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Antibacterial activity of *Flacourtia jangomas* and *Flacourtia sepiaria*

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Abstract

The chloroform soluble fraction of *Flacourtia jangomas* and *Flacourtia sepiaria* were subjected to *in vitro* antibacterial screening (by disc diffusion method) against two Gram positive and two Gram negative bacteria to know antimicrobial effectiveness. Chloroform fraction of *Flacourtia jangomas* showed good activity against all the tested bacteria and among them *E. coli* was found the most susceptible bacterium with the zone of inhibition was 14 ± 0.59 mm. *Flacourtia sepiaria* had no activity against *E. coli* and *Bacillus cereus.* In respect of the zone of inhibition of both plant fractions, *Flacourtia jangomas* was better activity than *Flacourtia sepiaria*. Among all tested extract, only *F. jangomas* extract showed significant MIC value, ranges of (0.325 to 5 mg/ml). In toxicity study, the chloroform fraction of *Flacourtia jangomas* showed toxic effect using brine shrimp lethality bioassay with LC_{50} values of 12.58 µg/ml.

Key-Words: *Flacourtia jangomas Flacourtia sepiaria,* antibacterial, MIC, Toxicity.

Introduction

Simultaneous with population explosion, virulent strains of microorganisms become more common and their increased attack accounts for increased mortality[1]. Bangladesh, being a country with high density of population, infectious diseases becomes a great challenge in the health and economic sector. To prevent infectious diseases, large number of antibiotics, mostly synthetic drugs, of different chemical nature has been developed in the last few decades. Now a days, a good number of antibiotics are found to be resistant. Inappropriate and injudicious uses of antibiotics or self treatment practices are some of the major reasons for rapid and widespread drug resistance[2]. In recent years, hospital environments become a major site for the emergence of multiple drug resistant strains that make the situation more dangerous[3].

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Moreover, theses synthetic antibiotics have many side effects including organ-damaging effects, histotoxicity, drug to drug interaction problems and so on[4]. Finding healing powers in plants is an ancient idea. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994[5]. This evidence contributes to support and quantify the importance of screening natural products. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flaonoids, etc, which showed wide range of *in vitro* antibacterial and antifungal activity[6,7]. So, the development of new antibacterial agents, the most feasible way to combat the problem of microbial resistance and for substitution with ineffective ones. Moreover, it is presumed that the broad spectrum effectiveness of plant species may provide a suitable basis for new antimicrobial therapies[8].

Bangladesh is a country with large genetic diversity of medicinal plants. Traditional medicine and modern have already identified the antimicrobial and other

medicinal values of most of these plants[9]. However, huge number of medicinal plants is yet remains unidentified. As a first step, screening of plants for their antimicrobial properties is helpful for identifying such potent plants. In the present study, *Flacourtia jangomas* and *Flacourtia sepiaria* was taken for investigation its antibacterial property against some pathogenic bacteria. *Flacourtia jangomas* and *Flacourtia sepiaria* belong to the family Flacourtiaceae. In India, dried leaves of *Flacourtia jangomas* are used to treat asthma[10] and a decoction of the leaves of the same species are used to treat diarrhoea and dysentery in the Malay Peninsula[11]**.** Two limonoids, i.e. limolin and jangomolide were reported from the stem and bark of *F. jangomas*. Leaves and bark are slightly acid and acrid and reported to be useful in diarrhea, piles, weakness of limbs, bleeding gums and stomatitis[12]. Leaves and stem also have anti-diabetic property[13]. Roots are important remedy for relieving toothache. Fruits are used in treatment of liver related disorders[14]. In Indo China the liquid from the infusion of the roosted or grilled leaves of *Flacourtia sepiaria* is given to woman after parturition[15]**.** In addition, considering the folk medicinal application of those plants, this work was set out in order to investigate the antibacterial activity of chloroform fraction of *Flacourtia jangomas* and *Flacourtia sepiaria* against some pathogenic bacteria. Toxicity profile was also investigated using brine shrimp toxicity assay.

Material and Methods

Plant materials

The root of plant *Flacourtia jangomas* and *Flacourtia sepiaria* were collected from the adjoining area of Rajshahi University Campus, Bangladesh during November to December 2006 and were identified by Taxonomist, Department of Botany and University of Rajshahi, Bangladesh where a voucher specimen number (Voucher No # 98) has been deposited.

Preparation of extracts

The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered plant (750gm) was taken in large glass bottle and extracted with Ethyl acetate: MeOH (7:3) for 7 days. The procedure was repeated twice using same solvent system for next 3 days. The extract was decanted first through a cotton plug and finally filtered through filter paper to get clear filtrate. The filtrate obtained by repeated maceration was evaporated under reduced pressure at 40°c using Rotary evaporator. It renders a gummy concentrate and air evaporated to solid mass. The net weight of dry extract was 5 gm. The dry plant extract (2gm) was dissolved in $CHCl_3$ and fractionated in a conical flask using $CHCl_3$ solvent system. Each fraction further evaporates using Rotary evaporator and then air dried to solid mass (60mg).

Test microorganisms

Strains, including fungi and bacteria both Gram positive and negative were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). *Bacillus cereus* ATCC 14579, *Bacillus megaterium* ATCC 6019, *Escherichia coli* ATCC 25922, *Shigella shiga* ATCC 9161, were used as test microorganism. All these bacterial are recommended by ATCC for their susceptibility assay. The strains are maintained and tested on Nutrient Agar (NA) for bacteria.

Screening of antibacterial activity

The chlororform fractions of the root of *Flacourtia jangomas* and *Flacourtia sepiaria* were tested for antibacterial activity by disc diffusion method[16]**.** By reconstituting with ethanol, two different concentrations of each fraction (125µg/disc and 250µg/disc) were prepared. The test microorganisms were inoculated into respective medium by spread plate method with 24h cultured bacteria, grown in nutrient broth media. After solidification the filter paper disc (5mm in diameter) impregnated with the extract was placed on test organism-seeded plates. As positive control, Standard Amoxicillin (10µg/disc) disc and as negative control, a blank disc impregnated with 10µl ethanol solvent was used. The antibacterial assay plates were incubated at 37^{o} C for 24h. The antibacterial activities of the fractions were then determined by measuring the respective zone of inhibition in mm. The extracts that showed antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample according to method[17].

Determination of Minimum Inhibitory Concentration (MIC)

MIC values were also studied for microorganisms, which were determined as sensitive to the extract in disc diffusion assay. In order to determine the MIC values, extract was dissolved in 10% DMSO to make a concentration of 100 mg/ml. The extract was diluted in a simple dilution manner to make concentrations in the range of 20, 10, 5, 2.5, 1.25, 0.625, 0.312 mg/ml. 0.1 ml of the extract was then added to each hole. The MIC was taken as the lowest concentration of extracts that caused a clear to semi clear inhibition zone around the hole. All the tests were repeated in triplicates.

Determination of Relative Percentage Inhibition The relative percentage inhibition with respect to positive control was calculated by using the following formula[18]. Relative percentage inhibition of the test extract = [{100 x (a - b)}/(c - b)]. Where, a: total area of inhibition of the test extract; b: total area of inhibition of the solvent; c: total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area = πr^2; where, r = radius of the zone of inhibition.

Brine Shrimp Lethality Bioassay

The toxic potnetiality of the plant crude extract and fractions were evaluated using Brine Shrimp lethality bioassay method[19] where 6 graded doses (viz,. 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml, 160µg/ml, 320µg/ml) were used. Brine shrimps (*Artemia salina* Leach) nauplii Ocean 90, USA were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 hours. The mature nauplii were then used in the experiment. DMSO was used as a solvent and also as a negative control. The median lethal concentration LC_{50} of the test sample after 18 hours was obtained by a plot of percentage of the dead shrimps against the logarithm of the sample concentration. Vincristin Sulphate, a well known anticancer drug was used as a reference standard in this case.

Statistical analysis

All assays were performed in triplicate under strict aseptic conditions to ensure consistency of all findings. Data of all experiments were statistically analyzed and expressed as the mean ± standard deviation of three replicate experiments.

Results and Conclusion

Table 1 expressed the comparative antibacterial activity (zone of inhibition) of chloroform fractions of root of *Flacourtia jangomas* and *Flacourtia sepiaria*. Chloroform fraction of *Flacourtia jangomas* showed good activity against all the tested bacteria and among them *E. coli* was found the most susceptible bacterium with the zone of inhibition was 14 ± 0.59 mm. In respect of *Shigella shiga* and *Bacillus megaterium*, (zone of inhibition were 10 ± 0.50 and 11 ± 0.50 mm, respectively), at the dose of 250 µg/ml, *Flacourtia jangomas* was shown the similar or little bit higher activity than the standard Amoxicillin (zone of inhibition were 10 ± 0.05 and 9 ± 0.03 mm for *Shigella shiga* and *Bacillus megaterium*, respectively). On the otherhand, *Flacourtia sepiaria* have showed moderate activity against all the tested bacteria with the zone of inhibition range was 7 ± 0.59 to 9 ± 0.82 mm, at the dose of 250µg/ml, whereas at the dose of 125 µg/ml, *Flacourtia sepiaria* had no activity against *E. coli* and *Bacillus cereus.*

Minimum inhibitory concentration (MIC) values of the $CHCl_3$ fractions of the root extract of *F. jangomas* and *F. sepiaria* against susceptible bacteria were represented in table 2. All the tested extracts showed significant variations in MIC values depending upon the test bacteria. Among all tested extract, only *F. jangomas* extract showed significant MIC value, ranges of (0.325 to 5 mg/ml).

The results of relative percentage inhibition are reported in Table 3. *F. jangomas* extract showed the maximum relative percentage inhibition against *E. coli* (34.82%) followed by *S. shiga* (20.45%) and *B. megaterium* (22.91%) at the dose of 250 µg/ml. However, the relative percentage inhibition ranges was 11.01% to 17.43%, at the dose of 250 µg/ml, for *P. sepiaria* whereas, no relative percentage inhibition was found against *E. coli* and *B. cereus* at the dose of 125 µg/ml.

Phytoconstituents such as saponin, phenolic compounds, flavonoids and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections[20]. Literature review revealed the methonolic extract of the *F. jangomas* have various secondary metabolites *viz* flavonoids, saponins and carbohydrate, steroids, tannins, and phenolic compounds[13]. Two limonoids, i.e. limolin and jangomolide were reported from the stem and bark of *F. jangomas*[12]. So the antibacterial activity showed by the extract may be due to the presence of steroids, flavonoids and saponin. Moreover, Srivastava *et al.*,[21] reported that the fruit extract of *F. jangomas* showed good activity against *Pseudomonas aeroginosa*, *Klebsiella pneumonia* and *Escherichia coli*. Our result also supported to this report.

The brine shrimp lethality bioassay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine shrimp, which could also provide an indication of possible toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay[19]**.** The toxicity exhibited by the chloroform fraction of *Flacourtia jangomas* and Ampicillin trihydrate was presented in figure 1. The median lethal concentration (LC_{50}) of chloroform fraction of *Flacourtia jangomas* was 12.58µg/ml compared with positive control Amplicillin trihydrate (11.48µg/ml). The variation in BSLA results may be due to the difference in the amount and kind of toxic substances (e.g. flavonoids, triterpenoids, or coumarins) present in the crude extracts[13]. Moreover,

this significant lethality of the crude plant fraction (LC_{50} values less than 100 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to priority for further fractionation and isolation of these bioactive compounds. Other cytotoxicity tests and specific bioassays may be done on the isolated bioactive compounds later.

In conclusion, the results of the present study, in agreement with other authors[21] indicate that the $CHCl_3$ fraction of *Flacourtia jangomas* exhibits interesting antimicrobial properties and also show potent toxicity. These results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity. Now our next aim is to explore the lead compound liable for aforementioned activity from this plant.

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References

1. Manisha V., Neha S. and Satish S. (2009). Antimicrobial Activity of Stem Bark Extracts of Nyctanthes arbortristis Linn. (Oleaceae). *International Journal of Pharmacognosy and Phytochemical Research*, **1(1):** 12-14.
2. Alanis A.J. (2005). Resistance to Antibiotics: Are We in the Post-Antibiotics Era? *Archives of Medical Research*, **36:** 697-705
3. Quadri L.E.N. (2007). Strategic Paradigm Shifts in the Antimicrobial Drug Discovery Process of the 21st Century. *Infectious Disorders – Drug Targets*, **7:** 230-237.
4. George S. and Benny P.J. (2010). Antibacterial Potency of Fruit Extracts of Flacourtia inermis against Multidrug Resistant Strains and Comparison of its Activity with that of Standard Antibiotics. *International Journal of Pharmaceutical Science and Biotechnology*, **1(2):** 96-99.
5. Cragg G.M., Newman D.J. and Snader K.M. (1997). Natural products in drug discovery and development. *Journal of Natural Products*, **60:** 52-60.
6. Dahanukar S.A., Kulkarni R.A. and Rege N.N. (2000). Pharmacology of medicinal plants and natural Products. *Indian Journal of Pharmcology*, **32:** S81-S118.
7. Cowan M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbilogical Review*, **12:** 564-582.
8. Kaushik P. (2003). Haridra (Turmeric): antibacterial potentials, *Chowkhamba Sanskrit Series office, K 37/99, Gopal Mandir Lane, Varanasi*, 16.
9. Preethi R., Devanathan V.V. and Loganathan M. (2010). Antimicrobial and Antioxidant efficacy of Some Medicinal Plants Against Food Borne Pathogens. *Advances in Biological Research*, **4(2):** 122-125.
10. Ahmad J., Wizarat D., Shamsuddin K.M., Zaman A. and Connolly J.D. (1984). Jangomolide, a novel limonoid from *Flacourtia Jangomas*. *Phytochemistry*, **23:** 1269-1270.
11. Burkill I.H. (1966). A Dictionary of the Economic Plants of the Malay Peninsula, *Ministry of Agriculture and Cooperatives, Kuala Laumpur*, 2444.
12. Kirthikar K.R. and Basu B.D. (1993). Indian Medicinal Plants. 2nd Edition. *Dehra dun, India*, 220
13. Singh A.K. and Singh J. (2011). Evaluation of anti-diabetic potential of leaves and stem of Flavourtia jangomas in streptozotocin – induced diabetic rats. *Indian Journal of Pharmacology*, **42(5):** 301-305.
14. Srivastava D., Prabhuji S.K. and Rao G.P. (2009). Taxonomic and Ethno-biological Status of Flacourtia jangomas (Lour.) Raeus. : An Endemic Nutraceutical Plant of Eastern U.P. *Medicinal Plants*, **1(1):** 49-53
15. Perry L.M. and Metzger J. (1980). Medicinal Plants of East and Southeast Asia. Attributed Properties and Uses. *The MIT Press Cambridge*, 155.
16. Olurinola P.F. (1996). A laboratory manual of pharmaceutical microbiology, *Printed by National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria*, 69.
17. Vadlapudi V., Bobbarala V., Penumajji S. and Naidu K.C. (2009). *Excoecaria allocha* L. Antimicrobial properties against important pathogenic microorganisms, *International Journal of Pharma Tech Research*, **4 (1):** 865-867.
18. Ajay K.K., Lokanatha R.M.K. and Umesha K.B. (2002). Evaluation of antibacterial activity of 3, 5-dicyano-4,6-diaryl-4-

ethoxycarbonyl-piperid-2-ones. *Journal of Pharmaceutical and Biomedical Analysis*, **27:** 837-840.

19. Mayer B.N., Ferrigni N.R., Potnam J.E., Jacobson L.B., Nicholas D.E. and Mclaughin J.L. (1982). Brine Shrimp: A convenient bioassay for active plant constituents, *Planta Medica*, **45:** 31-34.
20. Alam M.B., Hossain M.S. and Haque M.E. (2010). Evaluation of Antimicrobial and toxicity of different fractions of *Dillenia indica* Linn. Bark extract. *Journal of Global Pharma Technology*, **2(11):** 37-42.
21. Srivastava D., Sheikh S., Prabhuji S.K., Rao G.P., Tripathi A. and Sribastava R. (2010). Antibacterial properties of fruits extract of Flacourtia jungomas (Lour.) Raeus. *In International Conference on Challenging and Emerging Dimensions in Medicinal/Herbal Plants and their Products: A Global Perspective*; Technical Session -3/PP2, Abstract, 26.

Table 1: *In vitro* antimicrobial activity of *Flacourtia jangomas* and *Flacourtia sepiaria* extract on various bacterial by disc diffusion method

Test microorganisms	[a]Diameter of Zone of inhabitation (mm)				[b]Amoxicillin (10 µg/disc)
	F. jangomas		*F. sepiaria*		
	125µg/disc	250µg/disc	125µg/disc	250µg/disc	
Gram Negative					
Shigella shiga	8 ± 0.19	10 ± 0.50	7 ± 0.34	9 ± 0.82	10 ± 0.05
Escherichia coli	9 ± 0.56	14 ± 0.59	NA	8 ± 0.43	25 ± 0.89
Gram Positive					
Bacillus cereus	7 ± 0.50	8 ± 0.09	NA	7 ± 0.59	12 ± 0.02
Bacillus megaterium	8 ± 0.69	11 ± 0.50	8 ± 0.17	9 ± 0.18	9 ± 0.03

[a] Values of the observed diameter zone of inhibition (mm) excluding cap diameter. Incubation conditions for bacteria – 24 hours at 37^0C. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation.

[b] Reference standard

NA- Zone of inhibition < 5 mm consider as no activity.

Table 2: Minimum inhibitory concentration of $CHCl_3$ fractions of *Flacourtia jangomas* and *Flacourtia sepiaria* extract on various bacterial by agar diffusion method.

Extract	Minimum Inhibitory Concentration (MIC) (mg/ml)			
	Gram Positive		Gram Negative	
	B. cereus	*B. megaterium*	*S. shiga*	*E. coli*
F. jangomas	5	1.25	1.25	0.325
F. sepiaria	10	> 20	10	> 20

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Table 3: Relative percentage inhibition of fractions of *Flacourtia jangomas* and *Flacourtia sepiaria* extract on various bacterial

Test microorganisms	Relative percentage inhibition (in %)			
	F. jangomas		*F. sepiaria*	
	125µg/disc	250µg/disc	125µg/disc	250µg/disc
Gram Negative				
Shigella shiga	14.39	20.45	10.12	17.43
Escherichia coli	18.30	34.82	-	13.21
Gram Positive				
Bacillus cereus	10.45	13.29	-	11.01
Bacillus megaterium	13.97	22.91	13.29	16.49

Fig. 1: Toxicity assay of $CHCl_3$ fractions of *Flacourtia jangomas* by Brine shrimp lethality bioassay