**IJPSR (2014), Vol. 5, Issue 3** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 October, 2013; received in revised form, 10 December, 2013; accepted, 16 February, 2014; published 01 March, 2014

# A COMPARATIVE STUDY ON ANTIBACTERIAL ACTIVITIES AND CYTOTOXIC PROPERTEIS OF VARIOUS LEAVES EXTRACTS OF *AVERRHOA BILIMBI*

Md. Abdullah Aziz, Shahariar Rahman, Mahfuzul Islam and Anjuman Ara Begum*

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

**Keywords:**

*Averrhoa bilimbi*, Antibacterial activity, Zone of inhibition, Disc diffusion method, Brine shrimp lethality bioassay, $LC_{50}$

**Correspondence to Author:**

**Anjuman Ara Begum**

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

E-mail: anjumantanya@yahoo.com

**ABSTRACT:** The study was carried out to assess the antibacterial & cytotoxic activities of extracts from leaf parts of the plant, Averrhoa bilimbi. Ethanol, methanol and water were used as solvents and antibacterial & cytotoxic effects were measured using disc diffusion test & brine shrimp lethality bioassay (BSLB). The susceptibility of the microorganisms to the extracts of this plant was compared with standard antibiotic cefadroxil. Water extract exhibited more potent antimicrobial activity by inhibiting wide range of gram positive and gram negative bacteria in comparison to ethanolic and methanolic extract of leaves of *A. bilimbi.* On the other hand, the ethanolic extract of *A. bilimbi* leaves( EEL) was found to be the most toxic to Brine Shrimp nauplii, with $LC_{50}$ of 3.7 µg/ml whereas anticancer drug vincristine sulfate (VS) proved $LC_{50}$ value 1.73 µg/ml indicating that the potent cytotoxic compounds in this plant have affinity for non-polar solvents. The spectrum of activity observed in the present study may provide the indication that the plant could be a possible source to obtain new and effective antibacterial & cytotoxic agents. Hence further study should be needed for the isolation of the therapeutic antibacterials & cytotoxic agents from the leaves extract of *A. bilimbi.*

**INTRODUCTION:** The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is therefore, should be utilized to combat the disease causing pathogens [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(3).913-18

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(3).913-18

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increment has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects.

Therefore, there is need to search new infection-fighting strategies to control microbial infections [2]. BSLB represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties [3].

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drug [4, 5].

*A. bilimbi* of oxalidaceae family is medicinally used as a folk remedy for many symptoms. It is used as antibacterial, antiscorbutic, astringent, post–partum protective medicine. It is also used for the treatment of fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache, aphthous ulcer and as a cooling drink .The chemical constituents of A. bilimbi include amino acids, citric acid, cyanidin–3–O–h–D–glucoside, phenolics, potassium ion, sugars , vitamin A[6].

Previous phytochemical investigations showed that chloroform extracts of *A. bilimbi*'s leaf and fruit have antibacterial activity against the Gram positive *S. aureus, S. epidermis, B. cereus, K. rhizophila, C. diphteriae* and Gram-negative *S. typhi, C. fuendii, A. hydrophila* and *P. vulgaris*[7]. The present study was aimed to evaluate the comparative antibacterial screening of various extracts (ethanol, methanol and water) of leaves of *Averrhoa bilimbi* against some Gram-positive and Gram-negative strains of bacteria and also to evaluate the cytotoxic activity.

## MATERIALS AND METHODS:

**Collection and Identification of Plant Materials:** Fresh leaves of *A. bilimbi* were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in January, 2013 that was identified by the taxonomist of the department of Botany, Jahangirnagar University.

**Preparation of the Plant Extracts:** Dried plant material (180g) was used for extraction procedure. The extracts were all made with analytical grade solvents (Merck). The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water to remove the dust particles and then dried under shade for a period of 7 days. The dried plant materials were then ground into fine powders using a laboratory grinding mill. Plant samples were extracted with 800 ml of ethanol, methanol and water separately using a Soxhlet apparatus following hot extraction procedure. Then the extract was filtered using Whatman No.1 filter paper. The filtrates were then dried in hot air oven at 40$^{o}$C. Then the extracts were stored under refrigeration at 4$^{o}$C for further studies.

**Preliminary Phytochemical Screening:** For preliminary phytochemical screening all the extractives were subjected to various tests (**Table 1**) for determination of chemical nature of the extractives [8].

**Test Microorganisms and Preparation of Stock Culture:** The activity of plant extracts was tested on thirteen different organisms: four gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus*) and nine gram negative bacteria (*Salmonella typhi, Vibrio cholerae, Proteus mirabilis, Escherichia coli, Salmonella typhi, Serratia spp., Erwinia spp., Pseudomonas spp., Salmonella spp., Shigella boydii*) were kindly provided by the Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh and reconfirmed by gram staining and sub culturing in appropriate selective media.

**Preparation of Standard Culture Inoculum of Test Microorganisms:** Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

**Antimicrobial Assay:** Agar disc diffusion is a widely accepted in vitro investigation method for preliminary screening of test microorganisms [9]. The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. And they were allowed to cool under laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good

diffusion and then transferred to an incubator at 37°C for 24 hours. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

**Cytotoxic Assay:** The experiment was carried out using the method described by Meyer *et al* [10]. In brief, *Artemia salina* Leach (brine shrimp eggs) was allowed to hatch and matured as nauplii (Larvae) in seawater for 48 hrs at $25^0$C. Serially diluted test solutions were added to the seawater, containing 10 nauplii. After incubation for 24 h at $25^o$C, the number of survivors was counted. Vincristine sulfate was used as positive control.

**Statistical Analysis:** The $LC_{50}$ (50% lethal concentration, µg/ml) of different plant extracts were determined from duplicate experiments. $LC_{90}$, and other statistics at 95 percent fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated by using LDP Line software (trial version).

**RESULT AND DISCUSSION:** The crude extractives when tested with various chemical reagents demonstrated the presence of alkaloids, glycosides, glucosides, saponins, tannins, steroids, carbohydrates and flavonoids shown in **Table 1**.

The effects of antimicrobial screening of different extracts of *A. bilimbi* have been presented in **table 2**. The crude extracts of plant showed moderate antibacterial activity. All the plant extracts showed dose dependent inhibition. Among the extracts, Water extract showed maximum efficacy against most of the bacteria and satisfactory inhibition against Gram-positive bacteria selected. The standard, cefadroxil exhibited significant zone of inhibition against all the test organisms. Among them, Gram-positive bacteria were inhibited more than Gram-negaitive bacteria by cefadroxil, *B. subtilis* being the most.

Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics [11]. The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes [12]. Water extract of *A. bilimbi* has been shown superior antibacterial activity than other extracts. This may be due to the presence of alkaloid, steroid and some polyphenolic compounds present in this extract.

**TABLE 1: PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS OF LEAVES OF *A. BILIMBI.***

| Compounds | Observation of various extracts | | |
|---|---|---|---|
| | Ethanol | Methanol | Water |
| Alkaloid | + | + | + |
| Saponin | + | + | + |
| Tannin | + | + | - |
| Flavonoid | + | + | + |
| Steroid | + | + | + |
| Carbohydrate | + | + | + |
| Glycoside | + | + | + |

(+) = Present; (-) = absent

**TABLE 2: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS A. BILIMBI. AT DIFFERENT CONCENTRATION AND STANDARD AT 10mg/disc**

| Test Microorganism | Ethanolic Extract | | | Methanolic Extract | | | Water Extract | | | Cefadroxil (10mg/disc) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2mg | 4mg | 6mg | 2mg | 4mg | 6mg | 2mg | 4mg | 6mg | |
| | Diameter of Zone of Inhibition (mm) | | | | | | | | | |
| *B. subtilis* | 8 | 8.5 | 9 | 7 | 9 | 10 | - | - | 8 | 32.5 |
| *B. cereus* | - | - | - | - | 8 | 12 | 7 | 10 | 12 | 19 |
| *B. megaterium* | - | - | - | - | - | - | - | - | - | 13 |
| *S. typhi* | 8 | 9.5 | 14.5 | - | - | - | 9.5 | 10 | 10.5 | 10 |
| *V. cholerae* | - | - | - | - | 7.5 | 10 | 7 | 9 | 10.5 | 10 |
| *P. mirabilis* | - | - | 7 | - | - | 8 | 7 | 9 | 12 | 11 |
| *E. coli* | - | - | 10 | 6 | 7 | 8 | 7 | 8.5 | 11 | 10 |
| *S. aureus* | - | - | - | - | - | - | - | - | - | 20.5 |
| *Serratia spp.* | - | - | - | - | - | - | - | - | 9 | 12 |
| *Erwinia spp.* | 9 | 10 | 10.5 | - | - | 6 | - | - | - | 21.5 |
| *Pseudomonas spp.* | - | - | - | - | 7 | 11 | - | - | - | 17.5 |
| *Salmonella spp.* | - | - | - | - | - | 7 | 9 | 10.5 | 11 | 25 |

In case of Brine shrimp lethality bioassay, Ethanolic extract of leaves (EEL) was found to be the most toxic to Brine Shrimp nauplii, with $LC_{50}$ of 3.7 µg/ml whereas the anticancer drug vincristine sulfate(VS) showed $LC_{50}$ value 1.73 µg/ml. The high toxicity of EEL part probably attributed to the alkaloid that is confirmed in phytochemical screening. The order at which cytotoxic potential of the test samples decreased was as follows:

Vincristine sulphate > EEL > MEL > WEL. All the data found from brine shrimp lethality bioassay and statistical analysis are presents in **Table 3 to Table 10 and Figure 1-4**.

**TABLE 3: PROBIT ANALYSIS FOR ETHANOLIC LEAVES EXTRACTS**

| Conc. (µg/ml) | Log conc. | Treated | Response % | Corrected response % | Linear response % | Linear probit |
|---|---|---|---|---|---|---|
| 1 | 0.0000 | 20 | 35.000 | 31.579 | 31.8624 | 4.5284 |
| 5 | 0.6990 | 20 | 55.000 | 52.632 | 54.4324 | 5.1113 |
| 10 | 1.0000 | 20 | 65.000 | 63.158 | 64.1440 | 5.3624 |
| 20 | 1.3010 | 20 | 75.000 | 73.684 | 73.0190 | 5.6134 |
| 50 | 1.6990 | 20 | 85.000 | 84.211 | 82.7703 | 5.9453 |
| 100 | 2.0000 | 20 | 95.000 | 94.737 | 88.4135 | 6.1963 |
| 200 | 2.3010 | 20 | 95.000 | 94.737 | 92.5971 | 6.4473 |
| 500 | 2.6990 | 20 | 95.000 | 94.737 | 96.2192 | 6.7792 |

Where Conc. = Concentration.

**TABLE -4: $LC_{50}$, $LC_{90}$ CONTAINING UPPER LIMIT AND LOWER LIMITS, CALCULATED AND TABULATED CHI SQUARE ( $\chi^2$) VALUES FOR ETHANOLIC EXTRACTS OF LEAVES**

| $LC_{50}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | $LC_{90}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | Calculated ( $\chi^2$) | Tabulated ( $\chi^2$) |
|---|---|---|---|---|---|---|---|
| 3.6769 | 1.1671 | 7.3713 | 126.5538 | 56.4682 | 558.9732 | 1.4923 | 12.6 |

**TABLE 5: PROBIT ANALYSIS FOR METHANOLIC LEAVES EXTRACTS**

| Conc. (µg/ml) | Log conc. | Treated | Response % | Corrected response % | Linear response % | Linear probit |
|---|---|---|---|---|---|---|
| 1 | 0.0000 | 20 | 25.000 | 21.053 | 22.5616 | 4.2466 |
| 5 | 0.6990 | 20 | 55.000 | 52.632 | 47.0541 | 4.9261 |
| 10 | 1.0000 | 20 | 55.000 | 52.632 | 58.6546 | 5.2187 |
| 20 | 1.3010 | 20 | 75.000 | 73.684 | 69.5389 | 5.5111 |
| 50 | 1.6990 | 20 | 75.000 | 73.684 | 81.54 | 5.8982 |
| 100 | 2.0000 | 20 | 95.000 | 94.737 | 88.3058 | 6.1908 |
| 200 | 2.3010 | 20 | 95.000 | 94.737 | 93.0956 | 6.4834 |
| 500 | 2.6990 | 20 | 95.000 | 94.737 | 96.9189 | 6.8703 |

**TABLE 6: $LC_{50}$, $LC_{90}$ CONTAINING UPPER LIMIT AND LOWER LIMITS, CALCULATED AND TABULATED CHI SQUARE ($\chi^2$) VALUES FOR METHANOLIC LEAVES EXTRACTS**

| $LC_{50}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | $LC_{90}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | Calculated ( $\chi^2$) | Tabulated ( $\chi^2$) |
|---|---|---|---|---|---|---|---|
| 5.9574 | 2.6817 | 10.4723 | 124.0075 | 62.2552 | 390.6818 | 2.7703 | 12.6 |

**TABLE -7: PROBIT ANALYSIS FOR WATER EXTRACTION OF LEAVES**

| Conc. (µg/ml) | Log conc. | Treated | Response % | Corrected response % | Linear response % | Linear probit |
|---|---|---|---|---|---|---|
| 1 | 0.0000 | 20 | 15.000 | 10.526 | 3.29531 | 3.1576 |
| 5 | 0.6990 | 20 | 25.000 | 21.053 | 16.7397 | 4.0353 |
| 10 | 1.0000 | 20 | 25.000 | 21.053 | 27.8683 | 4.4133 |
| 20 | 1.3010 | 20 | 35.000 | 31.579 | 41.7304 | 4.7912 |
| 50 | 1.6990 | 20 | 35.000 | 31.579 | 61.4442 | 5.2909 |
| 100 | 2.0000 | 20 | 95.000 | 94.737 | 74.8198 | 5.6689 |
| 200 | 2.3010 | 20 | 95.000 | 94.737 | 85.2375 | 6.0468 |
| 500 | 2.6990 | 20 | 95.000 | 94.737 | 93.8958 | 6.5466 |

**TABLE 8: $LC_{50}$, $LC_{90}$ CONTAINING UPPER LIMIT AND LOWER LIMITS, CALCULATED AND TABULATED CHI SQUARE ($\chi^2$) VALUES FOR WATER EXTRACTS OF LEAVES**

| $LC_{50}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | $LC_{90}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | Calculated ($\chi^2$) | Tabulated ($\chi^2$) |
|---|---|---|---|---|---|---|---|
| 29.3285 | 9.8749 | 79.4404 | 307.5891 | 185.4408 | 3569.4549 | 17.0312 | 12.6 |

**TABLE -9: PROBIT ANALYSIS FOR VRINCHRINSTIN SULFATE.**

| Conc. (µg/ml) | Log conc. | Treated | Response % | Corrected response % | Linear response % | Linear probit |
|---|---|---|---|---|---|---|
| 0.06 | 0.7782 | 20 | 10.000 | 5.263 | 3.53089 | 3.1883 |
| 0.125 | 1.0969 | 20 | 15.000 | 10.526 | 7.83917 | 3.5835 |
| 0.25 | 1.3979 | 20 | 15.000 | 10.526 | 14.8460 | 3.9568 |
| 0.5 | 1.6990 | 20 | 25.000 | 21.053 | 25.1528 | 4.3303 |
| 1.0 | 2.0000 | 20 | 45.000 | 42.105 | 38.3465 | 4.7036 |
| 5.0 | 2.6990 | 20 | 65.000 | 63.158 | 71.5816 | 5.5705 |
| 12.5 | 3.0969 | 20 | 95.000 | 94.737 | 85.6282 | 6.0640 |
| 25.0 | 3.3979 | 20 | 95.000 | 94.737 | 92.4553 | 6.4373 |

**TABLE -10: $LC_{50}$, $LC_{90}$ CONTAINING UPPER LIMIT AND LOWER LIMITS, CALCULATED AND TABULATED CHI SQUARE ($\chi^2$) VALUES FOR VS**

| $LC_{50}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | $LC_{90}$ (µg/ml) | Lower limit (µg/ml) | Upper limit (µg/ml) | Calculated ($\chi^2$) | Tabulated ($\chi^2$) |
|---|---|---|---|---|---|---|---|
| 1.7338 | 1.1013 | 2.8621 | 18.7225 | 9.396 | 54.7015 | 3.3479 | 12.6 |

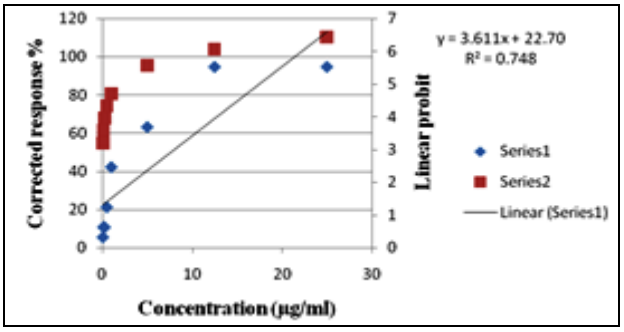


**FIG-1: CYTOTOTOXICITY EFFECT OF VS**

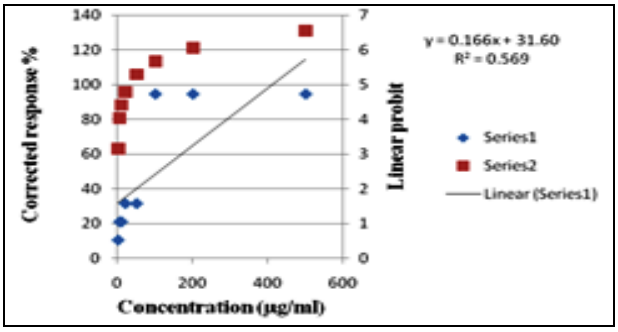


**FIG-4: CYTOTOTOXICITY EFFECT OF WEL**

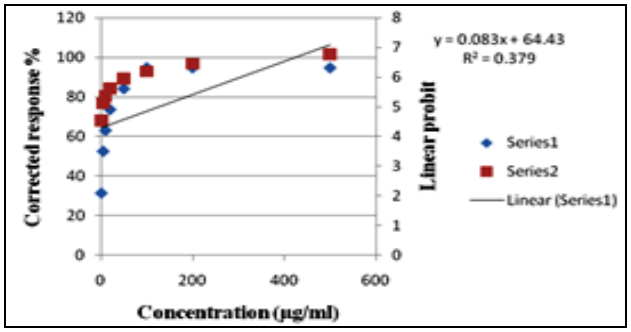


**FIG-2: CYTOTOTOXICITY EFFECT OF EEL**

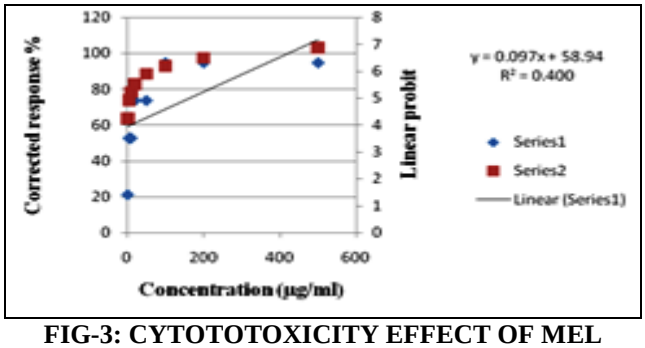


**FIG-3: CYTOTOTOXICITY EFFECT OF MEL**

From the above data, it is clear to us that as the polarity of solvent of extraction increases, brine shrimp toxicity decreases. This implies that most of the potent cytotoxic compounds in this plant have affinity for non- polar solvents and are more effectively extracted by these solvents. During toxicity evaluation of plant extracts by BSLB, *Meyer et al., 1982* described that $LC_{50}$ values lower than 1000 µg/ml are considered bioactive[10]. Concentration dependent increment in percent mortality of brine shrimp nauplii produced by the *A. bilimbi* indicates the presence of cytotoxic principles in these extractives. Preliminary phytochemical screening revealed the presence of alkaloids and steroids. So the observed cytotoxic action may be due to the presence of such compounds. There may be correlation between BSLB and antibacterial properties that could be deduced in this study..

**CONCLUSION:** In conclusion, the extracts of *A. bilimbi* have moderate & potent actions as antibacterial & cytotoxic agents. This finding provides an insight into the usage of the plant in traditional treatment of antibacterial, antiscorbutic, astringent, post–partumprotective medicine, itches, boils, syphilis, bilious colic, whooping cough, stomach ache, aphthous ulcer and other diseases associated with bacterial infection which could be of considerable interest to the development of new drugs through the isolation of active antibacterial & cytotoxic principles from the leaves of *A. bilimbi.*

**ACKNOWLEDGEMENTS**: The authors would like to thank Kalyan Kumar Biswas, Jahangirnagar University, Savar, Dhaka, Bangladesh for technical support throughout the study.

## REFERENCES:

1. Sen A and Batra A: Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia Azedarch* L. International journal of current pharmaceutical journal 2012; 4(2): 67-73.
2. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM and Lavekar GS: Antimicrobial activity of some Indian medicinal plants. African Journal of Traditional, Complementary, and Alternative Medicines 2007; 4 (3): 313 – 318.
3. McLaughlin JL: Crown gall tumors on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. Methods in Plant Biochemistry 1991; 6: 1-32**.**
4. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S and Verma RS: Antibacterial activity of the plant used in Indian herbal medicine. International Journal of Green Pharmacy 2010; 10: 22-28.
5. Warrier PK, Nambiar VPK and Ramankutty C. Indian medicinal plants, a compendium of 500 species. Orient Longman Ltd. Hyderabad, India, Vol. 5, 1995: 10-12.
6. Roy A, Geetha RV and Lakshmi T: Averrhoa bilimbi Linn–Nature's Drug Store; A Pharmacological Review. International Journal of Drug Development and Research 2011; 3 (3): 101-106.
7. Jais AMM, Henie EFP, Zainuddin ENHE, Zaiton H and Zakaria ZA: In vitro antibacterial activity of *Averrhoa bilimbi* L. leaf and fruits extracts. International Journal of Tropical Medicine 2007; 2: 96-100.
8. Ghani A. "Medicinal Plants of Bangladesh". 2nd Ed. The Asiatic Society of Bangladesh. Dhaka, Bangladesh. 2003: 31, 39-40, 418, 500-504, 589-580.
9. Bayer AW, Kirby WMM, Sherris JC and Turck M: Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology1966; 45: 493-496.
10. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL: Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-4.
11. Haslam E: Natural polyphenols (vegetable tannins) as drugs: possible mode of action. Journal of Natural Products 1996; 5: 205-215.
12. Scalbert A: Antimicrobial properties of tannins. Phytochemistry: 1991; 30: 3875-3883.

**How to cite this article**:
Aziz MA, Rahman S, Islam M and Begum AA: Comparative antibacterial screening and cytotoxic potential of various extracts of leaves of *Averrhoa bilimbi*. *Int J Pharm Sci Res* 2014; 5(3): 913-18.doi: 10.13040/IJPSR.0975-8232.5(3).913-18