Article published online: 2022-07-07

ARTICLE

Phytochemical Screening, Antibacterial and Cytotoxic Activity of Different Fractions of *Xylocarpus mekongensis* Bark

Arif Ahmed[1], Zubair Khalid Labu[2], Shubhra Kanti Dey[1], Arpona Hira[1], Mohamed Sariful Islam Howlader[2], Mohamed Hemayet Hossain[3] and Jimuty Roy[4]

[1]Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
[2]Department of Pharmacy, World University of Bangladesh, Dhaka-1205, Bangladesh
[3]BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh.
[4]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Corresponding author: Arif Ahmed Email: arif.061126@gmail.com
Published: 10 July 2013
Ibnosina J Med BS 2013,5(4):206-213
Received: 31 December 2012
Accepted: 09 March 2013
This article is available from: http://www.ijmbs.org

Abstract

Background: In Bangladesh, the tree *Xylocarpus mekongensis* (Lamk.) M. Roem. is used as the traditional medicine for the treatment of a number of ailments. **Objectives:** The present study was undertaken for antibacterial activity and cytotoxicity study of the methanol, ethyl acetate and chloroform extract of *X. mekongensis* bark. **Materials and methods:** Antimicrobial activity has been investigated against *Vibrio cholera*, *Shigella flexneri*, *Shigella boydii*, *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus* by disc diffusion and broth macrodilution assay. For cytotoxicity test, methanol, acetate and chloroform extract of bark of *X. mekongensis* were subjected to brine shrimp lethality bioassay. **Results:** The zone of inhibition has been observed with almost all bacteria with some exceptions. Minimum inhibitory concentrations (MIC) of the extracts were found to be significant. The LC_{50} values of these three extracts were found to be 1280, 320 and 320 μg/ml, respectively. **Conclusions:** Findings of the study justify the use of the plant in traditional medicine and suggests further investigation.

Keywords: *Xylocarpus mekongensis*, Phytochemical screening, Antimicrobial activity, MIC, Brine shrimp lethality

Introduction

Xylocarpus mekongensis (*X. mekongensis*) (Lamk.) M. Roem. belongs to Family-Meliaceae also commonly known as Possur in Bangladesh. It is a glabrous, medium-sized tree found in littoral forests of Bengal, Burma, the Andaman's, the Malay Peninsula and Archipelago, Australia, Fiji and Africa. In Bangladesh, this species grows in the north tract, remote from the sea and mainly in low lying swampy areas of the Sundarbans, the largest mangrove forest in the world (1). *X. mekongensis* is a perennial tree of 3 to 5 m tall. It is rare and grows just behind or sometime in association with *Rhizophora* sp. along the banks of the tidal canals and creeks where the salinity is low. Leaves compound with 1 or 2 pairs of leaflets, elliptic or oblong in shape,

obtuse at both ends. Leaves are pale to dark green. Bark reddish brown with thick flakes and buttress present. The pneumatophores are stout, stumpy and finger like. Flowers are inflorescence up to 8 cm or longer, with a distinct main axis and regular lateral cymes and the flowering season is July to September. The fruits are ball shaped, about the size of an orange fruit and green in colour when young becoming brown on maturity. The fruit contains 8 to 12 semi-triangular seeds. The fruiting period is October to November (2). This plant traditionally is used as an astringent and febrifuge. It is also used for the treatment of dysentery and diarrhea (3). Xyloccensin has been isolated from the bark of *X. mekongensis* (4). The bark and pneumatophore of *X. mekongensis* possess antimalarial, antidiarrhoeal and antinociceptive activities (4-6). Anti-inflammatory and antioxidant activities of the crude ethanolic extract of the kernel root of *X. mekongensis* was evaluated in different methods and the results suggest the probable anti-inflammatory and antioxidant activities of the ethanolic extract of *X. mekongensis* kernel root and justify its use in traditional medicines (7). Free amino acids, total methylated onium compounds (TMOC) and total nitrogen were investigated in young and old leaves of 22 mangrove species from Northern Queensland (Australia) and high proline accumulation was observed in *X. mekongensis* (8).

Materials and Methods

Plant material

The plant *X. mekongensis* was collected from Borigualani range of Sundarban of Khulna district, Bangladesh. The plant was collected on the 10 August, 2011 in the day time. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: DACB - 35372) and a voucher specimen was also deposited there. The necessary plant part (bark) were carefully cleaned and separated from other parts of the plant as well as from undesirable materials. After cutting into small pieces, these were dried under shade with ample aeration. After complete drying, the plant material was grinded into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered plant material was weighed using an electric balance, kept in a suitable airtight container and then stored in a dark, cool and dry place for further use.

Extraction

Methanol, ethyl acetate and chloroform are selected as soaking solvents to compare the different activities of the extracts like polar extracts (Methanol extract), moderately polar extract (Ethyl acetate extract) and non-polar extract (Chloroform extract). The powdered plant material (crushed barks) was macerated in methanol, ethyl acetate and chloroform respectively for three days with occasional shaking. It was then filtered through a piece of clean, white cloth and then through a cotton plug to remove the plant debris. The filtrate was evaporated using a rotary vacuum evaporator at a temperature of 50^{o} C to yield the crude extract.

Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent and Mayer's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, steroids with Libermann-Burchard reagent and sulphuric acid, gums with molish reagent and sulphuric acid and reducing sugars with Fehling's solutions A and B and Benedict's reagent (9-11).

Test microorganism

One Gram-positive bacterium, *S. aureas* and five Gram-negative bacteria, *V. cholera*, *S. boydii*, *S. flexneri*, *S. typhi* and *S. paratyphi* were taken for the test. The bacterial strains used for this investigation were obtained from the bacterial stocks preserved in animal cell culture laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Antimicrobial assay

The antibacterial activity was investigated using two methods: disc diffusion and broth macro-dilution assay (12-14). Reference microorganisms from the stock were streaked onto nutrient agar plates and the inoculated plates were incubated overnight at 37°C. Using a sterile loop, small portion of the subculture was transferred into test tube containing nutrient broth and incubated (2-4 h) at 37°C until the growth reached log phase. Nutrient agar media seeded with standard inoculums suspension was poured in Petri-dishes and allowed to solidify. Discs (BBL, Cocksville, USA) impregnated with methanol, ethyl acetate and chloroform extract (600 µg/disc), standard antibiotic disc (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and blank (solvent methanol, ethyl acetate and chloroform) discs were placed on the Petri-dishes with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Finally the inoculated plates were incubated

at 37° C for 18 h and the zone of inhibition was measured in millimeters. The broth macro-dilution assay was carried out to determine the minimum inhibitory concentration (MIC). Stock suspension of the extract was prepared in nutrient broth with tween-80 concentration not exceeding 5%. Serial dilution of the stock was carried out to obtain six different concentrations (8, 4, 2, 1, 0.5 and 0.25 mg/ml) in six vials containing 1 ml each. The same procedure was followed for the standard antibiotic solution of ceftriaxone to obtain six different concentrations (8, 4, 2, 1, 0.5 and 0.25 µg/ml) in six vials containing 1 ml each. Then 1 ml of freshly grown inoculum was added to each vial and incubated at 37° C for 12 h. After incubation period, the vials were checked for turbidity and the lowest concentrations of the extract/ standard showing no turbidity were regarded as the MIC of the test substance.

Brine shrimp lethality bioassay

In this assay, the eggs of *Artemia salina* were hatched for 24 h at room temperature (25-30 °C) in artificial sea water (20 g NaCl and 18 g table salt in 1L of distilled water) to obtain nauplii (shrimp larvae). Test samples (methanol, ethyl acetate and chloroform extract of bark of *X. mekongensis*) dissolved in DMSO was added in test tubes in such a way that the each contains 4 ml of sea water with sample concentrations of 5, 10, 20, 40, 80, 160, 320, 640, 1280 and 2560 µg/ml where the concentration of solvent should be not more than 5%. Same procedure was followed for the standard drug chloramphenicol. The final volume for each test tube was adjusted to 10 ml with artificial sea water with 10 living nauplii in each. The process also includes control test tubes containing 10 living nauplii in 10 ml of artificial sea water. The test tubes were observed and the number of survived nauplii in each test tube counted and the results were noted after a period of 24 h. The percentage of dead nauplii in the test and standard group was established by comparing with that of control group (15-16).

Results

Phytochemical screening

Phytochemical screening of the methanol and chloroform extract of bark of *X. mekongensis* indicates the presence of flavonoids, steroids, tannins and saponins and phytochemical screening of the ethyl acetate extract of bark of *X. mekongensis* indicates the presence of flavonoids, steroids, gums, tannins and saponins (Table 1).

Cytotoxic activity

In the antibacterial assay, the chloroform extracts (600 µg/ml) of *X. mekongensis* bark (CEXM) inhibited all bacteria. In disk diffusion assay, 600 µg/ml of the methanol and ethyl acetate extract of bark inhibited all the microorganisms except *S. flexneri*, *S. boydii* and *S. typhi* (Table 2). The highest zone of inhibition was 17 mm against *V. cholera 01* (0-395) Classical. Zone of inhibition for the standard Kanamycin discs (30 µg/disc) ranged between 20 to 23 mm (Table 2).The data obtained from broth macro dilution assay for determining MIC is presented in (Table 3). MIC of the methanol extract of bark was 8000 µg/ml for *S. boydii*, *S. flexneri* and *S. typhi*; 4000 µg/ml for *V. cholera 01* (Z-49) Inaba, *S. paratyphi* and *S. aureus*; 2000 µg/ml for *V. cholera 01* (B-15) Ogawa Elton, *V. cholera 01* (0-395) Classical and *V. cholera* 0139 Al-1852. MIC of the ethyl acetate extract of bark was 8000 µg/ml for *S. boydii*, *S. flexneri* and *S. typhi*; 4000 µg/ml for *S. paratyphi*; 2000 µg/ml for *V. cholera 01* (B-15) Ogawa Elton, *V. cholera 01* (Z-49) Inaba, *V. cholera 01* (0-395) Classical and *S. aureus*; 1000 µg/ml for *V. cholera 0139* Al-1852. MIC of the chloroform extract of bark was 4000 µg/ml for *S. boydii* and *S. flexneri*; 2000 µg/ml for *V. cholera 01* (B-15) Ogawa Elton, *V. cholera 01* (Z-49) Inaba, *S. typhi* and *S. paratyphi*; 1000 µg/ml for *V. cholera 01* (0-395) Classical, *V. cholera*

Table 1. Phytochemical constituents of extracts of *X. mekongensis* bark

Extracts	Steroids	Alkaloids	Reducing Sugars	Tannins	Gums	Flavonoids	Saponins
Methanol extract of *X. mekongensis* bark	+	-	-	+++	-	+	+
Ethyl acetate extract of *X. mekongensis* bark	+	-	-	+	+	+	+
Chloroform extract of *X. mekongensis* bark	+	-	-	+++	-	+	+

+= Positive result, – = Negative result, +++ = significantly positive

Table 2. Results of the disc diffusion assay of *X. mekongensis* bark

	Diameter of zone of inhibition (mm)			
	MEXM	EAXM	CEXM	Kanamycin (30 µg/ disc)
Gram negative bacteria				
Vibrio cholera 01 (B-15) Ogawa Elton	13	14	16	20
Vibrio cholera 01 (Z-49) Inaba	11	14	15	21
Vibrio cholera 01 (0-395) Classical	14	15	17	22
Vibrio cholera 0139 Al-1852	10	15	16	20
Shigella flexneri	0	0	8	20
Shigella boydii	0	0	10	22
Salmonella typhi	0	0	11	23
Salmonella paratyphi	10	11	14	20
Gram positive bacteria				
Staphylococcus aureus	11	14	16	21

MEXM =Methanol extract of bark of *X. mekongensis* (600 µg/ml), EAXM =Ethyl acetate extract of bark of *X. mekongensis* (600 µg/ml), CEXM =Chloroform extract of bark of *X. mekongensis* (600 µg/ml)

Table 3. MICs of *X. mekongensis* bark extracts

	Minimum Inhibitory Concentration (MIC)			
	MEXM	EAXM	CEXM	Ceftriaxone (µg/ml)
Gram negative bacteria				
Vibrio cholera 01 (B-15) Ogawa Elton	2000	2000	2000	1
Vibrio cholera 01 (Z-49) Inaba	4000	2000	2000	1
Vibrio cholera 01 (0-395) Classical	2000	2000	1000	0.5
Vibrio cholera 0139 Al-1852	2000	1000	1000	0.5
Shigella flexneri	8000	8000	4000	2
Shigella boydii	8000	8000	4000	2
Salmonella typhi	8000	8000	2000	1
Salmonella paratyphi	4000	4000	2000	1
Gram positive bacteria				
Staphylococcus aureus	4000	2000	1000	0.5

MEXM =Methanol extract of bark of *X. mekongensis* (µg/ml), EAXM =Ethyl acetate extract of bark of *X. mekongensis* (µg/ml), CEXM =Chloroform extract of bark of *X. mekongensis* (µg/ml)

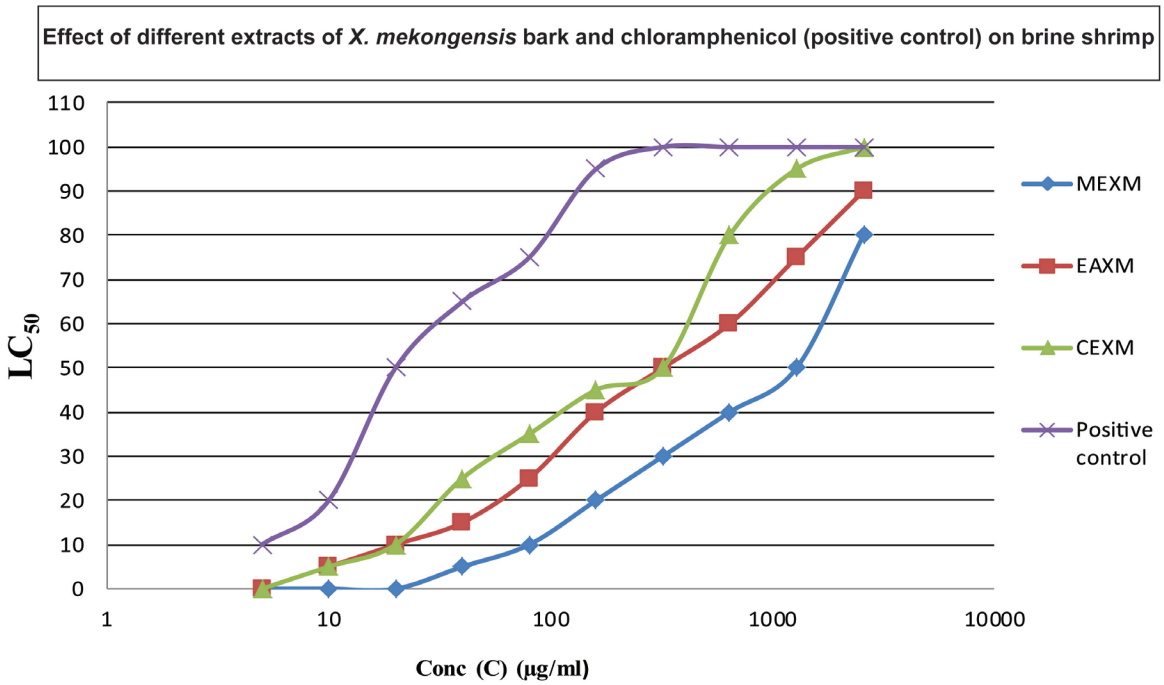

Figure 1. Mortality of nauplii by positive control and *X. mekongensis* bark extracts in brine shrimp lethality bioassay

Table 4. Effect of different extracts of X. mekongensis bark and chloramphenicol (positive control) on brine shrimp

Conc (C) (μg/ml)	% Mortality				LC_{50} (μg/ml)			
	MEXM	EAXM	CEXM	Positive control	MEXM	EAXM	CEXM	Positive control
2560	80	90	100	100				
1280	50	75	95	100				
640	40	60	80	100				
320	30	50	50	100				
160	20	40	45	95	1280	320	320	20
80	10	25	35	75				
40	5	15	25	65				
20	0	10	10	50				
10	0	5	5	20				
5	0	0	0	10				

MEXM =Methanol extract of bark of *X. mekongensis*, EAXM =Ethyl acetate extract of bark of *X. mekongensis*, CEXM =Chloroform extract of bark of *X. mekongensis*, Positive control= chloramphenicol.

0139 Al-1852 and *S. aureus*. In the brine shrimp lethality bioassay, the percent mortality the nauplii caused by the test extracts, as well as chloramphenicol is represented in (Table 4) and Fig.1. Probit analysis software LdP (LdP Line software, USA) was used to calculate the LC_{50} and was found to be 1280 μg/ml for the methanol extract of bark of *X. mekongensis*, 320 μg/ml for the ethyl acetate and chloroform extract of bark of *X. mekongensis* whereas 20 μg/ml for chloramphenicol (positive control).

Discussion

Highly significant degree of activity was observed against the test bacteria *V. cholera 01* (0-395) Classical with 17 mm in diameter followed by *S. flexneri* with 8 mm in diameter at 600 µg/ml of the chloroform extract; highest activity was observed against the test bacteria *V. cholera 01* (0-395) Classical and *V. cholera 0139* Al-1852 with 15 mm in diameter followed by *S. flexneri*, *S. boydii* and *S. typhi* with no activity at 600 µg/ml of the ethyl acetate extract and highest activity was observed against the test bacteria *V. cholera 01* (0-395) Classical with 14 mm in diameter followed by *S. flexneri*, *S. boydii* and *S. typhi* with no activity at 600 µg/ml of the methanol extract. A fluctuating trend of inhibition zone was found against some pathogens in the analysis. Similar fluctuation trend of inhibition zone was reported by Kunjal Bhatt *et al*., (2003) (17) and Uma and Sasikumar (2005) (18). This may be due to the fact that at higher concentrations, the rate of diffusion may perhaps be varied and hence, it might not be available to react with the microorganisms. In most of the bacteria examined, the methanol extracts of bark showed moderate antibacterial activity whereas ethyl acetate and chloroform extracts of bark showed relatively higher antibacterial activity in the assays. Although 600 µg/ml of the methanol and ethyl acetate extract of bark did not show antibacterial activity against *S. flexneri*, *S. boydii* and *S. typhi* but inhibited the same microorganism in broth marco dilution assay. However, the MIC was obtained at a higher concentration (8000 μg/ml) than the extract content in the disc (600 µg/ml). Therefore, concentration may play a role for the observed activity in latter experiment. Antibacterial activity offered by non polar compound(s) may also be a reason as it may fail to diffuse in agar media to exhibit antibacterial activity in disc diffusion assay (16). A difference in inoculums size used for the assay can lead to variable results for a given sample. In the present study we adjusted to keep the inoculums size as close to the recommended standard of 5×10^5 CFU/ml (14).

The plant is also reported to contain saponins, so there is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic (19). Phytochemical constituents such as tannins, flavonoids and several other aromatic compounds of plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (20-22). Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens (23).

The brine shrimp lethality bioassay is a rapid, simple and easily mastered technique for identifying biologically active compound present in a crude extract since it does not require aseptic techniques, inexpensive and very small amount of test material is needed (24). Result of the present study indicates that the bark extracts of *X. mekongensis* might have compounds with biological activity with actions like enzyme inhibition, ion channel interference, antimicrobial, pesticidal and/or cytotoxic activity (25-27). Both the test extracts and chloramphenicol showed a gradual increase in percent mortality of the shrimp nauplii with the increase in concentration. The LD_{50} obtained for the extracts was relatively low than that of chloramphenicol. However, it is still high as a crude extract and infers that there may be one or more compounds present in the extract having biological activity.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay and in the recent years several reports available on the antibacterial activity of plant extracts on human pathogenic bacteria (28). The beneficial effects of treatment can be obtained with the bark extract of *X. mekongensis* for various bacterial infectious diseases like dysentery, diarrhea. The broad antibacterial activities could be as a result of the plant secondary metabolites like flavonoids, tannins, steroids etc., present in the extracts. Usman and Osuji (2007) reported that tannins had been widely used topically to sprains, bruises and superficial wounds as such, it could be probable that tannins and other plant phenols from this extract were responsible for these broad activities (29).

In conclusion, the present study provides a rationale for the use of *X. mekongensis* bark in traditional medicine in Bangladesh. Further, it reflects a possibility for the development of more novel chemotherapeutic agents or templates from the plant so that the plant may serve for the production of improved therapeutic plant based drugs in future. But *in vivo* studies on the medicinal plant are necessary and should seek to determine toxicity of active constituents, their side effects, serum-attainable levels,

pharmacokinetic properties and diffusion in different body sites. The antibacterial activity could be enhanced if active components are purified and adequate dosage is determined for proper administration. It goes a long way in curbing administration of inappropriate concentration, a common practice among many traditional practitioners. This represents a preliminary report on the antibacterial activity of the medicinal plant *X. mekongensis* in Bangladesh and for rational use of the traditional plant it requires further scientific study as necessary on it.

Acknowledgement

We are thankful to Md. Hemayet Hossain, BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh for providing us with reagents and labs. Ceftriaxone was obtained from Drug International Ltd, Bangladesh.

References

1. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Allahabad: India; 1999.
2. Selvam V, Eganathan P, Karunagaran VM, Ravishankar T, Ramasubramanian R. Mangrove Plants of Tamil Nadu. M.S. Swaminathan Research Foundation, 3rd Cross Street, Institutional Area, Taramani, Chennai-600 113: India; 2004. p. 45.
3. Ghani A. Medicinal Plants of Bangladesh. 1st ed. Dhaka: Asiatic Society; 1998.
4. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manag. 2002;10:421-45.
5. Uddin SJ, Shilpi JA, Alam SMS, Alamgir M, Rahman MT, Sarker SD. Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus molucensis* in castor oil and magnesium sulphateinduced diarrhoea models in mice. J. Ethnopharmacol. 2005;101:139-43.
6. Uddin SJ, Shilpi JA, Rouf R, Ferdous MM, Nahar L, Sarker SD. Antinociceptive activity of some Bangladeshi medicinal plant extracts. Orient. Pharm. Exp. Med. 2006;6:96-101.
7. Jahan IA, Hossain MH, Nimmi I, Islam S, Kawsar MH, Islam MA. Evaluation of anti-inflammatory and antioxidant potential of the kernel root of *Xylocarpus mekongensis* (Lamk.) M. Roem. Orient. Pharm. Exp. Med. 2012;12:181-8.
8. Popp M, Larher F, Weigel P. Chemical Composition of Australian Mangroves III. Free Amino Acids, Total Methylated Onium Compounds and Total Nitrogen. Zeitschrift für Pflanzenphysiologie. 1984;114:15-25.
9. Ghani A. Medicinal Plants of Bangladesh. 1st ed. Dhaka: Asiatic Society; 1998. p.13.
10. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 13th ed, London: Cambidge University Press; 1989. p. 546.
11. Harborne JB. Phytochemal methods (A guide to modern techniques to plantanalysis). 3rd ed. London: Chapman and Hall;1984.
12. Cruickshank R. Medical Microbiology: A Guide to Diagnosis and Control of Infection. Edinburgh/London: E. and S. Livingstone Ltd.; 1968. p. 888.
13. Andrews JM. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 2001;48:5-16.
14. Ríos JL, Recio MC and Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol. 1988;23:127-49.
15. Ghisalberti EL. Detection and isolation of bioactive natural products. In: Colegate SM, Molyneux RJ, editors. Bioactive natural products- Detection, isolation and structural determination. CSIRO Livestock Industrie; 1993. p. 15-18.
16. Anderson JE, Chang CJ, McLaughin JL. Bioactive components of Allamanda schottii. J. Nat. Prod. 1998;51:307-8.
17. Kunjal Bhatt R, Gokani SJ, Snehal Bagatharia B and Virinda Thaker S. Antimicrobial activity of some medicinal plants: Comparision of methods employed and plants studied. As. J. Microbiol. Biotechnol. Env. Sci. 2003;5:455-62.
18. Uma C, Sasikumar JM. Antimicrobial activity of traditional medicinal plants from Southern Western Ghats. As. J. Microbiol. Biotechnol. Env. Sci. 2005;7:665-70.
19. Attele AS, Wu JA, Yuan C. Analgesic effects of different aucpoint stimulation frequencies in humans. Biochem. Pharmacol. 1999; 58:1685-93.
20. Doss A, Muhamed Mubarack H, Dhanabalan R. Antibacterial activity of tannins from *Solanum trilobatum* Linn. Leaves. Indian J. Sci. Technol. 2009;2:41-3.
21. Doss A, Vijayasanthi M, Parivuguna V, Venkataswamy R. Antimicrobial effects of the Flavonoid fractions of *Mimosa pudica* L. Leaves. J.P.R. 2011;4:1438-9.
22. Doss A, Parivuguna V, Vijaya Santhi M, Sruthi S. Antibacterial and preliminary phytochemical analysis of *Medicago sativa* L. against some microbial pathogens. Indian J. Sci. Technol. 2011;4:550-2.

23. Gopish KV, Kannabiran K. Antimicrobial activity of saponin fractions of the leaves *Gymnema sylvestre* and *Eclipta prostrate*. World J. Microbiol. Biotechnol. 2008;24:2737-40.
24. McLaughin JL, Rogers LL. The use of biological assays to evaluate botanicals. Drug Inf. J. 1998;32:513-24.
25. Borowitz JL, McLaughlin JL. Evidence for calcium channels in brine shrimp: diltiazem protects shrimp against cadmium. B. Environ. Contam. Tox. 1992;48:435-40.
26. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. Phytochem. Anal. 1991;2:107-11.
27. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45:31-4.
28. Satish S, Raghavendra MP, Raveesha KA. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Advances in Biological Research 2008;2:44-8.
29. Usman H, Osuji JC. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia leavis*. Afr. J. Trad. CAM. 2007;4:476-80.