species of Nepal are shown in Figs. 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, and 2.8.

Fig. 2.1 *Adiantum capillus-veneris*

Fig. 2.2 *Adiantum philippense*

Fig. 2.3 *Aleuritopteris bicolor*

Fig. 2.4 *Angiopteris helferiana*

Ferns are abundantly found in humid and shady forests. They are most diverse in the tropics, and in Nepal, in different climatic zones, they form an attractive component of the vegetation of its hills and forests showing different ecological habit as epiphytic, lithophytes, terrestrial, tree ferns, hanging club mosses, climbers, and hydrophytes (Gurung 1984; Hogan 2004). Fern flora, which usually prefers to grow on tree bases, tree trunks, branches, and tree tops clothed with mosses and leafy liverworts, are termed as the epiphytic ferns (Rajbhandary 2016). Of the epiphytes, the most common and widely distributed species are *Asplenium nidus, Drynaria quercifolia, Microsorium punctatum, Lepisorus thunbergianus, Pyrrosia lanceolata, Nephrolepis cordifolia*, etc. Fern and fern allies usually occur in rock crevices

Fig. 2.5 *Asplenium dalhousae*

Fig. 2.6 *Diplazium esculentum*

or mossy cliffs or those that occupy bare or humus-rich rocks in shady areas are called lithophytic ferns. The most common and widely distributed lithophytic species are *Adiantum capillus-veneris, Cheilanthes farinose, Onychium siliculosum, Odontosoria chinensis, Pteris vittata, Selaginella* spp., *Asplenium* spp., *Dryopteris cochleata*, etc.

The terrestrial area included exposed areas, shady areas, stream banks, and hill slopes. In evergreen and semievergreen forests where the forest flora is rich in humus and organic nutrients, some ferns and fern allies are found growing and they are called as terrestrial ferns. Some of the terrestrial ferns are *Lycopodium japonicum, Dryopteris juxtaposite, Osmunda japonica,* and *Nephrolepis auriculata*

Fig. 2.7 *Lygodium flexuosum*

Fig. 2.8 *Oeosporangium teinufolia*

(Rajbhandary 2016). Some species of climbing ferns found in in Nepal are *Lygodium flexuosum, L. japonicum, Stenochlaena palustris*, etc. Some ferns grow on ponds, puddles, banks of streams, ravines, waterfalls, and swampy areas: these are termed as hydrophytes. Some examples are *Isoetes coromandelina, Equisetum ramosissimum, Ceratopteris thalictroides, Marsilea minuta,* and *Diplazium polypodioides* (Kandel and Fraser-Zenkins 2020).

3 Bioactive Compounds

Secondary metabolites are important compounds which are produced to confer a selective advantage to the organism. The secondary metabolites, flavonoids, have anti-inflammatory properties through the inhibition of the cyclo-oxygenase pathways (Liang et al. 1999). *Selaginella* species has a large number of bioactive compounds, which contains alkaloid, phenols, sterol, aliphatic acid, and terpenoid. Biflavonoids, such as amentoflavone, sumaflavone, robustaflavone, ginkgetin, hinokiflavone, and isocryptomerin, are the most important valuable natural products of Selaginella (Li et al. 2014a). Some bioactive flavones such as amentoflavone, robustaflavone, biapigenin, hinokiflavone, podocarpusflavone A, and ginkgetin, in *Selaginella* sp., were reported to have antioxidant, antiviral, and anticancer activities (Shi et al. 2008; Liu et al. 2011; Li et al. 2014b). Flavonoids and phenolic compounds are the most common group of bioactive compounds present in plants, occurring in almost all plant parts, but mainly in photosynthesizing cells. These compounds are the main sources of coloration in blooming plants (Koes et al. 2005).

4 Antimicrobial Activity

Antimicrobial medicines can be grouped according to the microorganisms they act primarily against (Bhattarai et al. 2008, 2009). For example, antibiotics are used against bacteria, and antifungals are used against fungi. Recent studies comparing natural derivatives from plants with synthetic antimicrobials have shown that natural substances could be safer (Dorman and Deans 2000; Burt and Reinders 2003; Hygreeva et al. 2014).

There are many antibiotics currently available for the treatment of bacterial and fungal infections, but they are not always reliable against pathogenic organisms (Gearhart 1994). This situation has forced scientists to search for new bioactive compounds from plants (Kumar et al. 2006). In recent years, the search for phytochemicals with antioxidant, antimicrobial, or anti-inflammatory properties has been on the rise due to their potential use for the treatment of various chronic and infectious diseases (Halliwell 1996). Plant extracts have numerous secondary metabolites, having antimicrobial and antioxidant properties. Traditionally, the biomedical system, Ayurveda system, and Unani system of medicine all have suggested the medicinal use of ferns (Baskaran et al. 2018).

5 Activity of Ferns

Plant extracts have been widely used since ancient times for treating human illness. Numerous studies of the antimicrobial activity of different natural products have been reported (Bhattacharjee et al. 2006; Parekh and Chanda 2006, 2007). Although the medicinal value of pteridophytes has been known to traditional cultures for more than 2000 years, they are used only on a small scale in modern chemotherapy. Studies of the bioactivity of pteridophytes are still in their early years compared with angiosperms (Baskaran et al. 2018).

5.1 Activity Against Bacteria

The methanol extracts of *A. capillus-veneris* and *A. venustum* have been tested for their antimicrobial activity against five Gram-positive, six Gram-negative (including multiresistant *S. aureus*) bacterial and eight fungal strains using standard microdilution assay. Maximum activity was exhibited by *A. venustum* followed by *A. capillus-veneris*. The extract of *A. capillus-veneris* had a very low MIC value (0.48 μg/mL) against *Escherichia coli*, whereas *A. venustum* extract activity against *Aspergillus terreus* showed an MIC of 0.97 μg/mL (Singh et al. 2008).

The antagonistic potential of crude extract of *Adiantum philippense* was studied using agar cup/well diffusion method against food pathogens (*S. aureus, E. coli, P. aeruginosa*, and *S. flexneri*). Antibacterial activity results were portrayed in the form of zone of inhibition and revealed substantial antagonistic activity against all the tested bacterial strains. *S. aureus* was found to be more susceptible when compared to *E. coli, P. aeruginosa*, and *S. flexneri* (Adhan et al. 2020).

Adiantum caudatum was evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity toward bacterial species involved in skin diseases. The test organisms include *S. aureus, E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Serratia marcescens.* Water extracts did not show any antibacterial activity toward tested organisms; the same condition was observed with petroleum ether extracts. Acetone extract of *A. caudatum* showed moderate level of inhibition toward *S. aureus*; the plant showed lower level of inhibition toward *E. coli* compared to the other bacterial strains. *P. aeruginosa* and *S. marcesens* are the most sensitive organisms toward the methanol extract of the plant. The plant extracts did not show any antibacterial activity against *E. coli* (Thomas 2014). Petroleum ether extract contained nonpolar compounds dissolved in it, and these compounds did not have antibacterial activity. Likewise, water extract contained highly polar compounds and these compounds also showed lowest level of antibacterial activity. Methanolic extract of *A. caudatum* showed maximum action against *P. aeruginosa*, Gram-negative bacteria. The present antibacterial analysis of the plant supports the ethnobotanical importance of *A. caudatum*. The plant showed antibacterial activity in methanol extract. The methanol extract of the plant showed maximum level of activity toward *P. aeruginosa*. Petroleum ether and water extracts did not show any antibacterial activity toward any of the tested organisms. Methanolic extract of the plant exhibited minimum inhibitory concentration as 50 mg/ml and minimum bactericidal concentration as 25 mg/ml toward *P. aeruginosa* (Thomas 2014). Bioactivity studies showed strong antibacterial activity (MIC = 31.3–62.5 μg/mL) of *Blechnum orientale* against five Gram-positive bacteria (Lai et al. 2017).

The Malasar tribes in the Valparai hills, India, use *Dicranopteris linearis* due to their wound-healing activity (Santhosh et al. 2014). Also, wound-healing activity has been reported for *Nephrolepis cordifolia* (Upreti et al. 2009). The 1:10 dilution of the essential oil of *Equisetum arvense* was shown to possess a broad spectrum of a very strong antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Salmonella enteritidis* (Radulović et al. 2006). *Dryopteris cochleata* possesses antibacterial principles, soluble in acetone, which hinder the growth and multiplication of some multidrug-resistant bacterial strains (Thomas 2009).

Yenn et al. (2018) aimed to evaluate the antibacterial potential of *Helminthostachys zeylanica* on foodborne *Bacillus cereus*. The ethanolic extract showed significant inhibitory activity on *B. cereus* with a sizeable clear zone detected on disc diffusion assay. On broth microdilution assay, the MIC of the extract on *B. cereus* was 6.25 mg/ml and the MBC was 12.5 mg/ml. The inhibitory activity of the extract on *B. cereus* was bactericidal. In the growth dynamic study, the antibacterial efficacy of the extract was concentration dependent, where a lower colony-forming unit count was obtained with increased extract concentration. The GCMS analysis of the extract showed that the major constituents of the extract were phenol (36.26%) and quercetin (29.70%). This study is important as it shows the potential use of *H. zeylanica* as an effective agent to control *B. cereus*–related infections (Yenn et al. 2018).

On the other hand, *L. clavatum* was formerly reported to contain various phenolic acids such as dihydrocaffeic, vanillic, p-hydroxy-benzoic, syringic, p-coumaric, and ferulic acids (Towers and Maass 1965) and phenolic acids are known to display antimicrobial activity against a variety of microorganisms (Herald and Davidson 1983; Stead 1993). For instance, ferulic acid has been shown to have antimicrobial activity by several researchers against *S. aureus, B. subtilis, P. aeruginosa*, and *C. albicans* as well as *Listeria monocytogenes* (Fernandez et al. 1996; Kwon et al. 1997; Panizzi et al. 2002; Wen et al. 2003). In one study, syringic, caffeic, isovanillic, ferulic, and p-hydroxycinnamic acids were found to exhibit antimicrobial activity (Fernandez et al. 1996). For that reason, the presence of these compounds in this plant may explain their antibacterial activity. In the past, plants have afforded a number of anti-infective agents including emetine, quinine, and berberin (Iwu et al. 1999). The *Lycopodium* genus is also known to be rich in alkaloids with high toxicity (Ayer 1991). This may also contribute to the antimicrobial activity of the LC extracts (Ainge et al. 2002).

Antibacterial and antifungal activities of the *L. clavatum* extracts were tested against standard and isolated strains of the following bacteria: *E. coli, P. aeruginosa, P. mirabilis, A. baumannii, K. pneumoniae, S. aureus,* and *B. subtilis*. All the extracts possessed noteworthy activity against ATCC strains of *S. aureus* (Orhan et al. 2007a). Phytochemical analysis of water extracts of *Lygodium flexuosum* confirmed the presence of glycosides and carbohydrates, but alkaloids, tarpenoides, steroids, saponins, tannins, and flavonoids were absent (Nayak et al. 2013).

Verma et al. (2015) used four bacterial strains viz. *E. coli*, *K. pneumoniae, E. faecalis, S. aureus* for antimicrobial assay of the extracts and isolated compounds. Hexane and acetone extracts of *Selaginella bryopteris* and compounds isolated from it were tested for their antimicrobial activity, excluding (+)-syringaresinol, amentoflavone, due to paucity of the sample. The zone of inhibition and MIC values for extract as well as isolated compounds were measured against some Gram-positive and Gram-negative bacteria. No antimicrobial activity was observed in the n-hexane extract, but the acetone extract of *S. bryopteris* showed activity against all the microbes tested in this study. The maximum effect of the acetone extract was observed against *K. pneumoniae* and *S. aureus*. Usually, the n-hexane extract contains mainly nonpolar compounds such as oils and fats, while the acetone extract contains polar compounds, including phenols, glycosides, alkaloids, steroids, terpenoids, antioxidants, etc.

Vanillic acid is also the most potent antimicrobial agent present in *S. bryopteris*. It has activity against both Gram-positive and Gram-negative bacteria as well as against different fungal strains. Only β-sitosterol β-D-glucoside showed some activity against bacteria. Thus, it seems that addition of the glucoside moiety to β-sitosterol increases its potency as an antimicrobial agent (Verma et al. 2015).

The antimicrobial activity of *Tectaria macrodonta* may be attributed to various phytochemicals, namely, saponins, tannins, anthocyanin, flavonoid, phenol, and alkaloid (Masal and Dongare 2010). The aqueous, ethanol, and methanol extracts of the rhizomes of *T. macrodonta* were evaluated for its potential antibacterial properties. Potential antibacterial activities were exhibited by the methanol and ethanol extracts of *T. macrodonta* (Poudyali and Singh 2013).

5.2 Activity Against Fungi

Antifungal activities of the extracts of *Lycopodium clavatum* were tested against standard and isolated strains of *Candida albicans* and *C. parapsilosis*. The *L. clavatum* extracts showed reasonable antifungal effects (Orhan et al. 2007a, b).

Verma et al. (2015) used three fungal strains, viz. *C. albicans*, *C. krusie*, and *C. tropicalis*, for antifungal assay of the extracts and isolated compounds. Hexane and acetone extracts of *Selaginella bryopteris* and compounds isolated from it were tested for their antifungal activity. The zone of inhibition and MIC values for extract as well as isolated compounds were measured against some *Candida* species. No antimicrobial activity was observed in the n-hexane extract, but the acetone extract of *S. bryopteris* showed activity against all the microbes tested. It was concluded that Vanillic acid is the most potent antifungal agent present in *S. bryopteris* and β-sitosterol, β-sitosterol β-D-glucoside were effective against fungal strains (Verma et al. 2015).

5.3 Activity Against Viruses

Using vesicular stomatitis virus in monkey cell cultures as test organism, the extracts of *A. capillus-veneris* were found to exhibit antiviral activity (Husson et al. 1986). The antihyperglycemic and analgesic activity of the leaves of *A. philippense* has been studied by Tanzin et al. (2013). The whole plants of *A. capillus-veneris* were used by the tribes of the Valparai hills, Western Ghats, and Tamil Nadu, India, for their hypoglycemic and anticancer activity (Santhosh et al. 2014). Both leaves and rhizomes of *A. capillus-veneris* have been used in the preparation of herbal drugs for treating diabetes in India and Europe (Baskaran et al. 2018).

Some bioactive flavones, such as amentoflavone, robustaflavone, biapigenin, hinokiflavone, podocarpusflavone A, and ginkgetin, in *Selaginella* sp., were reported to have antivirus and anticancer activity (Ma et al. 2001; Shi et al. 2008; Liu et al. 2011; Li et al. 2014b). *Lygodium flexuosum* have shown significant effects against viral disease and jaundice. Butylated hydroxytoluene is primarily used as a food additive that exploits its antioxidant properties. It is also documented as an antioxidant additive in such diverse products as cosmetics and pharmaceuticals. It has been reported to have antiviral effects particularly in use against herpes family viruses and in combination with L-lysine and Vitamin C (Snipes et al. 1975; Brugh 1977; Kim et al. 1978; Richards et al. 1985; Pirtle et al. 1986; Chetverikova et al. 1989; Chetverikova and Inozemtseva 1996). Flavonoid derived from *Cheilanthes tenuifolia* possesses potent anticancerous and antioxidant activities that are responsible for their chemo-preventive potential (Jarial et al. 2018).

Lycopodium clavatum CHCl extract exerted good antiviral effect toward the DNA virus HSV (16–8 lg/ml) with the MNTC of 16 lg/ml, similar to that of acyclovir (16 to <0.25 lg/ml), except for its therapeutic range of LC-$CHCl_3$ was narrower. As to PI-3, LC-PE and LC-CHCl3-Alk exhibited some inhibition (16–4 and 32–4 lg/ml, respectively). In particular, the alkaloid fraction of *L. clavatum* showed quite similar anti-PI-3 effect and MNTC value to that of oseltamivir (32 to <0.25 lg/ml) (Orhan et al. 2007a, b).

6 Challenges

Pteridophytes have remained the exclusive domain of academicians, rarely heard outside the academic world. This chapter discusses about the antimicrobial properties of pteridophytes. Moreover, the bioactive compounds and traditional usage of Nepalese ferns have also been discussed. Various anthropogenic activities like deforestation for agricultural land expansion, logging, urbanization, and roads/trails building activities have resulted in tremendous pressure on the natural habitat of fern species.

Some of the ferns including tree fern are also not devoid of economic exploitation in some parts of the world, as it has high market demand due to its multiple socioeconomic uses viz. ornamental, horticulture, food, and medicinal uses (Chandra and Fraser-Jenkins 2008; Rout et al. 2009; Singh and Singh 2012), resulting in the rapid decline of the wild population resulted many species of tree ferns under threatened category in IUCN Red Data book and Appendix II of CITES. Tree ferns are being suffered from the unsustainable harvesting of fronds for food and fodder without being aware of the taking into consideration of its own natural viability challenges and its conservation status. Little knowledge on the importance of tree fern stands as one of the major factors for the declining its populations in that area. Currently, construction of rural roads in Nepal has become a major threat to the habitat as well as species itself as such construction practices are not well monitored. Keeping the aforesaid statements in view, the existing threats for tree fern expand across the social, economic, and environmental dimensions both globally and locally due to which they continue to be under great threat of extinction.

The family Cyatheaceae is listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), 1975 in order to protect these epibiotic tree ferns from being sold randomly and overexploited. It is also listed in threatened category of IUCN Red Data Book in 1998. Despite the family being listed in CITES and IUCN Red Data Book, it has not been assessed for special protection in Nepal (Thapa et al. 2017).

Very limited researches have been conducted for the fern conservation in Nepal. Researches on reproductive biology of fern in Nepal are almost null due to which there is a huge gap in scope for effective conservation and management of fern species. Beside the natural conditions to be considered for fern conservation, the actual threats from the human use in Nepal are still not known clearly. This is a huge knowledge gap to be addressed to identify the effective conservation measures for ferns. The awareness in communities on the importance and conservation of ferns is very limited. Despite their dependency on fern dominantly for food and fodder purpose and few for medicinal values, people lack strong rationale for initiating some effective and concrete conservation initiatives to protect the remaining natural patches. There is a crucial need of generation of knowledge on fern and its dissemination to the communities and developing a conservation and management plan. This step will provide a foundation on which the conservation efforts for protecting the tree fern species can be extended in the areas of the country and the region.

7 Opportunities

The pteridophytes constitute an important component of the Nepalese flora: the greatest number of species is recorded in Central Nepal. In recent years, the importance of ferns and their allies in plant science research has been increasing continuously (Baskaran et al. 2018); however, only a small number of species have been analyzed for the pharmaceutical property. In the context of Nepal, pteridophytes research is in preliminary phase. We hope that this chapter will encourage the Nepalese scientists to search more ferns for discovery of novel antimicrobials. Further research on high valued pteridophytes for exploration of chemical constituents and their commercialization into national level is recommended.

The present contribution attempts to describe the scope of pteridophytes in antimicrobial drug discovery, pharmacology, and ethnopharmacopoeias. Such initiative is crucial for conserving traditional knowledge and paving the way forward for antimicrobial drug discovery.

8 Conclusions

Some identified important isolated compounds from the Nepalese pteridophytes have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. In recent years, the importance of ferns and their allies in plant science research has been increasing continuously; however, only a small number of species have been analyzed for their pharmaceutical property. In the context of Nepal, pteridophytes research is in preliminary phase. We hope that this chapter will encourage the Nepalese scientists to explore more ferns that helps in the discovery of novel antimicrobial drugs for human benefits. A descriptive research on high valued pteridophytes for exploration of chemical constituents and their commercialization into national level is recommended.

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