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Chemical diversity analysis on some selected medicinally important pteridophytes of Western Ghats, India

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ABSTRACT

Objective: To study the phyto-constituents present in some selected medicinally important pteridophytes from the Western Ghats, India. **Methods:** The dried and powered leaves materials (50 g) were extracted successively with 250 mL of petroleum ether, ethyl acetate, methanol, chloroform, acetone, benzene and water by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40℃ using Rotary evaporator. The residues obtained were stored in a freezer −70℃ until further tests. Phytochemical screening of the extracts was carried out according to the standard methods. **Results:** The present study screened the phytochemical properties of ten plants with sixty six extracts and showed varied degree of phyto-constituents present. The presence or absence of the phyto-constituents depended upon the solvent medium used for extraction and the physiological property of individual taxa. **Conclusions:** From these results, it can be concluded that the ten plants extracts may be used as broad-spectrum antimicrobial, bioactive agent after extensive investigation.

1. Introduction

Phytochemicals are present in a variety of plants, and are utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables[1]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids[2]. In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12 000 have been isolated, a number estimated to be less than 10% of the total[3]. Active components are found in different parts and organs of plants, and change or modify the functions of human and animal organs and systems. These substances serve as molecules of plant defense against predation by microorganisms, insects and herbivores and at the same time also exhibit medicinal properties for treating several ailments. Scientific research has allowed us to discover a wide range of active components, of which the most important, as far as health is concerned, are essential oils, alkaloids, glycosides or heterosides, mucilage and gums, and tannins[1]. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. Therefore, to meet the increasing demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously increased. This prompted us to evaluate plants as the source of potential chemotherapeutic agent, antimicrobial agent and to evaluate their ethno medicinal use[4]. Recently, there

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has been growing interest in exploiting the active principles of flora owing to their natural origin, cost effectiveness and lesser side effects[5-15]. Although the medicinal value of the pteridophytes have been known to man for more than 2 000 years, compared to the angiosperms very little application has been found in modern chemotherapy and researches on the antibiotic activity of this plant group are still in their infancy. *Drynaria quercifolia* (*D. quercifolia*)(J.) Smith. Is reported to treat vitiated vata, pitta, inflammation, infection, typhoid, cough, arthritis, headache, diarrhea, ulcer, and migraine. Decoction of fresh or dried rhizome is used for hemoptysis. Besides, it is also used as an astringent or antihelminthic. The leaves are used as poultices, and to treat dyspepsia, phthsis and fever. In Bangladesh, rhizomes are used in the treatment of excited mental disorders. *D. quercifolia* mixed with the plant *Asparagus racemosus* is applied to the head for calming effect and to reduce hair loss. It is a potential resource for anti-bacterial, anti-fungal, anti-gonorrheal, antipyretic, antioxidant, anti-Inflammatory/ analgesic, anti-dermatophytic activity and small doses shows central nervous system depressant effect. The rhizome paste is applied for treatment of diarrhoea, typhoid, cholera, chronic jaundice, fever, head ache and skin diseases. Whole plant is reported to have anthelmintic, expectorant, tonic property, and is used in the treatment of chest and skin diseases. Epifriedelinol, beta-amyrin, beta-sitosterol, beta-sitosterol 3-beta-D-glucopyranoside, and naringin were isolated from methanolic extracts of *D. quercifolia* dried rhizome and the test was positive for coumarins and triterpenes. The rhizomes of the plant have antibacterial properties and are used traditionally for treatment of cough, tuberculosis and typhoid fever[16-21]. *Dicranopteris linearis* (*D. linearis*)(Gleicheniaceae), known locally to the Malay′s as Rasam′ has been used in the Malay′s traditional medicine as a cooling drink and also used to reduce fever. Young leaves are used as poultice, decoction or infusion for fever, anti-helmintic, diarrhea, bound externally to wounds, cuts, ulcers, broils, sores, women sterility, anti-asthmatic and to get rid of intestinal worms. Scientifically, *D. linearis* extracts have been reported to possess antinociceptive, anti-inflammatory, antipyretic, cytotoxic, anti-staphylococcal activity and anti-oxidant properties[22-26]. *Hemionitis arifolia* (*H. arifolia*) (Burm.) Moore (family: Hemionitidaceae) is used in folk medicinal practices to treat diabetes mellitus in certain remote villages of Trivandrum district, Kerala state. In ethno-medical practices, the same fern is also used to treat aches and burns. Leaf extract is applied to centipede bite and wounds and about 10 g of root powder is taken orally with water in empty stomach twice a day for 10 days for treatment of hypertension[27-30]. *Drymoglossum heterophyllum* (*D. heterophyllum*) (Linn.) Trimen paste is obtained by crushing pinnae applied externally in the form of poultice on fractured bones after setting up the bones. Bamboo splints are usually tired around so as to prevent dislocation of fractured bones[31]. *Ceratopteris thalictroides* (*C. thalictroides*) Linn. leaves are used for skin complaints. It is used as drawing agent in treatment of carbuncles. In China, it is used as styptic to stop bleeding. In India, leaf powder along with turmeric is applied to unhealed wounds. Young fronds are eaten as vegetables[32]. *Blechnum orientale* (*B. orientale*) L. leaves are traditionally used as poultice to treat boils, blisters or abscesses and sores as a diaphoretic, and to treat stomach pain, skin infections, ring worms, diarrhea, urinary bladder complaints, sterilization of women and to stop wound bleeding, In addition they possess anthelmintic, anti-fungal, anti-bacterial, antiviral and anti-cancer, cytotoxicity properties. The leaf is also boiled and eaten as vegetable by the natives[33-35]. Whole plants of *Pityrogramma calomelanos* (*P. calomelanos*) (L.) Link is used for kidney troubles. In Trinidad and Tobago, the whole plant is used for urinary problems, amenorrhea, cough, fever, flu, haemostatic, hypertension, menorrhagia, tuberculosis and high cholesterol. Decoction of the plant with roots of *Cynodon dactylon* and styles of *Zea mays* is used for kidney afflictions. Previous studies yielded complex flavonoids, including four flavanones, calomelanols G, H, I and J. P. caloelanos possess anti-tumor potential[36]. A number of publications are available on the phytochemical and antimicrobial activity of pteridophytes at the global level but there are only limited publications on the pteridophytic flora of Western Ghats, India. To supplement the research in this respect and keep the folkloric use of this species in treating infectious diseases stimulated the investigation of the phytochemical studies on *B. orientale L, C. thalictroides* (L.) Brong, *D. heterophyllum* (L.) Trimen, *D. linearis* Burm. f., *D. quercifolia* (J.) Smith, *H. arifolia* (Burm), *T. Moore, Lindsaea ensifolia* (*L. ensifolia*) SW, *Nephrolepis multiflora* (*N. multiflora*) (Roxb.) Jarrett, *Pteris confusa* (*P. confusa*) T.G. Walker and *P. calomelanos* (L.) from the Western Ghats, India.

2. Materials and methods

2.1. Plant collection

Healthy, disease free entire plants of *B. orientale* L, *C. thalictroides* (L.) Brong, *D. heterophyllum* (L.) Trimen, *D. linearis* Burm.f., *H. arifolia* (Burm), *T. Moore, L. ensifolia* SW, *N. multiflora* (Roxb.) Jarrett, *P. confusa* T.G. Walker and *P. calomelanos* (L.) and parts *i.e.* leaves and rhizomes of *D. quercifolia* (J.) Smith, were collected from Kakachi, Tirunelveli Hills (1 000 m) in the Western Ghats. Voucher specimen has been deposited in Centre for Biodiversity and Biotechnology, Department of Botany, Nesamony Memorial Christian College, Marthandam - 629 165, Tamil Nadu, India.

2.2. Extraction of the plant and preliminary phytochemical screening

The fresh materials were washed in tap water 5 min and dried using blotting papers. The washed plant materials were air and shade dried for two weeks and pulverized to powder using mortar. The dried and powered leaves materials (50 g) were extracted successively with 250 mL of petroleum ether, ethyl acetate, methanol, chloroform, acetone, benzene and water by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40℃ using rotary evaporator. The residues obtained were stored in a freezer −70℃ until further tests[37]. Phytochemical screening of the extracts was carried out according to the standard methods[38-43].

3. Results

In the present study, the phytochemical screening was performed with acetone, benzene, chloroform, ethanol, petroleum ether and aqueous extracts of the whole plants of *B. orientale*, *C. thalictroides*, *D. heterophyllum*, *D. linearis*, *H. arifolia*, *L. ensifolia*, *N. multiflora*, *P. calomelanos*, *P. confusa* and leaves and rhizomes of *D. quercifolia*.

Out of sixty six tested extracts, fifty nine extracts showed the presence of phenolics. Next to that, forty seven extracts were illustrated their existence of tannin. Twenty eight extracts showed saponin occurrence in the crude extracts of the selected plants. Steroid was present only in twenty extracts, followed by coumarin in nineteen extracts. Carboxylic acid showed its presence only in eleven extracts.

Table 1.
Preliminary phytochemical studies on *D. quercifolia*, *D. heterophyllum*, *D. linearis*, *B. orientale*, *C. thalictroides* and *H. arifolia*.

Plants name	Solvents	Alkaline	Phenol	Flavonoids	Saponin	Protein	Quinnone	Steroids	Tannin	Xanthoprotein	Carboxylic acid	Coumarin	Carbohydrates
D. quercifolia – *leaves*	A	–	+	–	–	–	–	++	++	++	–	–	+
	B	–	++	–	++	–	–	–	+	–	–	–	+
	C	–	–	–	++	–	–	–	–	–	+	–	+++
	W	–	++	–	–	–	–	–	+	+	+	+	+++
	E	–	++	–	–	–	–	–	+	–	++	–	++
	P	–	++	–	++	–	–	–	+	–	–	–	–
D. quercifolia – *rhizome*	A	–	+++	–	–	–	–	–	++	–	–	–	+
	B	–	+++	–	++	–	–	–	++	–	–	–	+
	C	–	++	–	–	–	–	++	++	–	–	–	+++
	W	–	+++	+++	–	–	–	+++	+++	–	–	–	–
	E	–	++	++	–	–	–	+++	+++	–	–	–	–
	P	–	++	–	++	–	–	++	++	–	–	–	–
D. heterophyllum	A	–	+	–	–	–	–	–	+	++	–	–	+
	B	–	++	–	+	–	–	+	+	–	+	++	++
	C	–	++	–	++	–	–	–	+	–	+	–	+++
	W	–	++	–	–	–	–	–	+	–	+	++	+++
	E	–	+	–	–	–	–	+	+	+	–	+	+++
	P	–	++	–	–	–	–	+	+	–	–	++	++
D. linearis	A	–	+++	–	–	–	–	+++	+++	+++	–	–	+
	B	–	+	–	+++	–	–	–	–	–	–	–	+
	C	–	++	–	++	–	–	–	–	–	–	+	++
	W	–	+++	–	–	–	–	++	+++	–	–	+	++
	E	–	–	–	–	–	–	+	+	++	++	+	+++
	P	–	++	–	–	–	–	–	+	–	–	–	+
B. orientale	A	–	++	–	–	–	–	+	+	++	–	–	+
	B	–	++	+	+++	–	–	–	++	–	+++	+	++
	C	–	+++	–	++	–	–	++	–	–	–	–	
	W	–	++	–	–	–	–	–	–	++	–	–	++
	E	–	+++	–	–	–	–	–	+	++	–	–	+
	P	–	+++	–	++	–	–	++	++	–	+	–	–
C. thalictroides	A	–	+++	–	–	–	–	++	–	–	–	–	–
	B	–	++	–	+++	–	–	–	–	–	–	–	++
	C	–	++	–	–	–	–	–	–	–	–	–	+++
	W	–	+++	–	–	–	–	–	++	–	–	–	++
	E	–	+++	–	–	–	–	–	+	++	–	–	+
	P	–	+++	–	+	–	–	–	++	–	–	–	+++
H. arifolia	A	–	+++	–	–	–	–	–	+++	–	–	++	+++
	B	–	++	–	+++	–	–	+++	–	–	–	–	+
	C	–	+	+	–	–	–	–	+	+++	–	+	++
	W	–	+	–	–	–	–	–	+	–	–	+	+
	E	–	++	–	++	–	–	–	+	+++	–	+++	+++
	P	–	+	–	++	–	–	–	+	–	–	+	+

A – Acetone; B – Benzene; C – Chloroform; W – Aqueous; E – Ethanol; P – Petroleum ether.

Table 2.
Preliminary phytochemical studies on *N. multiflora*, *L. ensifolia*, *P. calomelanos* and *P. confuse*.

Plants name	Solvents	Alkaline	Phenol	Flavonoids	Saponin	Protein	Quinnone	Steroids	Tannin	Xanthoprotein	Carboxylic acid	Coumarin	Carbohydrates
N. multiflora	A	−	++	−		−	−	−	+	−	−		++
	B	−	+	−	+++	−	−	−	−	−	−	+	−
	C	−	+	−	+++	−	−	−	−	−	+	+++	−
	W	−	++	−	−	−	−	−	+	−	+	++	+
	E	−	++	−	−	−	−	−	+	−	−	−	++
	P	−	++	−	−	−	−	−	−	−	−	−	−
L. ensifolia	A	−	+++	−	−	−	−	−	+++	−	−	−	+++
	B	−	++	−	+++	−	−	−	−	−	−	−	−
	C	−	−	+	++	−	−	−	−	−	−	−	++
	W	−	++	−	−	−	−	−	+	−	−	−	++
	E	−	+++	−	−	−	−	+++	++	−	−	−	+++
	P	−	+	−	+++	−	−	−		−	−	−	−
P. calomelanos	A	−	+	−		−	−	+	+	+++	−	−	+++
	B	−	+	−	+++	−	+	−	+	−	−	−	+++
	C	−	++	−	+++	−	−	+	−	−	++	−	+++
	W	−	−	−	−	−	−	−	−	−	−	+	+++
	E	−	++	−	−	−	−	−	+	−	−	−	++
	P	−	−	−	−	−	−	−	+	−	−	−	++
P. confusa	A	−	+	−	−	−	−	+	+	+++	−	−	+++
	B	−	+	−	+++	−	+	−	+	−	−	−	+++
	C	−	++	−	+++	−	−	+	−	−	++	−	+++
	W	−	−	−	−	−	−	−	−	−	−	+	+++
	E	−	++	−	−	−	−	−	+	−	−	−	++
	P	−	−	−	−	−	−	−	+	−	−	−	++

A − Acetone; B − Benzene; C − Chloroform; W − Aqueous; E − Ethanol; P − Petroleum ether.

Flavonoid was present only five extracts and quinnone was present in two extracts and alkaloid was present only one extract.

4. Discussion

The present study screened the phytochemical properties of ten plants with sixty six extracts and showed varied degree of phyto-constituents present. The presence or absence of the phyto-constituents depends upon the solvent medium used for extraction and the physiological property of individual taxa. There are some studies on phytochemistry and pharmacology of *B. orientale*, *D. linearis*, *H. arifolia*, and rhizomes of *D. quercifolia*[16-36], but there is no report on C. thalictroides, *D. heterophyllum*, *L. ensifolia*, *N. multiflora*, *P. calomelanos*, *P. confusa* and leaves of *D. quercifolia*. In the case of *D. quercifolia*, in addition to the previous observation on rhizomes, the present study revealed and supplemented the phytochemical properties of leaves of *D. quercifolia*.

The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins *etc*[44]. Recently, a number of plants have been reported for antimicrobial properties across the world[7-15,24,30,35,38,39,41]. This investigation screened ten plants from India for phytochemical properties. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences.

Many tannin-containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic[45-47]. In the present study we revealed the tannins (47/66 extracts) presence in all tested plants. Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include anti-microbial, anti-inflammatory, anti-feedent, and hemolytic effects[48,49]. Saponin is used as mild detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, antifungal, anti-inflammatory, weight loss, *etc*. In the present study we observed the saponin (28/66 extracts) also present in all the tested plants. Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and

antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. In the present study, steroid (20/66) is present in all the selected plants except *N. multiflora*. Coumarin has been used as anti-coagulant drugs and to treat lymphedema[50]. In the present study we observed the coumarin (19/66 extracts) presence in *B. orientale, D. heterophyllum, D. linearis, H. arifolia, N. multiflora, P. calomelanos, P. confusa* and leaves of *D. quercifolia*. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities[51]. In the present study we observed the flavonoids presence only in few plants *viz., B. orientale, H. arifolia, L. ensifolia* and rhizomes of *D. quercifolia*. Phenolic possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include anti-microbial, anti-inflammatory, anti-feedent, anti-viral, anti-cancer, and vasodilatory actions[52]. In the present study we observed phenolic presence in all tested plant with maximum percentage (59/66; 89%). Phytochemical studies on extracts of *B. orientale, C. thalictroides, D. heterophyllum, D. linearis, H. arifolia, L. ensifolia, N. multiflora, P. calomelanos, P. confusa* and leaves and rhizomes of *D. quercifolia* revealed the presence of carbohydrates, steroids, alkaloids, tannins, saponins, flavones, carboxylic acid, xanthoprotein and phenolic compounds. The antimicrobial activity of *B. orientale, D. linearis, H. arifolia* and rhizomes of *D. quercifolia* may be due to one/more group of above phyto-constituents. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Plant with antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products. From these results, it can be concluded that the selected ten plants extracts may be used as broad-spectrum antimicrobial, bioactive agent after extensive investigation. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

Conflict of interest statement

We declare that we have no conflict of interest.

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