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Antibacterial activity of medicinal plant *Cyclea peltata* (Lam) Hooks & Thoms

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ABSTRACT

Objective: To investigate the antibacterial activity of Padathaali (*Cyclea peltata*) against three gram positive and eight gram negative bacterial strains. **Methods:** The fresh whole plants were collected from Kerala, India. The dry crude nonpolar and polar extract of whole plant of *C. peltata* i. e. Petroleum ether, hexane, chloroform, ethyl acetate, acetone, methanol and aqueous extracts of five concentrations (1, 2, 5, 10 mg/ml) were used to investigate the antibacterial activity. NCCL standards were strictly followed to perform antibacterial disc susceptibility test using disc diffusion method. **Results:** All the extracts showed varying degree of inhibitory potential against all the tested bacteria. Methanol extract of plant had higher inhibitory action against *Staphylococcus aureus, Streptococcus haemolyticus, Klebsiella pneumonia* and *Proteus vulgaris*. Acetone extract of plant showed maximum inhibitory action against *Klebsiella pneumonia* and *Streptococcus haemolyticus*. **Conclusions:** The present investigation showed the effectiveness of crude extract of this plant against tested bacterial strains. This study further suggests the use of whole plant extract in treating disease caused by tested microbial organisms.

1. Introduction

The antibacterial activity have been screened in many plants because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, become an ever increasing therapeutic problem. Due to the indiscriminate application of antibacterial drugs most of the microbial organisms have developed high resistance to a good number of the commercial antibiotics. This coupled with other problems like the dangerous side effects of some commercial antibiotic drugs have led the scientists to think of other alternatives like new antimicrobial substitutions from other sources especially medicinal plants[1-3]. The presence of antimicrobial substances in the higher plants is well established fact and they provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[4-5]. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to obtain a thorough knowledge about their properties, safety and efficacy[6]. There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources.

This study describes the antibacterial properties of *Cyclea peltata*. *C. peltata* is a member of the family Menispermaceae, commonly known as 'Padathaali' or 'Padakkilangu'. The

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plant grows throughout India and Sri Lanka, up to 800–900m elevation. It is a slender twining shrub, frequently climbing up on tall trees and has tuberous roots. The flowers are yellowish in colour with drupaceous fruits. This plant is pungent and bitter in taste, and it has antipyretic and astringent properties[7]. The plant is used in traditional Ayurvedic medicine and the root of the plant is employed as an important ingredient of 'Hinguvachadi Choornam' which is used to treat gastric ulcer and allied stomach ailments[8]. The root of *C. peltata* is also used to treat jaundice and digestive disorders[9]. The plant parts are also used against malarial disease [10].

Due to the high medicinal value of this plant, National Medicinal Plant Board of India identified this plant as "medicinal plant species in high trade sourced from tropical forests"[11]. The tribal people "Kurichiya" in India use the tuberous roots of this plant along with a little salt to treat stomach pain[12]. There are several reports of the use of various parts of *C. peltata* by Indian indigenous communities like the Kani and Siddis tribes for various medicinal purposes to cure several diseases[13-16]. Pharmacological study of *C. peltata* was carried out by Kupchan et al. (1961) and isolated d-tetrandrine, dl-tetrandrine, disochondrodendrine, and fangchinoline from the roots and found that these compounds have activity similar to that seen with d-tubocurarine[17]. In another study, Kupchan et al. (1973) isolated five bisbenzylisoquinoline alkaloids from the roots of this plant. This includes cycleapeltine, cycleadrine, cycleacurine, cycleanorine, and cycleahornine chloride[18]. They determined the structures of these compounds also. The present investigation was carried out to test the antibacterial efficacy of the whole plant extract of *C. peltata* against some pathogenic bacterial strains.

2. Materials and methods

2.1 Plant material

The whole plant of *C. peltata* collected during August-September of 2011 from the campus of St. Thomas College, Palai, Kerala, India. The plant was authenticated by Department of Botany, where a voucher specimen was deposited.

2.2 Extraction procedure

The plant material was washed with water and shade dried at room temperature. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. 30 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using hexane, chloroform, ethyl acetate, acetone, methanol and water successively. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered and concentrated in rotary flash evaporator. The concentrated plant extract used for antimicrobial assays.

2.3 Test bacteria

A total of eleven bacterial species were tested in the present study. The gram positive species were *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Bacillus cereus* and gram negative species were *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Proteus rettigiri* and *Vibrio cholerae*. The species that were not purchased were originally isolated from clinical materials collected from patients and identified using standard biochemical tests. The bacterial strains were maintained on nutrient agar slants at 4℃.

2.4 Culture media and inoculums preparation

Nutrient agar /broth (Himedia, India.) were used as the bacterial culture medium in the bacterial assays. Loops full of all the bacterial cultures were inoculated in the 50 ml of sterile nutrient agar (NA) in 100 ml conical flask at 37 ℃ for 72 hrs.

2.5. Antibacterial activity

The extracts obtained were screened for their antibacterial activity in comparison with standard antibiotic penicillin (10 mg/mL) *in vitro* by disc diffusion method using various bacterial strains[19]. The paper discs (6 mm diameter, Whatman No. 1 filter paper) containing 1.0, 2.0, 5.0, 10.0 mg/ml plant extracts were dried and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface of the medium[20]. The plates were kept at room temperature for half an hour and subsequently incubated at 37 ℃ and observed for zone of inhibition after 24 hours. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each extract. The results were recorded by measuring the zone of growth inhibition surrounding the disc.

3. Results

C. pelteta is a well known medicinal herb used in ayurvedic and other traditional medicines for their effectiveness against wide range of diseases including skin infections due to the advantage of the diversity of secondary metabolites responsible for their antibacterial activity. The antibacterial activity of the ethanolic extract of whole plant of *C. pelteta* was

studied against both gram positive (*Staphylococcus aureus*, *Streptococcus haemolyticus* and *Bacillus cereus*) and Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Proteus rettigiri* and *Vibrio cholerae*) organism at 4 different concentrations (1.0, 2.0, 5.0 and 10.0 mg/ml) and the antibacterial activity was compared with that of the standard antibiotic penicillin (10 mg/mL).

The results of antibacterial screening of hexane, chloroform, ethyl acetate (EA), acetone, methanol, ethanol and water whole plant extracts of *C. peltata* are presented in Table 1 and 2. The results revealed variability in inhibitory concentrations of each extract against a given bacteria. The inhibition of bacterial growth was dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evidenced by the higher zone of inhibitions at higher concentrations of each extract. All extracts showed antimicrobial activity to at least six of the tested microorganisms. Among the various gram positive bacteria used, acetone extracts of *C. peltata* showed maximum activity (zone of inhibition 16.9 mm) against *S. haemolyticus* whereas it showed moderate activity against *S. aureus* and *B. cereus* (Table 1). Similarly among the eight gram negative bacteria tested, acetone extracts exhibited maximum activity (zone of inhibition 17.4 mm) against *K. pneumonia* (Table 2). Acetone extract was appeared to be the most effective extract. None of the water extracts showed any antibacterial activity. None of the chloroform extracts was active against any of the gram positive bacteria tested. The antibacterial activity was more prominent on the gram negative bacteria than the gram positive bacteria.

4. Discussion

In this study we have demonstrated the antibacterial activity of whole plant extract of *C. peltata* against a wide range of various bacterial strains which include gram positive and negative bacteria with the highest antibacterial activity being demonstrated against *K. pneumonia*. The antibacterial activity have been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, become an ever increasing therapeutic problem.

Table 1
Antibacterial activity of non-polar and polar extracts of *C. peltata*.

Extracts	Concentration of extract (mg/mL/disc)	Zone of inhibition (mm)		
		Gram positive bacteria		
		Staphylococcus aureus	*Streptococcus haemolyticus*	*Bacillus cereus*
Hexane	1	–	–	–
	2	8.0±0.03	–	–
	5	10.3±0.05	–	–
	10	13.2±0.02	–	–
Chloroform	1	–	–	–
	2	–	–	–
	5	–	–	–
	10	–	–	–
EA	1	8.4±0.04	–	–
	2	10.2±0.26	–	–
	5	14.6±0.12	10.4±0.14	–
	10	16.5±0.12	13.8±0.28	–
Acetone	1	–	7.4±0.43	–
	2	–	10.2±0.39	–
	5	7.8±0.03	13.3±0.67	10.4±0.44
	10	9.7±0.02	16.9±0.26	13.6±0.39
Methanol	1	8.5±0.36	–	–
	2	10.6±0.38	–	–
	5	13.8±0.02	10.7±0.45	7.5±0.48
	10	16.3±0.28	12.3±0.32	9.7±0.33
Ethanol	1	7.6±0.55	–	–
	2	9.5±0.38	–	–
	5	11.3±0.29	9.7±0.23	–
	10	12.6±0.15	11.8±0.16	10.4±0.27
Water	1	–	–	–
	2	–	–	–
	5	–	–	–
	10	–	–	–

Table 2
Antibacterial activity of non-polar and polar extracts of *C. peltata*.

Extracts	Concentration of extract (mg/mL/disc)	Zone of inhibition (mm)							
		Gram negative bacteria							
		Escherichia coli	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Seretia marcescens*	*Proteus rettigiri*	*Vibrio cholerae*
Hexane	1	–	7.3±0.33	–	–	7.3±0.24	8.3±0.56	7.3±0.34	–
	2	–	9.6±0.32	9.7±0.56	–	7.7±0.46	8.4±0.36	7.8±0.57	–
	5	7.6±0.22	12.3±0.57	10.4±0.46	–	8.4±0.29	9.3±0.18	9.0±0.39	–
	10	9.2±0.26	15.4±0.79	12.6±0.24	–	9.2±0.57	10.2±0.24	9.6±0.16	7.2±0.36
Chloroform	1	–	–	7.2±0.17	–	–		7.2±0.28	–
	2	–	–	8.3±0.38	–	–		7.9±0.25	–
	5	8.3±0.18	–	12.7±0.36	7.1±0.33	–		8.4±0.14	–
	10	11.3±0.57	–	14.5±0.29	7.8±0.24	7.2±0.64		9.7±0.56	8.6±0.28
EA	1	9.2±0.54	–	–	–	–	–	–	–
	2	11.2±0.43	–	–	7.2±0.26	–	–	–	–
	5	13.0±0.79	12.2±0.45	8.4±0.59	7.8±0.17	7.1±0.75	7.4±0.23	–	–
	10	16.2±0.24	14.5±0.54	12.3±0.36	8.5±0.58	7.3±0.23	8.3±0.42	8.0±0.37	–
Acetone	1	–	8.3±0.22	7.5±0.68	9.4±0.49	7.6±0.75	–	7.1±0.56	–
	2	–	12.3±0.42	9.6±0.77	9.3±0.37	7.9±0.38	–	7.5±0.37	–
	5	8.2±0.66	14.3±0.53	12.3±0.19	9.8±0.58	8.4±0.52	7.8±0.32	8.3±0.21	7.3±0.38
	10	12.1±0.78	17.4±0.35	14.5±0.33	11.8±0.23	8.8±0.69	8.4±0.56	9.5±0.12	7.7±0.25
Methanol	1	7.3±0.49	8.4±0.22	7.3±0.24	7.2±0.32	–	–	–	–
	2	9.4±0.18	10.5±0.55	9.4±0.44	8.2±0.52	–	–	–	–
	5	12.3±0.31	13.2±0.75	11.6±0.66	8.6±0.22	7.2±0.26	–	8.3±0.32	–
	10	14.0±0.22	16.2±0.82	14.3±0.29	10.6±0.12	7.8±0.14	7.5±0.51	8.7±0.54	8.4±0.45
Ethanol	1	–	7.0±0.24	–	–	–	–	–	–
	2	–	9.3±0.57	7.3±0.27	7.6±0.52	–	–	–	–
	5	8.3±0.32	12.3±0.63	10.8±0.18	8.4±0.62	7.3±0.23	–	8.3±0.44	7.3±0.26
	10	11.2±0.46	14.5±0.59	12.3±0.35	9.3±0.32	7.6±0.64	7.1±0.48	9.4±0.49	7.9±0.57
Water	1	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	–

The results are mean ±SD (n=10)

The presence of antimicrobial substances in higher plants is well established as they provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds. Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for other alternatives. Continued further research and exploration of plant derived antimicrobials is needed today since such principles represent the vast untapped source for medicine. Medicinal plants are important source for the development of potential new chemotherapeutic drugs and the in vitro antibacterial test form the basis. The broad spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in folklore medicine[21]. The antibacterial activity of the plants may be due to the presence of various active principles in them. Plant extracts often contains polyphenols and flavonoids which could be the antimicrobial components. The bioactivity of plant extracts is attributed to phytochemical constituents. Flavonoids are a major group of phenolic compounds reported for their antiviral[22], antimicrobial[23] and spasmolytic[24] properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties[25]. The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

In the present study the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains. Similar results were obtained by different workers in various systems[26-27]. The inhibitory effect of the extract on the growth of microorganisms could be attributed to the presence of some phytochemicals that were found present in the plant extract. The demonstration of antibacterial activity against both gram positive and gram negative bacteria by this plant may be indicative of the presence of broad spectrum antibiotic compounds[28-30]. The present study justifies the claimed uses of *C. peltata* in the traditional system of medicine to treat various infectious diseases caused by the

microbes. This study encourages the cultivation of this highly valuable medicinal plant to meet the increasing demand from traditional medicinal system.

Conflict of interest statement

We declare that we have no conflict of interest.

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