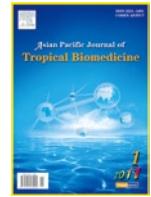




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Antibacterial activity of ethanolic extracts of selected medicinal plants against human pathogens

Renisheya Joy Jeba Malar T¹, Johnson M^{2*}, Mary Uthith M¹, Arthy A¹¹Department of Biotechnology, Noorul Islam College of Arts and Science, Kumaracoil–Tamil Nadu, India²Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Tirunelveli, Tamil Nadu, India

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ABSTRACT

Objective: To evaluate the antimicrobial potential of five medicinally important plants namely, *Curcuma mangga* (*C. mangga*) Valetton & Van Zijp, *Ficus racemosa* (*F. racemosa*) Roxb., *Vitex negundo* (*V. negundo*) L., *Ocimum basilicum* (*O. basilicum*) L., and *Etilingera elatior* (*E. elatior*) K. Schum. against the human bacterial pathogens. **Methods:** The *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus* (*S. aureus*) (ATCC 6538), *Salmonella typhi* (*S. typhi*) (MTCC 733), *Proteus vulgaris* (*P. vulgaris*), *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated from clinical samples. The bacteria were identified and confirmed by conventional microbiology procedure. Antimicrobial study was carried out by disc diffusion method against the pathogens by using the crude ethanolic extracts. **Results:** The results of the present study showed the presence of wide spectrum of antibacterial activities against all the above bacterial pathogens studied. The maximum zone of inhibition observed for each bacterium was as follows: *S. typhi* (12 mm), *K. pneumoniae* (13 mm), *P. vulgaris* (20 mm), *P. aeruginosa* (16 mm) and *S. aureus* (12 mm). **Conclusions:** The present study demonstrates that the *C. mangga*, *F. racemosa*, *V. negundo*, *O. basilicum*, and *E. elatior* are potentially good sources of antibacterial agents against the pathogens viz., *K. pneumoniae*, *S. aureus*, *S. typhi*, *P. vulgaris* and *P. aeruginosa*.

1. Introduction

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to search for biologically active principles in plants[1]. Medicinal plants represent a rich source of antimicrobial agents. Wide range of different parts of medicinal plants was used for extract as raw drugs and they possess varied medicinal properties. Some of these raw drugs are collected in smaller quantities by local use while many other raw drugs are collected in larger quantities and traded in market as raw material for many herbal industries[2]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity[3]. In addition, in developing countries, synthetic drugs are not only

expensive and inadequate for the treatment of diseases but also have adulteration and side effects. This kind of situation stresses the need to search a new drug for treating such infection[4]. Usually the discovery of new drug to cure pathogenic disorders is done by using pathogens obtained from commercial laboratories. The screening of drugs by using such microbial strains comparatively for many years may not be effective due to the evolution of resistant strains at high rate. So the antibacterial screening for any drug by using freshly collected clinical samples will be more potent. The revival of interest in plant derived drugs is mainly due to the current wide spread belief that "green medicine" was safe and more dependable than the costly synthetic drugs which may have adverse effect[1]. Hence, researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against microbial infection[5]. Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants growing wild in different parts of our country. In India several medicinal plants are used from ancient times to cure specific ailments[6]. Hence, in the present investigation, the antimicrobial potential of

*Corresponding author: Johnson M, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Tirunelveli, Tamil Nadu, India.

Tel: +91 97 86 92 43 34

Fax: +91 46 22 56 17 65

E-mail: ptcjohnson@gmail.com

five medicinally important plants viz., *Curcuma mangga* (*C. mangga*) Valetton & Van Zijp, *Ficus racemosa* (*F. racemosa*) Roxb., *Vitex negundo* (*V. negundo*) L., *Ocimum basilicum* (*O. basilicum*) L., and *Etilingera elatior* (*E. elatior*) K. Schum. has been evaluated against human bacterial pathogens isolated from clinical samples.

2. Materials and methods

Healthy and disease free plant parts i.e., roots of *C. mangga* Valetton & Van Zijp and leaves of *F. racemosa* Roxb., *V. negundo* L., *O. basilicum* (L.), and *E. elatior* K. Schum. were collected from wild. The fresh materials (200 g) were shade dried and powdered using the tissue blender. The powdered materials were soaked in 50 mL ethanol for 48 h at room temperature. After soaking, the extracts were filtered using Whatman 41 filter paper. The filtrates were collected, made up to known volume (50 mL) and stored in refrigerator at 4 °C. The ethanolic extracts were used for antibacterial studies. The *Salmonella typhi* (*S. typhi*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus vulgaris* (*P. vulgaris*), *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated from clinical samples[7]. The bacteria were identified and confirmed by conventional microbiology procedure. Stock cultures of *S. typhi*, *K. pneumoniae*, *P. vulgaris*, *S. aureus* and *P. aeruginosa* were grown in nutrient broth at 30 °C and were sub-cultured and maintained in nutrient broth at 4 °C. Antibacterial study was carried out by disc diffusion method[8] against the pathogens. The crude ethanolic extracts were used for bioassay against both gram negative and gram positive bacteria. Inoculum was prepared from the 24 h old culture of bacterial isolates in nutrient broth. Nutrient

agar plates were prepared and the inocula were seeded by spread plate method. Sterile discs with 6 mm in diameter were loaded with 100, 200 and 400 µL of plant ethanolic extracts and introduced into the sterile medium with the test organisms. The plates were incubated at 37 °C for 18–24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone. All the samples were done in triplicate. Both positive and negative controls were also determined. The ethanol was used as negative control to determine its effect on test organisms. Two common antibiotics viz., amoxicillin and tetracycline were used as positive control to compare its effect on test organisms with the plants extracts.

3. Results

Ethanolic extracts of five different plants were examined for the antibacterial activity against the isolated human pathogens and the results were given in Table 1. The antibacterial activity was observed in the ethanolic extracts of all the plants against all the tested bacteria with varied activity. The maximum zone of inhibition i.e. 12 mm for *S. typhi*, 13 mm for *K. pneumoniae*, 20 mm for *P. vulgaris*, 16 mm for *P. aeruginosa* and 12 mm for *S. aureus* was observed. The ethanol extracts of *C. mangga* showed the highest zone of inhibition against two pathogens viz., *P. vulgaris* (20 mm) and *P. aeruginosa* (16 mm), while the ethanolic extracts of *E. elatior* demonstrated the maximum zone of inhibition against another two different pathogens viz., *K. pneumoniae* (13 mm) and *S. aureus* (12 mm). The ethanolic extracts of *O. basilicum* showed the maximum activity against two pathogens viz., *S. typhi* and *S. aureus*.

Table 1

Antibacterial activity of ethanolic extracts of *V. negundo*, *F. racemosa*, *C. mangga*, *E. elatior* and *O. basilicum*.

Name of the plants	Ethanolic extracts (µL)	Zone of inhibition (mm)				
		<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
<i>V. negundo</i>	100	6	5	3	6	2
	200	8	7	5	11	4
	400	11	10	8	15	7
<i>F. racemosa</i>	100	5	4	7	3	2
	200	7	6	12	7	5
	400	9	9	19	9	9
<i>C. mangga</i>	100	6	3	8	6	3
	200	9	5	12	11	5
	400	12	8	20	16	10
<i>E. elatior</i>	100	6	6	4	3	2
	200	9	9	7	5	4
	400	13	12	10	7	7
<i>O. basilicum</i>	100	5	5	6	3	3
	200	8	8	11	5	7
	400	11	12	15	8	12

4. Discussion

Several workers have reported that many plants possess antimicrobial properties including the parts *i.e.* flower, bark, stem, leaf, *etc.* Recently, a number of plants have been reported for antimicrobial properties across the world[9–19]. In the present investigation, five medicinally important plants from India have been screened for antimicrobial potential. Rathish *et al*[20] reported the usage of ethanol as a solvent for the preparation of plant extract for antibacterial studies. In the present study, *in vitro* antibacterial efficacy of the crude ethanolic extracts of five plants was quantitatively assessed on the basis of zone of inhibition. All the plants studied in the present investigation exhibited varying degree of inhibitory effect against the selected bacterial human pathogens. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria[21]. In the present study the ethanol extracts of the selected plants also showed zone of inhibition against the isolated human pathogens with varied diameter. The result of the present study revealed that the *F. racemosa*, *C. mangga*, *V. negundo*, *O. basilicum* and *E. elatior* can be used in the treatment of boils, sores and wounds, since *P. aeruginosa* has been implicated as causative agents of these diseases. Based on the present study it also confirmed that the leaves of *F. racemosa*, *V. negundo*, *O. basilicum* and *E. elatior* and roots of *C. mangga*, can be used in the treatment of gastroenteritis, food borne illness, urinary tract infections, neonatal meningitis, nosocomial infections, wound, septicemia, pneumonias and from mild superficial skin infections to life-threatening systemic diseases which are caused by *P. vulgaris* and / or *S. pyogenes*. The pathogen *S. typhi* is known to cause fever and food borne illness. In the present study the ethanolic extracts of roots of *C. mangga*, leaves of *F. racemosa*, *V. negundo*, *O. basilicum* and *E. elatior* show the inhibitory activity against the bacterium *S. typhi* and thus the present study confirms the presence of active constituents present in these plants.

It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to identify the bioactive compound and to evaluate the mechanisms of action of *F. racemosa*, *C. mangga*, *V. negundo*, *O. basilicum* and *E. elatior* extracts on some organisms associated with human diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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