Antimicrobial Activity of Rhizome of *Christella dentata.* (forsk.) Brownsey & Jermy Against Selected Microorganisms

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Abstract

Men have boundless exposure to several microorganisms, which may cause different infections. These pathogenic organisms can develop resistance due to various reasons. Resistances to antibiotics are a big issue globally because antibiotic resistance treatment effects are reduced, resulting in increased morbidity and mortality worldwide. The need is to reduce the resistance and to develop a novel antimicrobial agent in a natural way. The present study aims to evaluate Chloroform's antifungal and antibacterial activity, ethyl acetate, PBHM, and deionized water extract of *Christella dentati*on: one gram-positive, four gram-negative, and one gram-negative fungal strain. The antimicrobial activity of *C.dentata* was tested by using the ager well diffusion method. The results showed that the plant exhibited antimicrobial activity in the chloroform and ethyl acetate extract. The chloroform and ethyl acetate showed a maximum level of antimicrobial activity, moderate level of inhibition displayed by PBHM and the lowest level of inhibition showed by deionized water extract. Most sensitive organism was *Pseudomonas*, *Candida albicans* and *S.aureus*. The study confirmed that all extracts showed different antimicrobial activity against tested organisms. The differentiating in the activity of these extracts could serve for the formulation of novel antimicrobial agents in future.

Keywords: *Christella dentation,* Chloroform's, ethyl acetate, PBHM, deionized water extract, antimicrobial activity

Tob Regul Sci.™ 2022; 8(1): 584-590
DOI: doi.org/10.18001/TRS.8.1.51

INTRODUCTION

Pteridophytes are an ancient group of non-seed-bearing plants, characterized as a significant group in biogeography as they contain a large number of relict and endemic taxa[1]. Nearly 12,000 taxa of pteridophytes are reported to be distributed on earth in different ecological niches. Out of these, 10,000 taxa are ferns and the rest fern allies[2]. Although pteridophytes are the second largest group, they represent only 5–7% of the total vascular plants but still play an important ecological role, particularly in the tropical vegetation[3].

The number of taxa of pteridophytes included within The Plant List belongs to 48 families and 587 genera. This list contains 47,439 scientific plant names of species rank for the pteridophytes. Of these, 10,620 are accepted species names (www.plantlist.com). The pteridophytes, distributed in the tropical, subtropical, and remote tropical islands and in different habitats or even in deeper rainforests, play a definitive role in constituting the strong carpet flora and undercover vegetation [2].*C. dentata* (Forssk.) Brownsey & Jermy (Family: Thelypteridaceae) has been reported extensively in West Pakistan and Kashmir [4].

It grows in the presence of high humidity and shade conditions[5]. *Christella dentata* possesses antifungal and antibacterial properties[6-8]. The leaves of *C. dentata* (Brownsey & Jermy) are dimorphic, evergreen, often closely placed 50 to 150 cm, fertile leaves with longer petioles and more contracted pinnae and stems short-creeping, 4-6 mm in diameter[1].The plant is edible[9]and is used to treat skin diseases in folk medicine[10].There is limited information about the traditional medicinal uses of these ferns, with one species, *Christella dentata*, reportedly used in Bangladesh as an antihyperglycemic and analgesia[8, 11, 12]. This study aimed to use (*Christella sp.)* for the antimicrobial activity against selected bacteria and fungi.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fresh plants of *Christella dentate* were collected in March 2019 from Bella (kolti zeri), a village in the Mansehra district. The antifungal and antibacterial activity of the rhizome of *Christella dentata* was performed in the laboratory of Hazara University located in Mansehra district of KPKthe rhizome of the plant is separated and washed with tap water and then with distilled water for proper decontamination. Then rhizome of the plant was shade dried in 20 -25 days at room temperature.

2.2. Preparation of plant extract

After the confirmation, the plant's rhizome was separated and washed with tap water and distilled water for proper decontamination. Then rhizome of the plant was shade dried in 20 -25 days at room temperature. For extraction, the shade dried rhizome of *Christella dentata* was converted into powder by an electrical grinding machine. The powdered form of rhizome was then weighed on a balance and stored in a sterile container for further use. Different solvents with different polarities were used for extraction methods, including Chloroform, ethyl acetate, and deionized water.

2.2.1.Chloroform and ethyl acetate extraction of *Christella dentata*

5g of powder drug of *Christella dentata* was taken in two flasks, and 100ml of ethyl acetate and chloroform solvent was added in each flask with the help 100ml glass cylinder and soaked for 7-8 days at room temperature. The solution was shaking periodically. After this, the extract was filtered by Whatman filter paper and transferred to a beaker. The filtrate was dried on a hot plate, and then

the powder was scratched from the beaker by a sterile blade and stored in a clean container for further experiment.

2.2.2.Deionized water extraction of *Christella dentata*

5g of rhizome powder of plant was added to the flask, and 100ml of deionized water was added. Initially, the solution was heated at 100ºc for a few minutes on a hot plate then the temperature was dropped to 80ºc for 40 minutes. Shaking was done through a magnetic stirrer. After 40 minutes of heating, deionized water gives brownish colour, showing that extraction was done properly. The solution was then filtered by Whatman filter paper. The filtrate was added to centrifuge tubes for centrifugation. The filtrate was centrifuged at 4000 rpm for 40 minutes. After centrifugation, the filtrate was stored in the container and supernatant (heavy biomass) in Eppendorf tubes for antimicrobial activity.

2.3. Pure culture collection

Pure culture of bacteria and fungus was collected from different research labs of Pakistan. Pure culture includes various microorganisms; Gram-negative bacteria; *E.coli, Pseudomonas aeroginosa, S. aureus, Shigella.* Fungal strain; *Candida albicans*.Gram-positive; MDR strain of *Salmonella typhi.*

2.4. In vitro antimicrobial assay

For antimicrobial activity, chloroform and ethyl acetate was dissolved in DMSO, and water extracts heavy biomass was dissolved in deionized water. Antimicrobial activity was checked through the Agar well diffusion method.

2.4.1. Agar well diffusion method

The pure cultured was subculture on nutrient media and incubated for 24 hours at 37ºc temperature. The fresh culture of microbes was inoculated on Petri dishes filled with the appropriate amount of nutrient agar using a streak plate method. After inoculation, three holes were made in Petri dishes containing bacterial culture, while four holes were made in fungus having a plate. For positive control drug of choice was used according to bacterial strain. For fungus, positive control *fluconazole* solution was prepared. For solution preparation, 500mg capsule of fluconazole is added to 1ml of deionized water and then added to the well with the help of a pipette and negative control of chloroform and ethyl acetate extract. DMS was used, and for plant heavy biomass and water extract, deionized water was used and added in well. The other two holes of both fungal and bacterial plates are filled with plant solvent extract at a concentration of about 50µl and 100µl for testing antimicrobial activity.

2.4.2.Determination of zone of inhibition

After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts and the antibiotics with slide calipers

3. RESULT

The antibacterial activity of *Christella dentata* was evaluated by Agar well diffusion method. The antimicrobial activity of various extracts of *Christella dentata* rhizome was studied in two different concentrations (50µl and 100µl) against five bacterial strains and one fungal strain. Bacterial strain

includes gram-positive *(Staphylococcus aureus*) gram-negative (*Shigella, E.coli*, MDR strain of *Salmonella typhi* and *Pseudomonas aeruginosa*). The fungal strain contains *Candida albicans* (Table .1).The antimicrobial potential of Chloroform, ethyl acetate, PBHM and deionized water extract was assessed in terms of zone of inhibition.

Table: 1. Antimicrobial effect of Rhizome of *C. dentate*.

Tested Microorganisms	Water Extract (µl)		PBHM (µl)		Chloroform Extract (µl)		Ethyl acetate Extract (µl)		Positive Control
	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	Antibiotics
	Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)
Candidaalbicans	-	-	2.6	2.9	3.8	4	3.6	3.9	Fluconazole= 3.7
E coli	1.9	2	2.1	2.4	2.8	3	2.9	3.3	Ciprofloxacin= 4
Pseudomonas aerugilosa	2	2.3	1.9	2.2	3.1	3.3	3	3.3	Gentamycin=2.8
Shigella	1.9	2.2	2	2.3	2.5	2.9	2.7	3.1	Cefotaxime=0.5
Staphylococcus aureus	2.4	2.7	2.6	2.8	3.3	3.8	3.8	4.1	Sulbactam= 3.4
Salmonella typhi	-	-	1	1.2	2.5	2.7	2.3	2.5	Cifotixin= 0.5
Negative Control	Deionized Water				DMSO				

3.1. Antibacterial results of chloroform and ethyl- acetate extract of *Christella dentata*

The present study demonstrates that chloroform and ethyl acetate showed a moderate level of inhibition to *E.coli, Shigella, Salmonella typhi,* and the most sensitive organisms toward ethyl acetate and chloroform extract of *C. dentata* were
Pseudomonas aeruginosa and *S.aureus* (Table 1.). Chloroform showed at 50µl and 100µl concentration about 3.3mm and 3.8mm biggest zone against *S. aureus* and 3.1mm and 3.3 against *Pseudomonas aeruginosa* (Fig.1). While ethyl acetate extract of *Christella dentata* showed the biggest zone of inhibition, about 3.8mm and 4.1mm towardS.aureus and 3.0mm, 3.3mm toward *Pseudomonas aeruginosa* (Fig.2).

Fig: 1. Antimicrobial activity of chloroform extract of *Christella dentata*

Fig: 2. Antimicrobial activity of ethyl acetate extract of *Christella dentate*

3.2. Antibacterial results of PBHM and deionized water extract of *Christella dentata*

Deionized water did not show antibacterial activity toward the MDR *Salmonella typhi* strain. In contrast, PBHM extract showed lower antibacterial activity towards *Salmonella typhi,* about 1mm and 1.2mm inhibition zones at 50µl and100 µl concentrate. Still, both showed a significant level of inhibition

to *E.coli, Salmonella typhi,Pseudomonas aeruginosa* and *Shigella* at 50µl and 100µl concentrations (Table.1). The biggest zone showed toward *S.aureus*by both PMHM and water. 2.4mm and 2.7 mm zone of inhibition to *S.aureus*are displayed by deionized water extract, and 2.6mm and 2.8mm zones are displayed by PBHM (Fig 3&4).

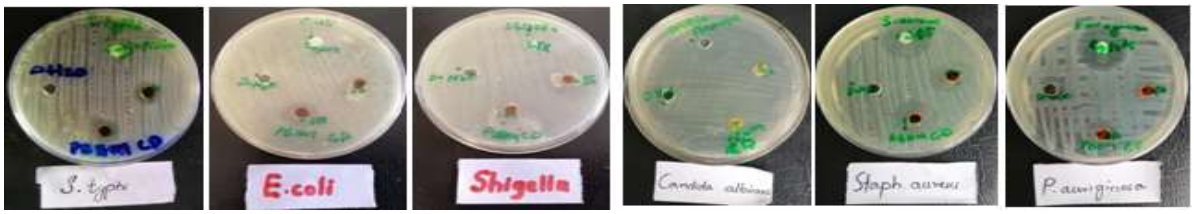

Fig: 3. Antimicrobial activity of PBHM extract of *Christella dentate*

Fig: 4. Antimicrobial activity of Deionized water extract of *Christella dentata*

3.3. Antifungal result of Chloroform, ethyl acetate, PBHM and deionized water extract of *Christella dentata*

The antifungal activity did not show by deionized water extract of Christella dental while ethyl acetate, Chloroform and PBHM showed significant antifungal activity at 50µl and 100µl concentrations (Table.1). The biggest zone of inhibition showed by Chloroform and ethyl acetate was 3.8mm and 3.6mm at 50µl concentration and 4mm, 3.9mm at 100µl concentration. The smallest antifungal zone showed by PBHM about 2.6mm at 50µl and 2.9mm at 100µl concentration (fig.5)

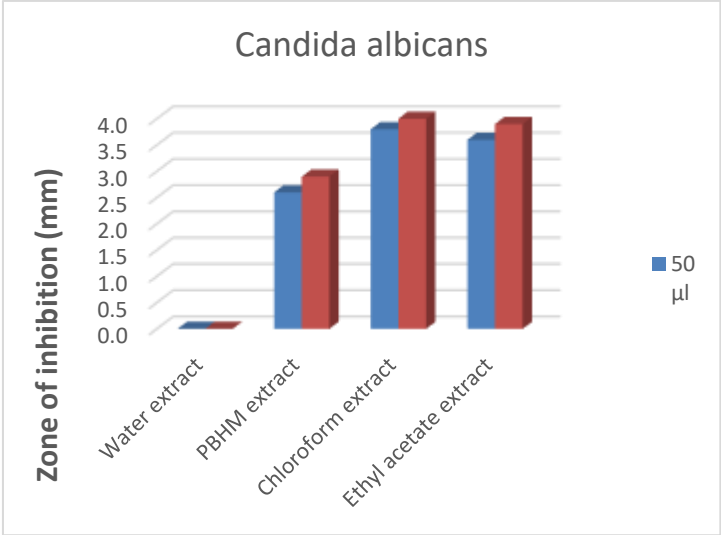

Figure 5: Water, PBHM, Chloroform, Ethyl acetate extracts of *Christella dentata* Rhizome activity against *Candida albicans*.

4. DISCUSSION

In Previous investigate in their article that *Christella dentata* rhizome part contains many secondary metabolites compared to other factors like leaves, so rhizome shows excellent antimicrobial activity compared to other plants parts. The above result shows that non-polar (chloroform) and semi-polar (ethyl acetate) extract show good antimicrobial activity towards bacterial strains such as *E.coli, Staphylococcus aureus* and *salmonella typhi*. The results were also similar to those of other studies that reported antimicrobial activity extract of *Christella dentata* frosk. Study in different seasons, but the difference in results could be due to the use of alcohol solvent [13][14] also demonstrate similar that *Christella* plant polar methanolic extract exhibit activity against *E.coli, S.aureus* but water extract did not show antibacterial activity. This difference in results may also be due to the use of different solvent systems[15] also showed that genus *Christella* exhibits antibacterial potential against *pseudomonas aeruginosa* and *S.aureus*. Thomas,2015 demonstrate that *S.aureus* and *Pseudomonas aeruginosa* are the most sensitive organisms to semi-polar extract of *Christella dentata* and *E.coli* are less sensitive organism. He also demonstrates that the water extract of *Christella* did not show antibacterial activity. The difference in this result could be due to concentration and solvents. The above result also showed that chloroform extract of thelypteris also has antifungal activity against Candida albicans and antibacterial activity against most pathogenic bacteria, including E.coli Pseudomonas *S.aureus, Shigella* etc. The results also have good agreement with the study reported by *[16]*. The study confirmed the therapeutic uses of Christella; thus, in search of a novel antimicrobial agent, the formulation consisting of different concentrations of these extracts could be proven good in the future.

5. CONCLUSION

In conclusion, the result confirms that the rhizome of *Christella dentata*plant has great potential against pathogenic microorganisms. Different solvent extracts of plants showed other antifungal and antibacterial activity. This difference in antimicrobial activity of extracts helps in the development of novel antibacterial agents in future. The next step is to isolate every active compound from the rhizome of *Christella* and to screen their bioactivity. The study also revealed

that chloroform and ethyl acetate extract exhibited the highest antimicrobial activity toward pathogenic *S.aureus, Pseudomonas aeruginosa* and *Candida albicans* than PBHM and deionized water extract. So the rhizome of plants could be a good source for formulating a novel antibiotic against pathogenic bacteria and fungi.

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