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Antimicrobial activity and Antioxidant activity of *Flacourtia jangomas* stem from Bihar, India

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Abstract

Flacourtia jangomas is mostly observed in Southeast Asia and East Asia. Its origin is wild and from tropical Asia, especially India. The Common names of *Flacourtia jangomas* are Coffee plum, Indian plum, Manila cherry and Paniala. Traditional uses of *Flacourtia jangomas* are that the fruits are used to cure diarrhea and nausea. The leaves and bark are used in bleeding gums and aching teeth, and the bark infusion is gargled to alleviate hoarseness. Flacourtia jangomas fruits are traditionally used to cure diarrhea and nausea. The leaves and bark are used in bleeding gums and aching teeth. Antibacterial activity of Ethyl acetate extract of the stem was determined by Agar diffusion method against Gram -positive bacteria viz., *Staphylococcus aureus*, *Bacillus polymyxa*, *Bacillus megaterium*, and Gram-negative bacteria viz., *E.coli*, *Salmonella typhi*, *Pseudomonas aeruginosa and Vibrio cholera*. The extracts shown zone of inhibition, ranging from 13 mm-28 mm. The extracts had the profound effect on Gram-negative bacteria with the highest zone of inhibition against *Pseudomonas aeruginosa* showing 28 mm followed by *Vibrio cholerae* with 20 mm. Antifungal activity was determined by Agar diffusion against four Fungal strains viz., *Aspergillus niger* ,*Aspergillus flavus*, *Trichoderma viridae* and *Neurospora* crassa. The extracts showed modearte inhibitory activity ranging from 2-15 mm. Antioxidant activity of *Flacourtia jangomas* stem extract was determined by Nitric oxide scavenging assay, the extract showed prominent antioxidant activity with Butylated hydroxy anisole (BHA) as a standard compound.

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Introduction

There is a huge demand for medicinal plants globally for the preparation of Herbal medicines [1]. Medicinal plants in India have been used for centuries to treat various diseases [2]. India is rich in species diversity, genetic diversity, and habitat diversity [3]. It is documented that conventional therapists used 2,500 plant species while 100 species of plants served as the regular source of medicine in India.100 species of plant has been used as the regular source and 2,500 plant species has been used as conventional therapist in india.[4].In developing countries Infectious disease represent the decisive cause of morbidity and mortality, due to this the pharmaceutical companies have been propelled to develop antimicrobial drugs, because of constant emergence of microorganism resistant to conventional antimicrobials [5]. Antimicrobial drugs along with the favorable effects of bacteria control, the antibiotics also cause various drug reaction being hypersensitivity and immunosuppression [6].Nowadays, presence of various multiple strains of antibiotic resistance microorganism has rapidly advanced the race to find potential compounds in plant for therapeutic, medicinal, aromatic and aesthetic uses [7,8]. It is important to investigate the composition activity and endorse the use of medicinal plant as potential sources of antimicrobial compound [9]. Due to the harmful effects and bacterial resistance caused by antibiotics, to conquer these problems it is need to find new antimicrobial drugs from plants.

Oxidative damage is caused by many microbial infections. In recent years, the studies on "oxidative stress" and its adverse effects on human health have become a subject of considerable interest [10,11]. Recently, there is a trend to find naturally occurring antioxidants, which are safe and effective to supplement processed food or pharmaceuticals and replace synthetic antioxidants which are being restricted due to adverse side effects elicited by them. In this regard, plants were found to be good source of naturally occurring antioxidants. Natural antioxidants, derived from plants, are secondary metabolites, mainly plant phenolics [12]. Synthetic antioxidants like Butylated Hydroxy anisole, Butylated hydroxyl toluene and gallic acid have been questionable to cause negative health effects, these synthetic antioxidant also have low solubility and moderate antioxidant [13,14]. Antioxidants from natural origins which is cheap and safe has been in trend nowadays because of the safety concerns of synthetic antioxidant [15]. Presence of Antioxidants such as carotenoids, vitamins, phenols, flavonoids and remote metabolites are found in various parts of plants [16,17] . In the present study we have investigated the antimicrobial potential and antioxidant potential of ethyl acetate extracts of Flacourtia jangomas stem.

Material and Methods

Collection of *Flacourtia jangomas* stem

Fresh *Flacourtia jangomas* stems were obtained from Munger, Bihar with latitude 25.3748° N, and 86.4735° E longitude and was authenticated by Dr. S.B.Padal, Department of Botany, Andhra University. Voucher specimen number-22229 and was deposited in Botany Department Herbarium, Andhra University, India.

Preparation of plant extracts:

Fresh *Flacourtia jangomas* stem was collected and washed thoroughly with distilled water 2-3 times and shade dried. Dried stems were powdered using electric pulverizers. The shade dried and powdered *Flacourtia jangomas* stem was filled in thimble of Whatman No 1 filter paper and extracted successively with Ethyl acetate in Soxhlet extractor for 48h. The solvent was concentrated under reduced pressure at 40° in Rotary evaporator and stored at 4° C in the airtight bottle for further use

Test Cultures:

The cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Test Bacteria:

Bacteria used for research are *Staphylococcus aureus MTCC 3160, Bacillus polymyxa(local isolate), Bacillus megaterium MTCC2444, Escherichia coli MTCC 723, Salmolella typhi MTCC 3216, Vibrio cholerae MTCC*3906, *Pseudomonas aeruginosa MTCC 7837*. These were maintained in nutrient agar slants.

Test Fungi:

Fungi used for research are *Aspergillus niger MTCC 1881, Aspergillus flavus MTCC 1883,Neurospora crassa MTCC 1855, Trichoderma viridae MTCC 2417*

Antibacterial activity

Antibacterial activity was performed by Agar diffusion method [18]. Initially, the stock cultures of bacteria were revived by inoculating in Nutrient broth and grown at 37°C for 18 hrs. The agar plates of the above media were prepared. Each plate was inoculated with 18-hour old cultures (100 µl, 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells of size 6mm was punctured by sterile cork borer and was filled with 100µl of *Flacourtia jangomas* stem ethyl acetate extract. Ciprofloxacin antibiotic was used as positive control (100µg) .All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone was noted in mm.

Antifungal activity

Antifungal activity was performed by Agar diffusion method [18]. Czapek Dox Agar media [19]. was used for the antifungal activity. Initially, the stock cultures were revived by inoculating in Czapek Dox broth media and grown at 27°C for 48 hrs. The agar plates of the Czapek dox media were prepared. Each plate was inoculated with 48-h old cultures (100 µl 10^{-4} CFU) and spread evenly on the plate. After 20 min, the well was punctured by sterile cork borer of 6mm size and was filled with 100 µl of *Flacourtia jangomas* stem ethyl acetate extract. Amphotericin was used as a positive control. All the plates were incubated at 27°C for 96 hours and the diameter of inhibition zone was noted in mm.

Antioxidant activity:

Assay of Nitric oxide scavenging activity

Nitric oxide scavenging assay was done by method described by Marcocci [20].The Nitric oxide scavenging assay was done by Griess reagent [21]. Antioxidant activity of *Flacourtia jangomas* stem extract was evaluated by Nitric oxide scavenging activity. Different concentrations (10µl, 50µl and 100µl) of *Flacourtia jangomas* stem extract and Butylated hydroxy anisole (BHA) were taken in different test tubes and made up to 3ml witho.1M phosphate buffer (pH 7.2).Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added (1ml) to each tube. The reaction mixture was incubated for 30 min at room temperature. A control without the test sample, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent
(1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1-Naphthylethylenediamine dihydrochloride).The absorbance of the *Flacourtia jangomas* stem extract was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ Nitric oxide radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD}) \times 100}{\text{Control OD}}$$

%-Percentage
OD-Optical Density

Statistical analysis

Statistical analysis was carried out by Kruskal-Wallis test. The level of significance was 5%.

Results

Flacourtia jangomas stem extract showed versatile potential against all the test bacteria as shown in table 2.The extracts showed highest antibacterial activity against *Pseudomonas aeruginosa* with 28 mm. Furthermore extracts showed prominent inhibitory activity against *Vibrio cholerae*, causes a dreadful disease Cholera with 20 mm zone of inhibition followed by *Salmonella typhi* with 18 mm zone of Inhibition. The extract also showed favorable antibacterial activity against *Bacillus megaterium* with 17 mm followed by both *Staphylococcus aureus* and *Bacillus polymyxa* with the similar zone of inhibition of 16 mm. Antifungal activity of the stem extracts was determined against fungal strains *Aspergillus niger*, *Aspergillus flavus Neurospora crassa* and *Trichoderma viridae* recorded as inhibition zone as inferred in table 1. The highest zone of inhibition was observed in *Aspergillus niger* with 15 mm , *Neurospora crassa* showed 5 mm zone of inhibition with least zone of inhibition for *Aspergillus flavus* showing only 2mm. The standard antifungal agent Amphotericin was used. *Flacourtia jangomas* stem extracts showed profound antioxidant activity done by Nitric oxide scavenging assay with Butylated Hydroxy Anisole (BHA) as standard compound and recorded as percentage depicted in table 3.Nitric oxide scavenging assay of the extracts for 10µl was shown 47.03, 50µl showed 59.46 and 100 µl gave the reading 65.41.The standard Butylated Hydroxy Anisole exhibited 50.81 for 10 µl , 77.30 for 50 µl and 89.73 for 100 µl .

Discussion

Flacourtia jangomas belongs to family *Flacourtiaceae* .The plant has been used traditionally for the treatment of different diseases in India [22]. *Flacourtia jangomas* contains bioactive component counting tannins, carbohydrates, fats, minerals, ascorbic acid, tartaric acids, proteins amino acids and phenolic compounds [23]. Due to the evolution of multidrug-resistant pathogens, the analysis for the novel cure all substitutes had gained acceptance of the capacity of medicinal plant extracts for curing infections [24, 25]. From my present work, the *Flacourtia jangomas* showed prominent antibacterial activity against 3 gram-positive and 4 gram-negative bacteria. All the bacteria were sensitive to the stem extracts with highest zones of inhibition for *Pseudomonas aeruginosa, Vibrio cholera* and *Salmonella typhi* and moderate zones of inhibition for *Bacillus megaterium*, *Bacillus polymyxa, Staphylococcus aureus*, *E.coli* and *Bacillus pumilis*. *Flacourtia jangomas* was investigated for antibacterial activity against both gram-positive and gram-negative bacteria by Pravin et al. in 2011.It showed that extract had profound inhibitory zones against *Shigella shiga* and *Bacillus megaterium* and poor activity for *Escherichia coli* [26]. In 2011, by Sarkar GC et.al chloroform fractions were studied against bacteria by Disc diffusion method, *E.coli* was shown the highest zone of inhibition of about 14±0.59 mm [27]. *Flacourtia jangomas* stem extracts also showed significant antifungal activity with the highest zone of inhibition for *Aspergillus niger* and *Trichoderma viridae* and poor activity for *Aspergillus flavus* and *Neurospora crassa*. The antioxidant activity was done by Nitric oxide scavenging assay. The standard used was Butylated hydroxyl anisole. The extract showed sharpened results. Talukdar et al in 2012 disclosed that ethanolic *Flacourtia jangomas* leaves extract (1mg/ml) showed higher scavenging activity than ascorbic acid [28]. Dubey et al in 2013 investigated antioxidant activity from unripe fruits of *flacourtia jangomas* [29]. Antimicrobial and antioxidant activity of *Flacourtia jangomas* done revealed it's potential in treating microbial infection and cancer as it can remove radicals causing cancer.

Ethnobotanical investigations have been found to offer important clues in the identification and development of traditionally used medicinal plants into modern drugs [30]. The present results show that *Flacourtia jangomas* stem can be used in various infectious diseases caused by both bacteria and fungi used in my study. The present study showed stem extract of *Flacourtia jangomas* had a strong antioxidative power on nitric oxide radicals and this could be attributed to the different phytochemical compounds present in these extract [31].

Conclusion

In present study antibacterial antifungal and antioxidant activity of *Flacourtia jangomas* was evaluated. Results revealed that *Flacourtia jangomas* have been reported with good antibacterial and antifungal activity. The stem showed profound broad-spectrum antibacterial activity which can be used for fighting against several dreadful diseases. The stem has significant potential for the development of new antimicrobial treatment and reduction of drug resistance. The stem was tested for antioxidant activity by Nitric oxide scavenging assay, the stem showed convincing antioxidant activity which can be associated with the presence of natural antioxidants. Further investigation can be done to identify novel bioactive components. The Stem extract is a potential therapeutic agent for the control of oxidative and non-oxidative damage caused by nitrogen species.

Microorganism	*Flacourtia jangomas* stem extract zone of inhibition in mm (100 µl)
Aspergillus niger	15
Aspergillus flavus	2
Trichoderma viride	14
Neurospora crassa	5

Table 1. Antifungal activity of *Flacourtia jangomas* Ethyl acetate (EA) stem extracts.

Microorganism	*Flacourtia jangomas* stem extract Zone of inhibition in mm (100 µl)
Staphylococcus aureus	16
Bacillus polymyxa	16
Bacillus megaterium	17
E. coli	15
Salmonella typhi	18
Pseudomonas aeruginosa	28
Vibrio cholera	20

Table 2: Antibacterial activity of *Flacourtia jangoams* Ethyl acetate (EA) stem extract

Concentration (µl)	% Nitric oxide scavenging activity	
	Flacourtia jangomas stem Plant extract	Butylated Hydroxy Anisole
10	47.03	50.81
50	59.46	77.30
100	65.41	89.73

Table 3:% Nitric oxide scavenging activity of *Flacourtia jangomas* Ethyl Acetate (EA) stem extract

Sl no.	Microorganism	Concentration (100µg) Zone of inhibition(mm)
01	*Staphylococcus Aureus*	31
02	*Bacillus Polymyxa*	32
03	*Bacillus megaterium*	20
04	*E.coli*	32
05	*Salmonella Typhi*	35
06	*Pseudomonas aeruginosa*	34
07	*Vibrio cholera*	34

Table 4: Standard Antibiotic values of Ciprofloxacin zones of inhibition (mm)

Sl no	Microorganism	Concentration 100 µg zone of inhibition (mm)
1	*Aspergillus niger*	2
2	*Aspergillus flavus*	-
3	*Neurospora crassa*	-

Table 5. Standard Antibiotic values of Amphotericin zones of inhibition (mm)

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