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Evaluation of antibacterial activities of ancient medicinal plants extracts against *E. coli* and *S. aureus*

N. Kumari, A. Sinha, P. R. Sahu and A. S. Khalkho

Department of Biotechnology, Marwari College, Ranchi, Jharkhand, India

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ABSTRACT

Herbs have always been the principal source of medicine in India. Medicinal plants have curatives properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more of its parts. This study was designed to investigate the antibacterial activities of plants namely Ficus geniculata (Putkal), Cassia tora (Chakor), Madhuca indica (Mahua), Pongamia pinnata (Karanj), Boerhaavia diffusa (Punarnava), Ficus religiosa(Peepal) and Moringa oleifera (Senjana). The plant extracts were prepared in two solvents viz. water and methanol. These extracts were tested for its antimicrobial potential against Escherichia coli and Staphylococcus aureus. Methods used to evaluate antimicrobial potential were agar well diffusion assay. Various concentrations of the extracts ranges upto 50mg/ml, 25mg/ml and 12.5mg/ml were prepared. The aqueous extract of Ficus religiosa at the concentration of 50mg/ml showed the highest inhibition zone as 1.3cm against E. coli. Preliminary results of antibacterial activity supported the traditional use of Ficus in folk medicine. These findings suggest a new pathway in elucidating a potential antibacterial agent from Ficus religiosa.

Keywords: Medicinal plants, Plant extracts, Antibacterial activity, Minimum inhibitory concentration

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INTRODUCTION

Urinary tract infections (UTIs) are the second most common type of infection in the world. Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. The uropathogens after attaching to the epithelial surface, subsequently colonizes and disseminates throughout the mucosa causing tissue damage. After the initial colonization period, pathogens can ascend into the urinary bladder resulting in symptomatic or asymptomatic bacteriuria. Further progression may lead to pyelonephritis and renal impairment[1].UTIs are among the most common medical conditions in female requiring medical treatment. Around 6-10% of all young females demonstrate bacteriuria [2]. Most of the urinary tract infections are caused by gram-negative bacteria like *Escherichia coli*, *Klebsiella* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Serratia*. The treatment mainly involves use of antibiotics but the pathogenic bacteria are becoming increasingly resistant to antibiotics [3]. The indiscriminate use of antibiotics has led to evolution of multi-drug pathogens. This necessitates the search for alternative compounds having antimicrobial property. Therefore emphasis has been laid over medicinal plants [4].

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs [5]. The symptoms of acute bacterial intestinal infection are usually mild to moderate, and spontaneous remission occurs but

in some cases, the disease can cause rapid deterioration of a patient's condition [6].Within a decade more than 100,000 persons with acute gastrointestinal infection were reported in one of the national surveillance programs for communicable diseases [7].

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Recognition of the medical and economic benefits of plant-based medicines is growing in both developing and industrialized countries, although it varies greatly from country to country [8]. Earlier studies have reported many significant antibiotic effects in various plant extracts [9-10]. The present study aimed to characterize the antimicrobial potential of seven ayurvedic herbs against the urinary tract infection and gastrointestinal infection pathogens, *viz*., *Ficus geniculata* (Putkal), *Cassia tora* (Chakor), *Madhuca indica* (Mahua), *Pongamia pinnata* (Karanj), *Boerhaavia diffusa* (Punarnava), *Ficus religiosa*(Peepal) and *Moringa oleifera*(Senjana).

MATERIALS AND METHODS

Selection of plants

Fresh plant/plant parts were collected randomly from suburbs of Ranchi, Jharkhand, India. The details of the species, family, common name and part used are given in Table 1. Plants were compared with specimens registered at NBPR Palandu, Namkum, Ranchi, Jharkhand. Fresh plant materials were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Plant extract

Selected plant material were dried under shade and crushed to prepare a coarse powder. The sieved powder (50 g) was extracted with methanol solvent (500 ml) by using Soxhlet extractor for 48-52 h. After complete extraction, the methanol and aqueous solvent was evaporated by using rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801) under reduced pressure to obtain methanol crude extract (4.88 g). Crude extracts were filtered separately through Whatman No. 41 filter paper to remove particles. The particle free crude extract was evaporated completely by using rotary evaporator under reduced pressure to obtain dry crude extracts. The residue left in the separatory funnel was re-extracted twice follow the same procedure and filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure. Later two solvents were used to redissolve the extracts, namely, methanol and distilled water, for further analysis.

Methanol extract

A 50g of dried leaf powder were taken in a separate container. To this 250ml of methanol was added and kept for 24 h with periodic shaking then filtered and the filtrate was collected. The procedure was separated three times with fresh volume of methanol. The filtrates were pooled.

Aqueous extract

A 50g of dried leaf powder were taken in a separate container. To this 250 ml of distilled water was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

Test microorganisms

The standard microorganisms were obtained from Feischer Scientific Laboratory ltd. India National Chemical Laboratory, Pune, India and clinically isolated microorganisms were obtained from Department of Microbiology, RIMS, Ranchi, Jharkhand. The bacterial strains were revived and grown in the nutrient broth. Further, maintained on nutrient agar slants at 4^{o}C.

Antimicrobial screening

Medium-Muller Hinton Agar (3.8 gm/100 ml of distilled water) was prepared, autoclaved at 121^{o}Cfor 15minutes at 15lbs and poured in sterile petri plates up to a uniform thickness of approximately 5-6cm and the agar was allowed to set at ambient temperature. The microorganisms were inoculated in nutrient broth and incubated at 37^{o}C. The well was made in the MHA medium after inoculation with microorganisms. Later wells were loaded with antibiotics and allowed to diffuse in the medium. The zone of inhibition (ZI) of bacterial growth around each well is measured and the susceptibility is determined.

Determination of minimum inhibitory concentration by micro-dilution assay
The minimum inhibitory concentration (MIC) was measured as the lowest concentration of the compound to inhibit the growth of microorganisms [11].MIC values were determined by broth dilution assay of micro dilution assay. Varying concentrations of the extracts (50mg/ml,25mg/ml and 12.5mg/ml) were prepared. A 0.1ml of standardized test organism of controls were equally set up by using solvents and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration of the respective plant extract.

Statistical analysis
The mean values will be compared using respective standard error (SE) followed by statistical comparison between control and test groups for evaluation of significant changes in values by Student's *t*-test. $P<0.05$ will be considered as significant. Results were expressed as mean value ± SE of growth inhibition zones diameters obtained with different extracts of various plants whose amount was sufficient to perform three repetitions.

RESULTS AND DISCUSSION

Many of the existing synthetic drugs cause various side effects. Hence, plant based drug development could be useful in meeting this demand for newer drugs with minimal side effects. Plants are important source of potentially useful chemicals for the development of new chemotherapeutic agents. The first step towards this goal is generally the in vitro antibiotic activity assay. Several studies have reported that many plants possess antibiotic properties including the parts i.e. flower, bark, stem, leaf, etc[12]. Recently, a number of plants have been reported for antibiotic properties across the world [13].

In present study the antibacterial analysis of different plants extracts *viz*., *F.geniculata*, *C.tora*, *M.indica*, *P.pinnata*, *B.diffusa*, *F. religiosa*, *M.oleifera* of aqueous and methanolic extracts were estimated against the antibiotic gentamycin on *E.coli* and *S.aureus*. The aqueous extract of seeds of*P.pinnata* showed the least activity at concentration of 12.5 mg/ml on both bacterial strain(Table 2).While the mehanolic extract of seeds of plant *M.indica* showed the least activity at 12.5mg/ml on both tested organisms (Table 3).

According to our study the aqueous extract of leafs of *F. religiosa* showed the highest antibacterial activity at the concentration of 50 mg/ml which was recorded as 1.3cm. The zone of inhibition of *F. religiosa* was even greater than that of Gentamycin which was recorded as 0.9 cm against *E. coli*. However, its activity against *S. aureus* was lower. Since the aqueous extract of leafs of F.religiosa is higher at 50 mg/ml than the positive control, thus this plant could be a better alternativefor many antibiotics against *E. coli* related infections. While the rest of extracts of all plants showed insignificant activity on both tested organisms. Therefore, plants which showed less activity than that of Gentamycin can be assumed not as effective as normal antibiotics. The differences in the antimicrobial efficacy could be due to variable distribution of phytochemical compounds in different parts. A recent study carried out by Supriya and Harshita (2013)showed that extracts of dried powdered leaves of *Ficusreligiosa* in petroleum ether, chloroform, methanol and water were quite effective in inhibiting growth of *E.coli* and *S.aureus*[14].It was found that chloroform extract showed good antimicrobial activity measuring zone of inhibition close to 16 mm against *E.coli*. The result was found in accordance with our results which also indicated greater zone of inhibition (>1 cm) for *F. religiosa* than other investigated plant extracts.

The antibacterial activity of *F. religiosa* leaves had tested against various bacteria like *P. vulgaris*, *E. coli*, *B.subtilis*, *S.aureus*, *P.aeruginosa* and *K. pneumonia*. The current findings supported the earlier work on chloroform extract of *F. religiosa* leaves extract [15]. However, present findings contradict with the earlier reports on diethyl ether and methanol extracts of *F. religiosa* leaves which showed maximum inhibition on *S.aureus* followed by *E.coli* and *P.aeruginosa*[16].

Our findings were found partially contradicting to the study conducted by Ramakrishnaiah and Hariprasad (2013) who investigated the antimicrobial activity of methanolic extract *F.religiosa*(bark and leaves),on three bacteria *E. coli*, *P.aeruginosa* and *S.aureus* and one fungus (*Aspergillusniger*) by disc diffusion method [17]. The methanolic extracts of leaves and bark showed antimicrobial activity against all three bacteria. Whereas, in our study antimicrobial effect of *F.religiosa* was only prominent against *E. coli*. Interestingly, against *S. aureus* the antimicrobial effect of *F.religiosa* was significantly limited. Study by Ramakrishnaiah and Hariprasad (2013) also indicated that at lower concentrations methanol extracts showed less antimicrobial activity and showed higher

activity at higher concentrations [17]. Our result also revealed less or no antimicrobial activity at lower concentrations. Our study has also been found in accordance with study carried out by Chavan(2011) [16]. The study by Chavan (2011) investigated antibacterial activity of *C. tora* and found that most of the microbial strains were resistant to its effects. Our study also indicated similar results, however, at higher concentrations the antimicrobial activity of *C. tora*was slightly effective.

Table 1: Ethnobotanical information of medicinal plant used for the study

Plant Species	Family	Common Name	Part Used	Abbreviation
Ficusgeniculata	Moraceae	*Phutkal*	Leaf	Sample 1
Cassia tora	Cesalpinaceae	*Chakor*	Leaf	Sample 2
Madhucaindica	Sapotaceae	*Mahua*	Bark	Sample 3
Pongamiapinnata	Fabaceae	*Karanj*	Seed	Sample 4
Boerhaaviadiffusa	Nyctaginaceae	*Punarnava*	Root	Sample 5
Ficusreligiosa	Moraceae	*Pippal*	Leaf	Sample 6
Moringaoleiferaa	Moringaceae	*Moringa*	Leaf	Sample 7

Table 2:Antibacterial activity (ZI) of aqueous plants extract recorded by agar well diffusion method

	Concentration (mg/ml)	Sample1 (cm)	Sample2 (cm)	Sample3 (cm)	Sample4 (cm)	Sample5 (cm)	Sample6 (cm)	Sample7 (cm)	Gentamycin (cm)
E.coli	50	0.41±0.01	0.32±0.02	0.46±0.03	0.27±0.01	0.44±0.03	1.3±0.05*	0.25±0.01	0.9±0.05*
	25	0.23±0.01	0.29±0.01	0.33±0.01	0.19±0.01	0.32±0.02	0.97±0.03*	0.15±0.01	
	12.5	0.19±0.02	0.17±0.01	0.12±0.01	0.18±0.01	0.11±0.01	0.49±0.03	0.29±0.01	
S.aureus	50	0.35±0.01	0.34±0.02	0.38±0.02	0.29±0.01	0.32±0.02	0.46±0.04	0.27±0.02	0.53±0.04
	25	0.26±0.01	0.19±0.02	0.31±0.02	0.15±0.01	0.21±0.01	0.35±0.02	0.19±0.02	
	12.5	0.12±0.01	0.14±0.01	0.21±0.01	0.11±0.01	0.12±0.01	0.25±0.01	0.18±0.02	

**P<0.05*

Table 3: Antibacterial activity (ZI) of methanolic plants extract recorded by agar well diffusion method

	Concentration (mg/ml)	Sample1 (cm)	Sample2 (cm)	Sample3 (cm)	Sample4 (cm)	Sample5 (cm)	Sample6 (cm)	Sample7 (cm)	Gentamycin (cm)
E.coli	50	0.43±0.02	0.46±0.02	0.29±0.01	0.45±0.01	0.41±0.03	0.5±0.01	0.32±0.02	0.9±0.05*
	25	0.34±0.03	0.23±0.01	0.26±0.01	0.42±0.03	0.37±0.01	0.36±0.02	0.25±0.01	
	12.5	0.31±0.03	0.12±0.02	0.17±0.01	0.39±0.01	0.28±0.01	0.23±0.01	0.1±0.01	
S.aureus	50	0.42±0.04	0.2±0.01	0.29±0.02	0.33±0.02	0.39±0.02	0.15±0.01	0.19±0.01	0.53±0.04
	25	0.35±0.02	0.12±0.01	0.15±0.01	0.27±0.01	0.17±0.01	0.27±0.02	0.36±0.02	
	12.5	0.26±0.01	0.11±0.01	0.11±0.01	0.18±0.01	0.11±0.01	0.35±0.01	0.41±0.02	

**P<0.05*

CONCLUSION

Present study evidently prove that *Ficus religiosa* is a better herbal alternative to many chemical antibiotics available in the market. Our study also indicates that use of ancient herbal medicine in development of alternative antibiotics is highly likely to reduce the emergence of antibiotic resistant microbes.

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