Evaluation of Phytochemicals and Antimicrobial potential of Aerial part of *Christella parasitica* (L.) H. Lév.

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ABSTRACT

Plants are the main sources of secondary metabolites and its extracts were used in Traditional medicinal systems for curing various ailments. Among the plant resources, Pteridophytes were underutilized medicinal plants that possess high value of bioactive compounds. In the present study, Phytochemical evaluation and antimicrobial potential of aerial part of *Christella parasitica* (L.) H. Lév. belonging to the family Thelypteridaceae was carried out. The powdered plant materials were subjected to successive solvent extractions and to evaluate their potential antimicrobial efficacy against human pathogens. The detection of phytocompounds in powdered samples was examined under normal and UV light ranges at 265 nm and 365 nm. Different fluorescence characters were indicates the presence of potential phytcompounds in the selected plant. Further, the extracts were tested against different human pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* and two fungi *Fusarium oxysporum* and *Candida albicans*. The results revealed that petroleum ether extract at 50mg /ml concentration was inhibited the growth of *Staphylococcus aureus* at the maximum of 14.4 ± 1.3 mm zone of inhibition. Therefore, the studies explored that petroleum ether extract of *C. parasitica* have more potential biological activities.

Keywords: Antibacterial activity, *Christella parasitica*, ethyl acetate, phytochemical and Pteridophytes

INTRODUCTION

Natural biodiversity has provided the foundation for all agricultural, horticultural plants and domesticated animals. Plants are one of the key elements of medicines. India is one of the twelve mega-biodiversity countries of the world, having rich vegetation with a wide variety of plants having a lot of medicinal value. These plants have found application processes in the pharmaceutical, cosmetic, agricultural, and food industries [1]. In India, the Pteridophytes have been found to grow in almost all climatic zones under different habitats and are represented by approximately 1200 species falling under 191 genera [2]. *Dicranopteris linearis*, the rhizome is used as an anthelmintic in Assam while the fronds are used for asthma in Madagascar [3]. The preliminary phytochemical screening in *C. parasitica* and found lowest phytoconstituents in high polar solvents [4]. In addition to that the species *C. dentata* extracts by chloroform and alcohol have more effective antimicrobial activities against different pathogens. But, the low polar solvent extracts showed instability in drug delivery. The pathogens are causing several diseases. The *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* are most common disease causing microorganism and still there are lack of drug molecules for therapeutics. There several strategies have followed to inhibit or control the activities of microbes. The synthetic antibiotics are effectively control the growth of pathogens indeed it causes more side effects. Therefore, the alternative methods are used to inhibit the growth of microorganism by using plant extracted compounds. Several plant extracts from different parts contains various secondary metabolites such as alpha-amyrin, beta-amyrin, lupeol, and 2-hydroxy-4-methoxy benzoic [5]. *C. dentata* extracts proved that more phytcompounds and these compounds are potential antimicrobial properties [6]. The genus *Christella* rhizomes are having more antioxidant and antimicrobial activities due to the presence of rich bioactive compound lupeol. Hence, in the present study aims to extract the various phytocompounds from the aerial part and analyse their chemical structure by analytical characterisation techniques.

The antimicrobial efficacy of extracted compounds was tested against various human pathogens.

MATERIALS AND METHODS

Plant materials selected for the present study:

The experimental plant *Christella parasitica* (L.) H. Lév. was collected from Manjolai of Tirunelveli district. Identification of plant was confirmed with the help of Pteridophytic flora [7] and submitted the plant specimens in the Xavier's College Herbarium (XCH 4231) at St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Preparation of Plant Powder

The fresh aerial part of the plant *C. parasitica* was collected and washed thoroughly to remove impurities. After that it was cut into pieces and shade dried at room temperature (25-30^{o}c), for two to three weeks. The dried materials were ground to fine powder for further analysis. After that it was cut into small pieces and shade dried at room temperature (25-30^{o}c), for two to three weeks. The dried materials were ground into fine powder for further analysis.

Fluorescence analysis

The fluorescence analysis of the powdered drug was done according to the methods described by Chase and Pratt [8]. Fine powder and their extracts were examined under visible light and UV light. These powdered materials were also treated with various reagents such as 50% HNO_3, acetone, ethyl alcohol, 50% H_2SO_4 1N HCl and 1N aqueous NaOH and colour changes were recorded.

Phytochemical analysis

Extraction

About 30 gm of dried plant powder *C. parasitica* was used for various successive solvent extractions (Petroleum ether, Ethyl Acetate, and Aqueous) at different volume and increasing polarity index. The individual fractions were collected and concentrated to obtain crude extracts. The aqueous extract was prepared directly by boiling the powder with distilled H_2O. These extracts were concentrated and kept in brown bottles for further use [9].

Preliminary phytochemical screening

The plant extracts were tested for the presence of bioactive compounds such as terpenoids, alkaloids, glycosides, steroids, phenols, tannins, flavonoids and saponins by standard methods of Yadav and Agarwala [10].

Qunatitative analysis

Estimation of Total Phenols

100 mg of plant material was homogenated with 10 ml of 80% ethanol. The homogenate was centrifuged at 3000 rpm for 5mins and the collected supernatant was made up to a known volume. The aliquot was taken from the supernatant and 0.5 ml folin- phenol reagent and 1 ml of 20 % of Na_2CO_3 were added. The final volume was made up to 5 ml with distilled water. The mixer was shaken well and kept in a boiling water bath for a minute. Then the tubes were cooled in running tap water and the resulting blue color was measured at 630 nm against blank [11].

Estimation of Total Flavonoids

10 gm of the powdered plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125 nm). The filtrate was later transferred into a previously weighed China dish and evaporated to dryness over a water bath. The final residue was weighed [12].

Calculation: Total Flavonoid = Final weight – Initial weight

Antimicrobial Activity

Antibacteial activity

The antibacterial activity of petroleum ether, ethyl acetate and aqueous extracts of *Christella parasitica* was evaluated by measuring the zone of inhibition. For antibacterial activity, the gram-positive bacteria such as *Bacillus subtilis* (MTCC2385), and gram-negative bacteria such as *Escherichia coli* (MTCC 25923), *Salmonella typhi* (MTCC 2915), *Pseudomonas aeruginosa* (MTCC 2945) were used. The bacterial strains were obtained from the Department of Botany, Bharathiar University, Tamil Nadu, India. Muller-Hinton broth was incubated at 37±1.5°C for 18 h. After incubation period the microbial strains were smeared on sterile Muller-Hinton agar plates. The negative control used in the study was the respective solvents and the positive control was Chloramphenicol. The plates were incubated at 37°C for 18 h. Antibacterial activity was done by well diffusion method [13].

Antifungal activity

The standard strain used for the study is *Fusarium oxysporum* and *Candida albicans*. This was grown on potato dextrose agar (PDA) media (Hi Media Laboratories Pvt, Ltd; Mumbai, India.) overnight at 37° C for 24 hrs and 48 hrs. 3-5 colonies of the standard strain of *Fusarium oxysporum* and *Candida albicans* were suspended in 2 ml of sterile normal saline and vortexed. The turbidity of the homogenous suspension was adjusted to approximately 0.5 McFarlands standards. The negative control used in the study was the respective solvents and the positive control was Clotrimazole [14]

RESULT AND DISCUSSION

Fluorescence analysis of plant powder

Fluorescence analysis of *C. parasitica* plant powder was carried out after treated with different chemical compounds such as, acetone, petroleum ether, methanol, benzene, chloroform, ammonia, acetic acid, sulphuric acid, ethanol, nitric acid, iodine, 0.1N HCl, 0.1N NaOH, distilled water, ethyl acetate and plant powder to study the fluorescence characters was observed under visible light and UV light (254 nm and 366 nm), which shown predominantly fluorescence effect in the plants. Some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostic evaluation [15]. The fluorescence powder characteristic of C. *parasitica* was displayed in Table 1.

Table 1: Fluorescence analysis of *C. parasitica*

S. No	Solvents and Chemicals	*C. parasitica*		
		Normal Light	UV Light	
			265nm	365nm
1.	Acetone	Dark green	Light green	Dark green
2.	Petroleum Ether	Light green	Pale yellow	Green
3.	Methanol	Green	Light green	Dark green
4.	Benzene	Light brown	Light green	Dark green
5.	Chloroform	Pale yellow	Light green	Green
6.	Ammonia	Brown	Light yellow	Dark green
7.	Acetic Acid	Light brown	Green	Dark Green
8.	Sulphuric Acid	Brown	Light green	Green
9.	Ethanol	Green	Light green	Dark green
10.	Nitric Acid	Orange	Brown	Dark green
11.	Iodine	Brown	Light brown	Dark green
12.	0.1 N HCl	Light brown	Light green	Dark green
13.	0.1 N Na OH	Dark green	Light green	Green
14.	5% $FeCl_3$	Brown	Green	Dark green
15.	Dis. Water	Green	Light green	Dark green
16.	Ethyl acetate	Light brown	Light green	Green
17.	Powder	Green	Light green	Dark green

The *C. parasitica* powder treated with acetone showed dark and light green colour under normal and UV light (265nmand 365nm). Petroleum ether and methanol treated powder showed light green, pale yellow, green and dark green colours under normal and UV lights. Light brown and dark green colours observed under normal light and 365nm UV light when the powder treated with benzene. Ethanol treated powder showed green and light green under normal and 265nm UV light respectively. Nitric acid treated powder showed orange colour under normal light and brown colour under 265nm of UV light. Iodine treated powder showed brown colour under normal light and light brown colour under 265nm and 365nm was observed under light and dark green in UV light. Similar to the present study, Kala *et al.* [16] previously applied fluorescence characters as a tool to characterize the different medicinal plants of South India. In Pharmacognostic evaluation of crude drugs, fluorescence analysis was an important parameter [17], which revealed the presence of natural chromophores of the drug source and helps to identify the similar drugs in the pharmaceutical industries.

Preliminary phytochemical analysis

Phytochemical screening is important method to identify new source of therapeutically and industrially valuable compound having medicinal significance to make the best and judicious use of available natural wealth. When the environmental condition is not favour for plants they produce secondary metabolites [18]. The secondary metabolites produced against the various adverse environmental conditions are alkaloids, flavonoids, tannin, saponins, phenols steroids, quinines, etc [19]. The preliminary phytochemical analysis of *C. parasitica* was showed the presence and absence of different phytoconstituents such as carbohydrates, glycosides, sterols, tannins, terpenoids, alkaloids, amino acids, protein, saponin and phenols in extracted samples (Table 2).

Table 2: Qualitative analysis of phytochemical constituents in different extract of *C. parasitica*

S. No	Phytochemicals	*C. parasitica*		
		Petroleum Ether	Ethyl acetate	Distilled water
1.	Carbohydrates	+	++	-
2.	Anthocyain	-	+	+
3.	Alkaloids	+	++	-
4.	Steroids/Phytosteroids	++	++	+
5.	Tannins	++	+	++
6.	Saponins	+	+	-
7.	Flavonoids	+	++	-
8.	Quinones	+	+	+
9.	Glycosides	++	+	-
10.	Cardiac Glycoside	++	++	-
11.	Terpenoids	+	+	+
12.	Phenols	+	+	+
13.	Protein/Amino acid	+	+	-
14.	Anthraquinone	+	-	-
15.	Phlobatannins	+	+	+
16.	Coumarins	-	+	-

(+) Presence of chemical compound, (-) Absence of chemical compound (++) Based on the intensity of characteristic colour.

Tannins, Steroids/Phytosteroids, Quinones, Terpenoids and Phenols are present in all the solvent extracts. Phlobatannins is present in petroleum ether, ethyl acetate and distilled water extracts. Protein/Amino acid, Cardiac Glycoside, Flavonoids, Carbohydrates and saponins are present in petroleum ether and ethyl acetate extracts. Anthraquinone was absent in petroleum ether extract. Coumarins are absent in petroleum ether and distilled water extracts. Anthraquinone are absent in ethyl acetate and distilled water extracts. Coumarins are absent in ethyl acetate extract only. The water extracts showed limited bioactive compounds including glycosides, saponins and protein, etc. shown in *C. parasitica*. Muraleedharannair *et al*. [19] evaluated the phytoconstituents of *Adiantum* and *Christella sp.* provides chemical marker and inter-specific variation between the medicinally important geniuses. Rajesh *et al*. [20] evaluated qualitative and quantitative phytochemical analysis of some important Pteridophytes *Actinopteris rediata*, *Drynaria quercifolia* from Western Ghats.

Quantitative analysis

Quantification of total flavonoids and phenols

The quantitative analysis of secondary metabolites such as alkaloids, flavonoids, sterols, glycosides, (poly) phenols and terpenoids were reported in *C. parasitica*. *C. dentatus*, *N. acutifolia* and *M. punctatum* showed the presence of total phenol and flavonoids [21]. But, in this study we identified high value of flavonoids and phenols contents in petroleum ether, ethyl acetate and water extracts of *C. parasitica* respectively in Table 3.

Table 3: Flavonoids and Phenols contents in the different extract of *C. parasitica*

S. No	Solvents	*C. parasitica*	
		Flavonoids (mg/g)	Phenols (mg/g)
1.	Petroleum ether	33.21±2.1	47.25 ±3.23
2.	Ethyl acetate	65.45±4.81	58.34±1.18
3.	Water	28.32±2.2	8.63±7.42

The different solvent extract was estimated the total flavonoid content of *C.parasitica* extracted samples. Among all extracted samples, ethyl acetate extract of *C. parasitica* shows highest flavonoids content (65.45±4.81 mg/g). The minimum quantity of flavonoids was recorded in the water extract of the same plant (28.32±2.2 mg/g). Ethyl acetate extract of *C. parasitica* showed maximum total phenolic content (58.34±1.18 mg/g) and petroleum ether showed 47.25±3.23 mg/g. But, total phenols content was observed less quantity in water extract that containing only 8.63±7.42 mg/g in *C. parasitica*. Flavonoids are important secondary metabolites soluble in water free radical scavengers that protect

the cells from oxidative cell damage [22]. Flavonoids are important secondary metabolites play important role in anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer activity [23]. Some earlier studies have also reported the presence of flavonoids *Equisetum arvense* [24], various types of flavonoids such as astragarin, kaempferol glucoside and kaempferol rutinoside have been reported in *C. parasitica* [25]. Phenolics are the most widespread secondary metabolites in the plant kingdom and have received much attention as potential natural antioxidants in terms of their abilities to act as both efficient free radical scavengers and metal chelators [26]. Phenols were present in all the solvents except distilled water of *Dicranopteris linearis* extract and indicate that this plant may be used as an anti-microbial agent [27].

Antimicrobial activity

In this study, different solvent extract of *C. parasitica* against four bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and two fungal strains (*Fusarium oxysporium* and *Candida albicans*) showed more or less sensitivity based on these extracts concentration and their bioactive compounds.

Antibacterial activity

The antibacterial activity of the different solvent extracts of *C. parasitica* was determined against some human pathogens. All the concentration of different solvent extracts was compared with the Chloramphenicol, a standard antibacterial drug. The zone of inhibition of *C. parasitica* against gram positive and gram negative bacterial strains were depicted data is shown in Table 4.

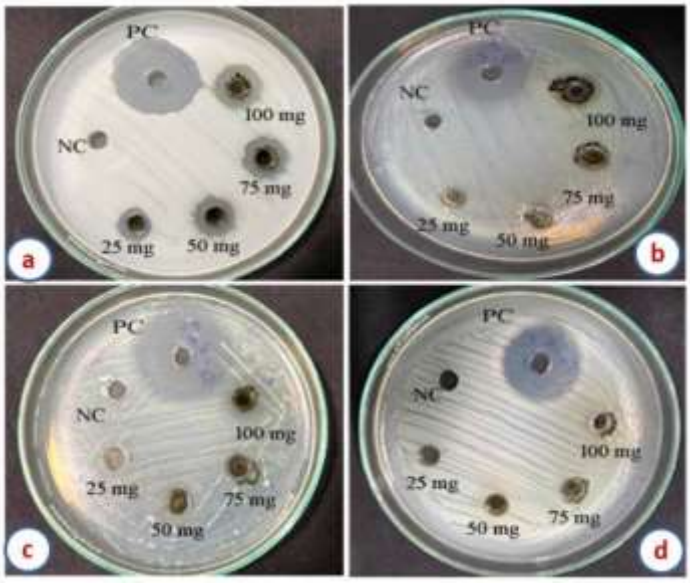
Table 4: **Antibacterial activity of *C. parasitica* extract using well diffusion assay method**

Bacterial Strains	Zone of Inhibition in different solvent extracts (mm)				
	C. parasitica				
	Petroleum ether	Ethyl acetate	Distilled Water	Negative control	Positive Control (Chloramphenicol)
Escherichia coli	2.4 ± 0.07	4.6 ± 0.3	9 ± 0.8	-	12.4 ± 1.2
Klebsiella pneumonia	3.2 ± 0.6	3.6 ± 0.2	5.2 ± 0.7	1.5± 0.05	15.2± 0.8
Bacillus subtilis	4.2 ± 0.5	7.4 ± 0.4	2.5 ± 0.4	-	19.8 ± 1.3
Staphylococcus aureus	6.4 ± 1.3	7.8 ± 0.4	5.1 ± 1.3	-	24.2 ± 0.5

± Standard Error, - Absent

Note: Values are means of three independent analysis of the extract ± standard deviation (n=3).

Figure 1: Antibacterial assay – Zone of inhibition of in ethyl acetate extract of *C. parasitica*



a. *Bacillus subtilis*, b. *Escherichia coli*, c. *Staphylococcus aureus*, d. *Klebsiella pneumonia*
1. Positive control-Chloramfenicol, 2. Negative control-Solvent.

The results showed that the highest inhibition zones were recorded in petroleum ether extraction of *C. parasitica* at a dose of 50 mg/ml concentration against *Staphylococcus aureus* (14.4 ± 1.3a). The lowest concentration of *Escherichia coli* and *Klebsiella pneumoniae* were obtained 2.4 ± 0.07d and 3.2 ± 0.6c zone of inhibition. The major compounds in *Christella* species are flavonoids groups such as rutin and quercitrin that have more antimicrobial activities against general infectious bacteria such as *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* [28]. The *isocoumarin bergenin* also has antimicrobial activities against the *Candida* species and some *Aspergillus* species [29]. The different solvent extraction of *C. parasitica* were inhibited both gram positive and gram negative bacteria at different concentrations and observed for better clear zone of inhibition shown in (Figure 1).

Antifungal activity

The result of antifungal activity was recorded in petroleum ether, ethyl acetate and water extract of *C. parasitica.* The petroleum ether extract fraction of C. *parasitica* was tested against fungus *Fusarium oxysporium* and *Candida albicans*. All the concentration of different solvent extracts was compared with the Fluconazole, a standard antifungal drug. The minimal inhibitory activities of different extracts of *C. parasitica* were recorded. Significant antifungal results were obtained by different extracts at different concentration and the zone of inhibition was recorded in Table 5. Among the two fungal strains, the higher zone of inhibition was recorded in *Candida albicans* that was 5.5±0.4 mm in *C. parasitica* plant extracts and no zone of inhibition was found on water extraction.

Table 5: **Antifungal activity of *C. parasitica* extract using well diffusion assay method**

Fungal Strains	Zone of Inhibition in different solvent extracts (mm)				
	C. parasitica				
	Petroleum ether	Ethyl acetate	Distilled Water	Negative control	Positive Control (Fluconazole)
Fusarium oxysporum	-	9 ± 0.4	-	-	8.4 ± 0.9
Candida albicans	3.2	5.5 ± 0.4	-	-	28.1 ± 0.4

± Standard Error, - Absent

Note: Values are means of three independent analysis of the extract ± standard deviation (n=3).

Similar results were found in *C. dentata* which possess antifungal and antibacterial activities [30, 31]. Recently there has been great effort to find candidates from natural products to effectively control infectious strains. For instance, flower extracts of *Calotropis procera* [32] and *Delonix regia* [33] have been reported to exhibit antimicrobial activity against infectious strains. Extracts of various plants containing flavonoids and isocoumarins have also been previously reported to possess antimicrobial activity. These findings indicated that the ethyl acetate extract of *C. parasitica* was more potent when compared with standard antibacterial and antifungal drugs. Similarly, higher antifungal activity was recorded in methanolic extracts of *N. cordifolia* which was evident by its potential inhibitory activity against fungal spore germination and radial growth of all fungi [34]. Duraipandiyan and Ignacimuthu [35] reported that potent antibacterial and antifungal activities on *Toddalia asiatica* in hexane, ethyl acetate, methanol, and water extracted fraction.

CONCLUSION

The study confirmed the presence phytoconstituents and antimicrobial potential of aerial part of *C. parasitica*. The preliminary phytochemical experiment strongly revealed that the plant contains phenol, steroid, flavonoid, tannin and terpenoid content sufficiently through its colour intensity. These extracted compounds have more potential antimicrobial activity. Therefore, the present investigation will pave a new way to develop drugs by purification from potent medicinal plants of *C. parasitica* further used in pharmaceutical applications.

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