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Preliminary phytochemical investigation and Antibacterial activities of several species of pteridophytes in South India

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Abstract

Pteridophytes are the first vascular land plants. Which have also been proved for the presence of antimicrobial substances against a number of pathogenic bacteria and is mainly due to the presence of various secondary phytochemicals viz. alkaloids, terpenoids, flavonoids, tannin, saponin and others. In the present study to investigate antibacterial properties of the selected pteridophytes obtained from Kerala and Tamilnadu against human pathogens. Leaf extracts of *Elaphoglossum nilgiricum; Crepidomanes plicatum; Adiantum raddianum; Pityrogramma austroamericana; Pseudocyclosorus ochthodes; Dryopteris cochleata; Athyrium solenopteris; Microlepia speluncae; Lindsaea odorata* and *Pyrrosia heterophylla* were collected, air dried and extracted with 80% of methanol. Antibacterial potential of leaf extracts were tested by the agar-well diffusion method against *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhimurium*. The results of the present study was observed that methanolic extracts of *Adiantum raddianum* and Elaphoglossum nilgiricum had the best antibacterial activity against *Salmonella typhimurium.* The conclusion of the present study observed that phytochemical analysis of the all the plant extracts have confirmed the presence of glycosides and carbohydrates, terpenoids, steroids, saponins, tannins, and flavonoids. These active compounds of the all the plant extract have responsible for the may be acted as antibacterial activity of the present study.

Keywords: Ferns, pteridophytes, phytochemicals, antibacterial activity, leaves, agar media

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1. INTRODUCTION

Ferns have been widely used by humans as ornamental plants, in domestic utensils, in handicrafts, as components of cosmetic formulations and foodstuffs, and for medicinal purposes (Morais-Braga et al., 2012a). Several species of the Pteridophytes are reported for the several medicinal properties (Sunil Kumar Verma and Shalu Kanwar,2020.) Literature review of pteridophytic species have revealed that substances in ferns exhibit diverse pharmacological properties such as cytotoxicity (Radhika et al., 2010), hepatoprotective activity (Wills and Asha 2006), antihyperglycemic activity (Zheng et al., 2011a, b), leishmanicidal activity (Socolsky et al., 2015), trypanocidal activity (Morais-Braga et al., 2013a, b), anti-nociceptive activity, anti-inflammatory activity (Yonathan et al., 2006), immunomodulatory activity (Wu et al., 2005), and chemopreventive effects (Wills and Asha, 2009). The antimicrobial properties of several ferns studied by several authors (Kumar and Kuushik. 1999, Parihar and Bohar. 2002;2003; Samir Kumarpal ,2013). Antimicrobial activity of three plant parts like rhizome, rachis and frond extracts of *Athhyrium filix-femina*(L.)Roth*., Dicranopteris linearis* (Burm.f.) Underw*., Pleopeltis macromarpa* (Bory ex Willd.)Kaulf. were tested and results of the frond and rhizome extracts of these species show good antimicrobial activity than rachis (Samir Kumarpal,2013), Recently, three pteridophytic species of *Cyathea brunoniana* and *Pronephrium nudatum* possesses antibacterial activity against *Staphylococcus aureus* (Kathakali Nath et al.,2017). In the present study to evaluate the antibacterial potential of several pteridophytes species of collected from the Western Ghats region of Tamilnadu and Kerala.

2. MATERIAL AND METHODS

2.1 Collection of study materials

The study plants were collected from the different locality of western ghats of Tamilnadu and Kerala regions. The species of pteridophytes were identified by regional pteridophytic flora.

2.2 Extraction and identification of Phytochemicals

The fresh leaf of these species were washed under running tap water, shade dried at room temperature and powdered. The powdered plant samples (50 g/250 mL) were extracted successively with 80 % methanol for using Soxhlet apparatus at 64.96 °C for 4 h (Elgorashi and Van Staden,2004). For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4 °C for further use. The investigation of preliminary qualitative phytochemical analysis was carried out to according to Trease and Evans ,(2002); Harborne, (1984). Methods.

Table-1: Collection of pteridophytes species

Sl.No	Pteridophytes species	Family
1	Elaphoglossum nilgiricum	Dryopteridaceae
2	Crepidomanes plicatum	Hymenophyllaceae
3	Adiantum raddianum	Pteridaceae
4	Pityrogramma austroamericana	Pteridaceae
5	Pseudocyclosorus ochthodes	Thelypteridaceae
6	Dryopteris cochleata	Dryopteridaceae
7	Athyrium solenopteris	Athyriaceae
8	Microlepia speluncae	Dennstaedtiaceae
9	Lindsaea odorata	Lindsaeaceae
10	Pyrrosia heterophylla	Polypodiaceae

2.3 Antibacterial activity

The tested bacteria were maintained for Mueller Hinton Agar/Broth. Prior to pouring into Petri plates all media were sterilized at 121° and 15 lbs for 20 min. To evaluated the antibacterial activity of theses extracts were done by Agar disc diffusion method followed by Murray et al., (1994). A final inoculum of 100µl suspension containing 10^8 CFU/ml of each bacterium was used. The bacterium was spread on Mueller Hinton Agar (MHA) medium. The disc (6 mm diameter) was impregnated with 10 µl of 10 mg/ml extracts and placed on the seeded agar. The tested plates were incubated at 37°C for 24 h for bacterial pathogens growing for a period required for a visible growth(Beneke et al.,1918). Each set of experiment was performed in triplicates.

3. RESULTS AND DISCUSSION

3.1 Preliminary investigation of phytochemicals

The results of preliminary active compounds of selected medicinal pteridophytes species were given in the table-2. The active compounds present in the extracts of these selected fern were 4 h Soxhlet apparatus packed for 80% methanol. Phytochemical studies on ferns have revealed that they contain a wide range of alkaloids (Dong et al., 2012), flavonoids (Xia et al,. 2014), polyphenols (Socolsky

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et al., 2012), terpenoids (Socolsky et al., 2007), and steroids (Ho et al., 2012). According to Dolly Rani et al., (2010) studied on the phytochemical tests were indicated the presence of all the metabolites such as flavonoids, tannins, alkaloids, reducing sugars, triterpenoids and steroids in the three pteridophytes, *P. nudum*, *N. biserrata* and *N. cordifolia*. From experiments on screening of total polyphenol contents of 37 ferns and fern allies, *Polystichum lepidocaulon and Polystichum polyblepharum* were reported to have more than 13% of total polyphenols from dried materials of both fronds and rhizomes. In addition, fronds of *Davallia mariesii* and rhizomes of *Cyrtomium fortune, Dicranopteris pedata, Athyrium niponicum* and *Dryopteris nipponensis* showed more than 10% of total polyphenols from dried materials (Goswami et al.,2016).

Table-2: Collection of pteridophytes species

Sl.No	Pteridophytes species	Available of Active compounds					
		Alkaloids	Flavonoids	Terpenoids	Glucosides	Tannin	Saponin
1	Elaphoglossum nilgiricum	+	+++	+++	+++	+	+
2	Crepidomanes plicatum	+	+++	+++	+++	+	+
3	Adiantum raddianum	+	+++	+++	+++	+	+
4	Pityrogramma austroamericana	+	+++	+++	+++	+	+
5	Pseudocyclosorus ochthodes	+	+++	+++	+++	+	+
6	Dryopteris cochleata	+	+++	+++	+++	+	+
7	Athyrium solenopteris	+	+++	+++	+++	+	+
8	Microlepia speluncae	+	+++	+++	+++	+	+
9	Lindsaea odorata	+	+++	+++	+++	+	+
10	Pyrrosia heterophylla	+	+++	+++	+++	+	+

3.2 Antibacterial activity

Ferns produce a wide array of secondary metabolites endowed with different bioactivities that could potentially be useful in the treatment of many diseases (Cao et al.,2017). Flavonoids are a class of natural phenolic compounds synthesized in plants as bioactive secondary metabolites (Nabavi et al., 2018) that are responsible for the characteristics of flavor, color, and pharmacological activities (Scarano et al., 2018). In the present study was antimicrobial potential of selected ferns extracted with 80% of methanol and their extracts active against bacterial pathogens observed results were represented in the table-3. Maximum antibacterial activity of both species of *E. nilgiricum* and *A. raddianum* extracts were active against *Salmonella typhimurium. Previous studies, t*he water extracts of *P. nudum* was least effective in inhibiting the growth of *P. aerugenosa* and most effective against *Salmonella typhimurium* and *Neprolepis biserrata* was most effective against *Salmonella typhimurium* (Dolly Rani et al., 2010). Earlier studies, *Adiantum* species has been reported to contain most significant antimicrobial properties(Singh et al.,2008). Previous studies, extracts of many different ferns have been tested for activity against bacteria, fungi and viruses (Zhou and Li, 1998; Xu et al., 2005). Water extracts of *P. multifida* showed remarkable antibacterial activity against *Staphylococcus aureus* (Zhou and Li, 1998; Kubo et al., 1992). In addition, water extracts and alcohol extracts from 20 species of medicinal pteridophytes were tested for bacteriostatic activity against *S. aureus*. Water and alcohol extracts of *P. ensiformis* Burm. and *P. semipinnata* exhibited particularly strong activity in these experiments (Cai et al., 2003). The inhibitory effects of polysaccharides from eight species of pteridophytes against *S. aureus* was tested using the disk agar diffusion method, revealing that polysaccharide extracts of *P. aquilinum*, *P. vittata*, *P. multifida* exhibited clear inhibitory effects against bacteria and fungi including *E. coli*, *P. vulgaris*, *S. aureus*, *S. cerevisiae*, and *Penicillium sp.* (Xu et al., 2005). Finally overall result was observed that 80% off methanol extract of all the ferns were the most effective in inhibiting growth of all the tested bacterial strains. In conclusion, the present study of the phytochemical study revealed the presence of tannins,

Pteridological Research ISSN : 2319-1919 (Print)

saponins, glycosides and flavonoids were present in the pteridophyte which are compounds capable of causing varied physiochemical and pharmacological effects. Further studies will be carried on the isolation of active principles of pteridophyte species of western ghats of Kerala and Tamilnadu.

Table-3: Collection of pteridophytes species

Sl.No	Pteridophytes species	Zone o Inhibition (mm)			
		Tested bacterial strains			
		Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Salmonella typhimurium*
1	Elaphoglossum nilgiricum	12	15	13	17
2	Crepidomanes plicatum	9	10	9	12
3	Adiantum raddianum	14	15	14	17
4	Pityrogramma austroamericana	9	9	11	12
5	Pseudocyclosorus ochthodes	12	9	9	11
6	Dryopteris cochleata	11	12	11	9
7	Athyrium solenopteris	12	15	11	13
8	Microlepia speluncae	12	13	12	14
9	Lindsaea odorata	9	11	9	10
10	Pyrrosia heterophylla	11	10	13	9

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41.