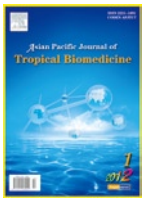
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



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Anti-bacterial studies on *Peristrophe bicalyculata* (Retz.) Nees

Janakiraman N[1], Sahaya Sathish S[1], Johnson M[2*]

[1]*Department of Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous), Tiruchirappalli-620 002, Tamil Nadu, India*
[2]*Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 23 February 2012
Received in revised form 3 March 2012
Accepted 2 April 2012
Available online 28 April 2012

Keywords:
Peristrophe bicalyculata
Antibacterial
Phytochemistry
Secondary metabolites

ABSTRACT

Objective: To study the phytochemical and antibacterial efficacy of *Peristrophe bicalyculata* (Retz.) Nees. (*P. bicalyculata*) extracts against the selected pathogens. **Methods:** The preliminary phytochemical screening was performed by Brindha *et al.*, method. The antibacterial efficacy of the ethanolic, acetone and chloroform extracts of *P. bicalyculata* were tested against *Bacillus cereus (B. cereus)*, *Enterococcus aerogenes (E. aerogenes)*, *Escherichia coli (E. coli)*, *Salmonella typhi (S. typhi)* and *Staphylococcus aureus (S. aureus)* by disc diffusion method. **Results:** The results of the phytochemical screening revealed the presence of various secondary metabolites like steroids, alkaloids, phenols, flavonoids, saponins and tannins. Ethanolic extract of *P. bicalyculata* was most effective against *E. coli*, *B. cereus* and *S. typhi*. Highest zone of inhibition (18 ±0.8 mm) was observed against *E. coli*. **Conclusions:** Thus, this study reveals a significant scope to develop a novel broad spectrum of antimicrobial drug formulation.

1. Introduction

Medicinal plants have been used for centuries as remedies for human diseases and other organisms because they contain certain components of therapeutic value. There are more than 35 000 plant species being used in various human cultures around the world for medicinal purposes[1]. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Recently some higher plant products have attracted the interest of microbiologist and pharmacologist to search for phytochemicals for their use as antimicrobials. Such plant products would be biodegradable and safe to human health[2,3]. In recent years, pharmaceutical companies have spent substantial time and money in developing therapeutics based upon natural products extracted from plants[4,5]. This situation has forced scientists to search for new antimicrobial substances in various sources like medicinal plants[6]. Numerous studies have been carried out on various natural products screening their antimicrobial activity[7-10]. The selection of crude plant extract for screening the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds[11]. Some organisms have developed resistance to the existing antibiotics; therefore the development of bacterial resistance to the presently available antibiotics has necessitated the research for new antibacterial agents[12]. *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae) (*P. bicalyculata*) is an erect, hispid herb or under shrub, 60-120 cm height found in forest undergrowth, hedges and waste band almost throughout India. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea. This plant is used by the traditional healers for curing many skin related problems; it is also used as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellant. This plant is also used for horse feed and ploughed into the soil as green manure[13]. Although undocumented, the plant is used in

*Corresponding author: Johnson M, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India.
Tel: + 91 97 86 92 43 34
Fax: + 91 462 2561 765
E-mail: ptcjohnson@gmail.com

South West Nigeria in the treatment of hypertension and other cardiovascular diseases. It was recently discovered to have hypolipidemic effects[14] and such effects are known to protect against cardiovascular diseases, including hypertension. With this knowledge the present research work is aimed to determine the phytochemical activity and antimicrobial properties of *P. bicalyculata* against selected bacterial pathogens.

2. Materials and methods

The fresh plants of *P. bicalyculata* (Retz.) Nees. were collected from the natural habitats of Tiruchirappalli district, Tamil Nadu. The whole plant samples were washed thoroughly for 3 times in running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The plants were cut, shade dried, grinded into fine powder and stored in air tight polythene bags until use. 20 g of each powdered plant material was extracted separately in the ratio of 1:6 at room temperature using various organic solvents namely ethanol, acetone and chloroform with gentle stirring for 72 h. The complete extraction was carried out with the following solvents in the increasing order of polarity. The extracts were filtered through Whatmann No. 1 filter paper and then transferred to glass vials and kept at 4 ℃ before use. The preliminary phytochemical constituents were qualitatively analyzed using Brindha *et al*[15] method. Antibacterial assay was carried out using disc diffusion method. Five bacterial strains were used namely *Bacillus cereus* (*B. cereus*), *Enterobacter aerogens* (*E. aerogens*), *Escherichia coli* (*E. coli*) , *Salmonella typhi* (*S. typhi*) and *Staphylococcus aureus* (*S. aureus*). The bacterial strains were preserved in the nutrient agar at 4 ℃ were revived in nutrient broth (liquid medium) and incubated at (37±1) ℃ for overnight. The diluted bacterial culture was placed on Nutrient agar medium and spread throughout the plate using sterile glass 'L' rod. The sterile filter paper disc of 6 mm diameter soaked with plant extract was placed on the surface of the medium and incubated at 37 ℃ for 24 h. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Chloramphenicol was used as positive reference standard. Each and every test was performed in triplicates.

3. Results

The phytochemical constituents present in the plant sample are represented in Table 1. The result of the antibacterial efficacy of the ethanolic, acetone and chloroform extracts of *P. bicalyculata* is tabulated in Table 2. Among the tested extracts, the ethanolic extract exhibited high degree of inhibition followed by chloroform and acetone. The zone of inhibition of various extracts of *P. bicalyculata* was compared with standard antibiotic disc (chloramphenicol). The ethanolic extracts of *P. bicalyculata* showed the highest zone of inhibition against *E. coli* (18 mm) followed by *B. cereus* (17 mm) and *S. typhi* (14 mm). The ethanolic extracts of *P. bicalyculata* were failed to show the inhibition against *E. aerogenes* and *S. aureus*. The acetone extracts of *P. bicalyculata* illustrated maximum zone of inhibition against *S. aureus* (14 mm) followed by *B. cereus* (11 mm) and *S. typhi* (10 mm). The acetone extracts of *P. bicalyculata* were failed to demonstrate the inhibition against *E. aerogenes* and *E. coli*. The chloroform extracts of *P. bicalyculata* showed highest zone of inhibition against *S. aureus* (14 mm) followed by *E. aerogenes* (13 mm) and *B. cereus* (12 mm). The chloroform extracts of *P. bicalyculata* were failed to show the inhibition against *E. coli* and *S. typhi*.

Table 1
Phytochemical screening of ethanolic, acetone and chloroform extracts of *P. bicalyculata* (Retz.) Nees.

Metabolites	Ethanol	Acetone	Chloroform
Steroids	+	+	+
Triterpenoids	–	–	–
Sugar	+	+	+
Reducing sugar	–	–	–
Alkaloids	+	–	–
Phenols	+	–	–
Catechine	–	–	+
Flavanoids	–	+	+
Saponins	+	–	–
Tannins	+	–	–
Anthroquinone	–	–	+
Aminoacids	+	+	–

Table 2
Antibacterial activity of different solvent extracts of *P. bicalyculata* against different bacterial pathogen.

Test bacteria	Zone of inhibition in mm (mean ± SD)						
	Ethanolic extract		Acetone extract		Chloroform extract		Chloramphenicol (30 μg/disc)
	Experimental (30 g/disc)	Negative control	Experimental (30 g/disc)	Negative control	Experimental (30 g/disc)	Negative control	
E. aerogenes	–	–	–	–	13.00± 0.04	7.00 ± 0.09	15.00 ± 0.08
S. aureus	–	–	14.00± 0.21	7 .00± 0.04	14.00 ± 0.08	8.00 ± 0.04	10.00 ± 0.80
B. cereus	17.00 ± 0.16	8.00 ± 0.08	11.00 ± 0.20	7.00 ± 0.04	12.00 ± 0.21	7.00 ± 0.04	11.00 ± 0.16
E. coli	18.00 ± 0.80	7.00 ± 0.07	–	–	–	–	12.00 ± 0.04
S. typhi	14.00 ± 0.08	7.00 ± 0.04	10.00 ± 0.12	8.00 ± 0.09	–	–	12.00 ± 0.12

4. Discussion

The beneficial medicinal effects of plant materials typically result from the secondary metabolites present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are distinctive to a particular plant species or group, reliable with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct[16]. The possibilities for the higher antibacterial activity of ethanolic extract are the nature of biologically active compounds and stronger extraction capacity that may yield a greater number of active constituents[17].

All the three extracts of *P. bicalyculata* showed the inhibition against *B. cereus*. This research finding gives a scope to further screen the chemical constituents of the extracts which will be very useful to combat the common food borne infections like severe nausea, vomiting and diarrhoea caused by *B. cereus*. *E. aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections. Some strains become very treatment resistant, majority of them are sensitive to most antibiotics. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic uremicsyndrome, peritonitis, mastitis, septicaemia and Gram negative pneumonia. Infection of *S. typhi* leads to the development of typhoid, or enteric fever. Other symptoms include constipation or diarrhoea, enlargement of spleen and possible development of meningitis. *S. aureus* can cause a range of illnesses from minor skin infections to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis.

The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolites[18]. The ethanolic extracts of *P. bicalyculata* showed the maximum bio-efficacy compared with other solvents due to the presence of more number of metabolites (7/12) such as steroids, phenols, alkaloids, saponins and tannins (Table 1). Giwa et al[19] reported the phytochemical and antimicrobial activity of *P. bicalyculata* from Nigeria. They recorded the antimicrobial activity against *S. aureus*, *Klebsiella* spp, *P. aeruginosa*, *Aspergillus niger*, *Asperigillus clavatus* and *Rhizopus stolonifer*. In addition to Giwa *et al*[19] observations in the present study we reported the antibacterial activity against the pathogens *E. aerogenes*, *E. coli*, *S. typhi* and *B. cereus*. Alkaloids are chemical constituents from plants that can work on the nervous system of the human body and used for analgesic, antispasmodic and bacterial effects[20]. The presence of alkaloids in the present study augments the use of alkaloid in global pharmaceutical market. Tannins have antibacterial, antiseptic, astringent, antiulcer and antiviral properties. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity[21]. Flavonoids in intestinal track lower the risk of heart disease. As antioxidants flavonoids from these plants provide anti-inflammatory activity[22]. The antimicrobial activities of phenolic compounds may involve multiple modes of action. For example, oils degrade the cell wall, interact with the composition and disturb cytoplasmic membrane[23], break membrane protein, interfere with membrane integrated enzyme[24], cause leakage of cellular components, coagulate cytoplasm, lessen the proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanism for energy production and metabolism, alter nutrient uptake and electron transport. The results of present study confirm the presence of phenol, tannins, alkaloids, flavonoids, saponins, steroids etc. which will increase the folkloric usage of this plant. It suggests that *P. bicalyculata* possess antibacterial properties and can be used as astringent, antispasmodic, antipyretic, antiseptic, anti-inflammatory, anticancer and antiviral agents in new drugs for the therapy of infectious diseases caused by pathogens in the near future.

In general, Gram-negative bacteria were more resistant to antibiotics than Gram-positive bacteria[25]. The resistance of gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside[26]. However, the gram positive bacteria do not possess such outer membrane and cell wall structures[27]. In contrary, the present study results showed that the gram positive bacteria are more susceptible to the crude extracts than gram negative bacteria. These results suggest the possible exploitation of this plant in the management of the infectious diseases. Further purification of the extract may yield a novel antibacterial drug to treat various diseases like diarrhoea, mastitis, septicaemia, pneumonia, bacteremia, meningitis, nosocomial infections, urinary tract infections etc.

In conclusion, it is hoped that *P. bicalyculata* has certain bioactive principles and medicinal values. The chemical compounds present in this plant forms the characteristic nature of its medicinal uses. Thus, it leads to the establishment of new compounds which is used to formulate more potent antimicrobial drugs of natural origin with fewer side effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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