Journal of Natural Products and Resources 1(1) (2015) 17–18

ISSN: 2455-0299

Contents List available at JACS Directory

Journal of Natural Products and Resources

journal homepage: http://www.jacsdirectory.com/jnpr

Antibacterial Evaluation of Fronds of *Christella dentata* (Forssk.) Brownsey & Jermy

T. Thomas*

Post Graduate and Research Department of Botany, St. Thomas College Palai, Pala – 686 574, Kerala, India.

ARTICLE DETAILS

Article history:
Received 06 September 2015
Accepted 27 September 2015
Available online 01 October 2015

Keywords:
Christella dentata
Antibacterial Activity
Disc Diffusion
Phytochemicals

ABSTRACT

Christella dentata is a common pteridophyte. Fronds of *Christella dentata* are evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacteria. Antibacterial activity was primarily evaluated by disc diffusion method. The results indicated that the plant exhibited antibacterial activity in acetone extract. The acetone extract of the plant showed maximum level of activity towards *Staphylococcus aureus*. Medium polar compounds are extracted during acetone extraction and these compounds are responsible for antibacterial activity. Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. Flavonoids and phenols were observed in various extracts. Flavonoid content in acetone extract of the plant may be one of the reasons for their antibacterial activity. Acetone extract of the plant exhibited minimum inhibitory concentration as 50 mg/mL and minimum bactericidal concentration as 100 mg/mL towards *Staphylococcus aureus*. The plant showed lower level of inhibition towards *Klebsiella pneumoniae* compared to the other bacterial strains.

1. Introduction

Pteridophtes are primitive vascular plants, which grow well in terrestrial habitat. Pteridophyte plants have medicinal values [1]. Plants are known to have defence systems against phytopathogenic bacteria [2]. The plant selected for study is *Christella dentata* (Forssk.) Brownsey & Jermy. It belongs to the family *Thelypteridaceae*; its synonym is *Polypodium dentatum* Forssk. The plant is a common medium sized terrestrial herb. It occurs in wide range of habitats from plains to forests of high altitude. The plant is distributed in all districts of Kerala. Its global distribution is throughout the tropics and subtropics of the world [3]. The plant has not much medicinal values, but the plant is used as a cushion for cattle by Raji tribes of Kumaon [4]. Since *C. dentata* is a common species and due to this reason, the plant is selected for evaluating its antibacterial potential. Widespread use of antibiotic medicines in human being helps to develop drug-resistant bacteria. These drug-resistant bacteria stand as a major problem in hospital acquired and community pathogens world-wide. Present study aims to evaluate antibacterial potential of the plant in various solvents extracts of increasing polarity towards pathogenic bacteria.

2. Experimental Methods

2.1 Preparation of Plant Extract

Fresh specimens of *Christella dentata* (Forssk.) Brownsey & Jermy. were collected in the month of January from Vagamon, Kottayam District, Kerala. A voucher specimen (SS 1553) was deposited at the herbarium of St. Thomas College, Palai. The air-dried fronds of the plant material (50 g) was ground and utilized for preparing extracts. Soxhlet extracts of petroleum ether, acetone, methanol and water were made successively [5] with a yield of 0.56%, 2.7%, 3.6%, and 0.6% respectively.

2.2 Microorganisms Used

The test organisms were *Staphylococcus aureus* subsp *aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomomas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109) and *Serratia marcescens* (MTCC 6164). All these bacteria are involved in various skin infections [6]. The test organisms were collected from the culture collection of the institute of Microbial Technology (IMTECH), Chandigarh. The bacteria sub cultured on nutrient agar slants, incubated at 37 °C for 24 hours and stored at 4 °C in the refrigerator to maintain the stock culture.

2.3 In Vitro Antibacterial Assay

Preliminary antibacterial activity was performed by disc diffusion method as indicated by Bauer et al., [7]. Sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2) poured into sterile petridish and after solidification, the bacteria (1 mL broth of approximately 10^5 CFU) were swabbed with a sterile needle under aseptic conditions. Sterile discs prepared using Whatman No. 4 filter paper of 6 mm diameter used for the study. The original solvents in which the extracts prepared were utilized as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/mL. About 20 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were placed after drying them in an incubator at 40 °C to remove any trace of solvent. The plates incubated at 37 °C for 24 hours to obtain inhibition zones. Experiments conducted in more than three replicates and average inhibitory zone diameter was determined.

2.4 Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was done by incorporating various amounts (400–0.39 mg/mL) of the extract into sets of test tubes with the culture media [8]. About 50 µL of the bacterial broth culture was added into each of the test tubes. The bacterial cultures containing the plant extracts were incubated at 37 °C for 24 hours. Test tube containing only the growth medium and each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not show any visible growth when compared to that of the control tubes.

2.5 Minimum Bactericidal Concentration (MBC)

Samples from the tubes in previous studies, which did not show any visible growth after a period of incubation, were subcultured onto a freshly prepared nutrient medium [9]. The minimum bactericidal concentration was considered as the lowest concentration of the extract

*Corresponding Author
Email Address: tojidr@yahoo.com (T. Thomas)

that did not yield a single colony on the nutrient agar plate after 24 hours incubation period.

2.6 Preliminary Detection of Phytochemicals

The crude samples were subjected to phytochemical screening for the presence of alkaloid, phenolics, terpenoids and flavonoids using the method of Harborne [10].

3. Results and Discussion

Petroleum ether and water extracts did not show any antibacterial activity towards tested organisms. Acetone extract of *C. dentata* showed moderate level of inhibition towards *Staphylococcus aureus*, The plant showed lower level of inhibition towards *Escherichia coli* compared to the other bacterial strains (Table 1). The plant extracts did not show any antibacterial activity towards *Klebsiella pneumoniae. Staphylococcus aureus* and *Pseudomonas aeruginosa* and are the most sensitive organisms towards acetone extract of the plant. No control discs exhibited antibacterial activity. The phytochemical evaluation of *C. dentata is* shown in the Table 2. Table 3 shows the results of antibacterial assays of pathogenic organisms towards standard antibiotics. Amoxycillin and chloramphenicol were not acting against *Pseudomonas aeruginosa.* Chloramphenicol was not effective towards *Klebsiella pneumoniae.* Active acetone extract of the plant was evaluated for minimum inhibitory concentration and minimum bactericidal concentration towards *Staphylococcus aureus*. The extract exhibited minimum inhibitory concentration as 50 mg/mL and minimum bactericidal concentration as 100 mg/mL towards *Staphylococcus aureus*.

Table 1 Antibacterial activity of fronds of *Christella dentata*

Name of plant	Extract used	Inhibition zone (mm) Value = Mean ± SD				
		Pseudomonas aeruginosa (MTCC-741)	*Staphylococcus aureus* (MTCC-96)	*Klebsiella*	*Escherichia coli* (MTCC-443)	*Serratia marcesens* (MTCC-97)
Christella dentata	Petroleum ether	-	-	-	-	-
	Acetone	12.2 ± 0.35	17.24 ± 0.25	-	9.4 ± 0.29	9.7± 0.21
	Methanol	-	-	-	-	8.20.± 0.23
	Water	-	-	-	-	-

(-) = No inhibition of growth. Value = Mean ± SD; Disc diameter 6 mm

Table 2 Results of phytochemical evaluation of fronds of *Christella dentata*

Name of plant	Plant extracts	Flavonoids	Alkaloids	Phenols	Sterols, steroid, phenol and poly phenol
Christella dentata	Petroleum ether	+	-	-	-
	Acetone	+	-	-	+
	Methanol	-	-	+	-
	Water	-	-	+	-

Value = '+' : Present '–' : Absent

Table 3 Antibacterial action of standard antibiotics

Name of Antibiotic (Con. 25 µg/Disc)	Inhibition zone (mm) Value = Mean ± SD		
	MTCC – 109	MTCC – 96	MTCC – 741
Streptomycin	25.2 ± 0.22	19.4 ± 0.36	18.4 ± 0.28
Amoxycillin	36.4 ± 0.24	35.3 ± 0.31	-
Chloramphenicol	-	24.4 ± 0.27	-

(-) = No inhibition of growth. Value = Mean ± SD; Disc diameter 6 mm

Non polar compounds were eluted during petroleum ether and acetone extraction. Out of these, acetone extract showed antibacterial activity. Water extract contained highly polar compounds and these compounds did not show antibacterial activity. Since water extraction was done after methanol extraction, most of the polar compounds would be removed along with methanol and few polar compounds might be left after methanolic extraction. This might be the reason for poor performance of water extracts. Medium polar compounds are soluble in acetone extract and these compounds have considerable level of antibacterial activity, while methanol extract contained polar compounds and they showed poor antibacterial potential. Acetone extract of *Christella dentata* showed maximum activity towards against *Staphylococcus aureus*, gram-positive bacteria. *Staphylococcus aureus infections are* common in cutaneous infections. They are often observed in nosocomial infections and its infection is common in-patients receiving treatment of severe common burns or other traumatic skin damage and in people suffering from cystic fibrosis. This pathogen colonises the lungs of patients and increasing mortality rate of individuals with the disease [11]. Flavonoids and phenols observed as general feature the plant extracts. None of the extracts showed the presence of alkaloids. Flavonoid content observed in acetone extract of the plant; it might be one of the reasons for its antibacterial activity. Antibacterial potential of the plant could not be equated with the potential of standard antibiotics, but the plant possessed some kind of antibacterial compounds with lower levels of potential. Based on the preliminary investigation, the plant could not be recommended for future isolation of potential antibacterial compounds.

4. Conclusion

Christella dentata was evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacterial species. The plant showed antibacterial activity in methanol extract. The methanol extract of the plant showed maximum level of activity towards *Staphylococcus aureus.* Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. The presence of flavonoids and phenols observed in various extracts. Acetone extract of the plant exhibited minimum inhibitory concentration as 50 mg/mL and minimum bactericidal concentration as 100 mg/mL towards *Staphylococcus aureus*.

References

[1] L. Singh, S. Somarjit, S. Kumarsingh, E. Jadu, Ethnobotanical uses of some Pteridophytic species in Maipur, Indian Fern J. 18 (2001) 14-17.

[2] E. Klein, D.L. Smith, R. Laxminaraya, Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005, Emerg. Infect. Dis. 13 (2007) 1840–1846.

[3] P.S. Easa, Biodiversity documentation for Kerala Part 5: Pteridophytes, Kerala Forest Research Institute Peechi, Kerala, 2003.

[4] P.C. Pande, Y.P.S. Pangtey, Studies on ethnobotany-1 on some less common edible and economic ferns of Kumaon region of Western Himalaya, J. Eco. Tax. Bot. 2 (1987) 81-85.

[5] M.P. Raghavendra, S. Satish, K.A. Raveesha, *In vitro* evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*, J. Agr. Sci. 2 (2006) 77-88.

[6] R.G. Valia, A.R. Valia, IADVL Textbook of Dermatology, 3rd Ed., Bhalani Publishing House, Mumbai, 2008.

[7] A.W. Bauer, M.D.K. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by standardized single disc diffusion method, Am. J. Clin. Path. 45 (1966) 493-496.

[8] A.L. Barry, Antimicrobic susceptibility tests: principle and practice, Lea and Febiger, Philadelphia, 1976.

[9] V.O. Ratimi, B.E. Laughon, J.S. Barlet, H.A. Mosadomi, Activities of Nigerian chewing sticks extracts against *Bacterioides gingivalis* and *Bacterioides melaninogenicus,* Antimicrob. Agents Chemother. 32 (1988) 598-600.

[10] J.B. Harborne, Phytochemical methods, Chapman and Hall Ltd, London, 1973.

[11] M.T. Madigan, J.M. Martinko, J. Parker, Brock biology of microorganisms, 9th Ed., Prentice Hall International Inc, New Jersey, 2000.