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# EVALUATION OF THE ANTIBACTERIAL, CYTOTOXIC AND INSECTICIDAL ACTIVITIES OF *HIBISCUS SABDARIFFA* BARKS

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**ABSTRACT**

The present study was designed to investigate the antibacterial, cytotoxic and insecticidal activities of the methanol (85%) extract of barks of *Hibiscus sabdariffa (MEHS).* Antibacterial tests were done against six Gram-positive and eight Gram-negative bacteria using disc diffusion method. The extract showed the highest activity against *Escherichia coli* (14± 0.21 mm) followed by *Shigella dysenteriae* (13± 0.09), *Sarcina lutea* (13± 0.13mm), *Shigella boydii (*12± 0.12mm) and *Staphylococcus aureus (*12± 0.12mm) whereas inactive against *Bacillus subtilis, B. megaterium, B. anthracis* and *B. cereus.* The cytotoxic activities of crude extract was determined using Brine Shrimp lethality Bioassay and $LC_{50}$ values of standard Vincristine sulphate as a positive control and the crude extract were found to be 0.21±0.19µg/ml and 9.605±0.21µg/ml respectively. In insecticidal study, MEHS showed the significant activity with 100% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml with 12 hours and also showed the activity in a dose dependent manner.

**Key words**: *Hibiscus sabdariffa*, Disc diffusion, Brine Shrimp lethality, Insecticidal activity.

## INTRODUCTION

Globalization interferes with infectious disease control at the national level while microbes move freely around the world, unrestricted by borders, human responses to infectious diseases and are conditioned by jurisdictional boundaries [1]. According to WHO, important progress has been made in controlling major infectious diseases. About 43% of total deaths occurred in developing countries due to infectious diseases in recent years [2]. Bangladesh, being a country with high density of population, infectious diseases becomes a great challenge in the health and economic sector. Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This results in the need of higher dose use with increased risk of drug toxicity or consideration to change the regimen. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation force scientists to search for new antimicrobial substances. Similarly, freedom from insect invasion and contamination has become an important consideration in storage of grain and to maintain high quality food product by preventing them from attack of the most frequently invading organisms [3]. Nearly one thousand species of insects have been associated with store products throughout the world. *Tribolium castaneum* (Herbst) is considered to be a major pest of stored grains. In Bangladesh *Tribolium castaneum* is abundantly found in stored grains of different cereals. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problem such as disturbances of the environment, increasing cost of application, pest resurgence, resistant to pesticides and lethal effects on non-target organism in addition to direct toxicity to users [4]. So there is an immediate need to develop safe alternatives with low cost, easy to use and friendly to the environment. Various medicinal plants and plant products have been used for years in daily life to treat diseases all over the world. Medicinal plants are important source of traditional and synthetic medicines containing different types of organic compounds having therapeutic properties. In Bangladesh thousands of species

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are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [5]. Hibiscus is one of the most common flower plants grown worldwide. There are more than 300 species of hibiscus around the world. One of them is *Hibiscus sabdariffa* Linn which is a member of the plant family, Malvaceae. It is commonly referred to in English as 'Roselle', and in Bangladesh, it is known as Chukair or mesta [6]. The plant is mainly cultivated for its leaves, stem barks, seeds and calyces [7]. *H. sabdariffa* has been reported to possess biological activities like antihypertensive, anticancer, antihyperlipidemic, antioxidant, anticonvulsant, anxiogenic, analgesic, CNS-depressant, serotoninergic activities, reducing oxidative liver damage, anti-inflammatory, antimicrobial and hypoglycemic activity [8, 9, 10, 11]. *H. sabdariffa* fruit has been used in medicine for its antiinflammatory, antioxidant and anticholesteromia properties [12, 13]. It also reported that *H. sabdariffa* extracts inhibit cytotoxicity and genotoxicity of hepatocytes as well as inhibit xanthine oxidase activity. Increased consumption of *Hibiscus sabdariffa* is thought to prevent certain degenerative diseases. The physico-chemical characteristics revealed that Roselle are health protective foods containing a number of essential nutrients such as vitamin A, vitamin C, minerals, carotene and dietary fiber [14]. Based on these reports our studies have been designed to examine the antibacterial, cytotoxicity and insecticidal activities of methanol extract of *H. sabdariffa* barks as there are no scientific reports of barks.

## MATERIALS AND METHODS

### Plant Material

The barks of *H. sabdariffa*, were collected from Gazipur, Dhaka, Bangladesh and were identified by the experts of Bangladesh National Herbarium, Dhaka, where voucher (verifier) specimen have been kept for further reference. The collected plant parts were dried under shade and pulverized (fine-grained) into coarse powders with the help of a suitable grinder. The powders were stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

### Preparation of the Extract

About 120gm of powdered material of each individual plant was taken in a clean, flat bottomed glass container and soaked in 200ml of 85% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. The semi-solid mass of each individual extract was stored in a closed container for further use and protection.

### Screening of Antibacterial Activity

The antibacterial activity of the plant extract was performed by disc diffusion technique [15] which is highly effective for rapidly growing microorganisms. All the microorganisms used in this study were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in respective solvent (1ml) to attain a concentration of 500µg/disc. The test microorganisms were inoculated into respective medium by spread plate method with 24h cultured bacteria, grown in nutrient agar medium. After solidification the filter paper disc (5mm diameter) impregnated with sample, the standard antibiotic (Kanamycin-30µg/disc) as positive and as negative controls, a blank disc impregnated with 10µl respective solvent was used.

The MEHS was tested against six Gram- positive and eight Gram negative (*Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Bacillus anthracis, Staphylococcus aureus, Sarcina lutea, Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* & *Shigella boydii*) bacteria. The arrangement of discs was such that they were not closer than 15mm to the edge of the plate to prevent overlapping the zone of inhibition. Then the plates were left in a refrigerator at 4°C for 12-18hrs in order to diffuse the material from the discs to the surrounds media in the Petri dishes. The Petri dishes were then incubated at 37°C for 24 hrs to allow the bacterial growth. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm. After incubation the antibacterial activities of the test material were determined by measuring the respective diameter of the zone of inhibition in terms of millimeters (mm) with a transparent scale and the experiment was done in triplicate.

### Screening of Cytotoxicity Activity

The Cytotoxic activity of the plant extract was evaluated using Brine Shrimp lethality bioassay method [16]. Here simple zoological organism (*Artemia salina*) was used as an expedient monitor for the screening. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48hr to mature shrimp called nauplii. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50µl in 5ml solution) plus sea water (3.8% NaCl in water) to attain concentrations – 2.5µg/ml, 5µg/ml, 10 µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80 µg/ml, 100 µg/ml, and 120µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Then matured shrimps were applied to each of all experimental vials and control vial. The number of the nauplii that died after 24 hr was counted. The median lethal concentration $LC_{50}$ of the test sample after 24 h was obtained by a plot of percentage of the dead shrimps against the logarithm of the sample concentration. Vincristine sulphate was used as a reference standard in this case.

## SCREENING OF INSECTICIDAL ACTIVITY

### Collection of Test Insects

The present experiment was carried out using insects *Tribolium castaneum* which were collected from

the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh.

**Insecticidal Assay**

To conduct surface film activity test 60 mm petri dishes were taken for the extracts and their replication. The sample solutions were prepared (2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml and 50 mg/ml) by dissolving the extract into respective solvent. Then they were poured into the lower part of the petri dish and allowed them to dry out. Then insects were released in each of the treated petri dish. A control experiment applying only the solvent into the petri dish, was also set at the same time under the same conditions [17]. After completing all the arrangements, treated petri dishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed first after 30 minutes from the start and then after 1, 2, 4, 8, 12 and 48 hrs of exposure and the data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recover the insects if occurred. The mortality records of the *Tribolium castaneum* adults were corrected by the Abbott's formula [18].

$P_r = (P_o - P_c \setminus 100 - P_c) \times 100$

$P_r$ = Corrected mortality%; $P_o$ = Observed mortality%; $P_c$ = Control mortality%, sometimes called natural mortality%.

**Statistical Analysis**

All assays were performed in triplicates 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.

**RESULT AND DISCUSSION**

Table 1 shows the antibacterial activity (zone of inhibitions) of the MEHS barks. The extract at a dose of 500µg/disc showed moderate antibacterial activity against *Salmonella paratyphi, Pseudomonas aeruginosa*, *Vibrio mimicus* and *Vibrio parahemolyticus* with the zone of inhibition range 8± 0.15 to 10± 0.23 mm. The highest zone of inhibition was found against *Escherichia coli* (14± 0.21 mm) followed by *Shigella dysenteriae* (13± 0.09), *Sarcina lutea* (13± 0.13mm), *Shigella boydii (*12± 0.12mm) and *Staphylococcus aureus (*12± 0.12mm) where as the lowest activity was shown against *Salmonella typhi* (7± 0.11mm). On the other hand MEHS barks showed no activity against *Bacillus subtilis*, *B. megaterium*, *B. anthracis*, and *B. cereus.*

Plant extracts having the compounds tannins [19, 20], flavonoids [21, 22], saponins [23, 24], terpenoids [25], alkaloids [26, 27] have been documented for antimicrobial activities. Previous Phytochemical studies of this plant revealed that cardiac glycosides, alkaloids, saponins, flavonoids [28] as well as steroids [29] are the main chemical constituents of *Hibiscus sabdariffa*. So, the antimicrobial activity showed by the extract of *H. sabdariffa* may be due to presence of such type of phytoconstituents. Antibacterial effects of this plant extracts against *E. coli*, *Pseudomonas aeruginosa Shigella dysenteriae* and *S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhoea in man and skin diseases [28].

The result of Brine Shrimp lethality bioassay is given in Table 2. The MEHS barks were found to be potent against brine shrimps with $LC_{50}$ value of 9.605±0.21µg/ml. The Brine Shrimps lethality was found to be concentration-dependent.

The brine shrimp lethality assay (BSL) has been used extensively in the primary screening of the crude extracts as well as the isolated compounds to evaluate the toxicity towards brine shrimps, which could also provide an indication of possible cytotoxic properties of the test materials [16]. It has been established that the cytotoxic compounds generally exhibit significant activity in the BSL assay, and this assay can be recommended as a guide for the detection of antitumor and pesticidal compounds because of its simplicity and low cost [30]. Earlier reports in several plant extracts showed a good correlation of this bioassay with the cytotoxic activity [30]. The assay also exhibited a good correlation with cytotoxicity in cell lines such as 9KB, P388, L5178Y and L1210 [31, 32]. In the present study the extract of MEHS displayed considerable general toxicity & different mortality rate towards shrimp nauplii (Table 2).

The mortality rate of nauplii was found to be increased in concentration of each of the samples. The inhibitory effect of the extract might be due to the toxic compounds present in the extract such as saponins, alkaloids and cardiac glycosides that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs [33]. So the cytotoxic effects of the plant extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extract [34]. The brine shrimps lethality further supports the antibacterial activities of *H. sabdariffa* on some pathogenic organisms observed in this study.

For insecticidal activity MEHS barks have shown 100% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml in 12 hours. Since the extract have strong insecticidal activity, dose dependent activity was done, where 6 graded doses (viz. 50, 40, 20, 10, 5 and 2.5 mg/ml) were used and the percentage of mortality were 100, 98.53, 92.66, 80.53, 53.66, and 38.33% at the dose of 50, 40, 20, 10, 5 and 2.5mg/ml, respectively (Fig 1).

It is noteworthy that carbohydrates, saponins, phytosterol, phenol, flavonoids and tannins have possessed larvicidal activity [35]. Therefore, saponins, flavonoids, cardiac glycosides as well as other secondary metabolites of this investigated plant may explain the toxic effect in the studied insects.

**Table 1. *In vitro* antimicrobial activity of MEHS barks**

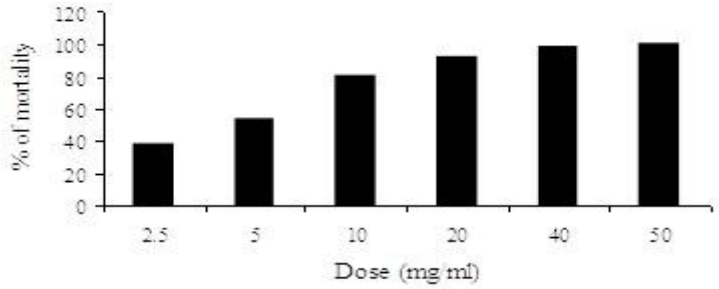
| | Diameter of zone of inhibition (mm) | |
|---|---|---|
| **Bacterial Strain** | **[b]Std. (30µg/disc)** | **[a]MEHS (500µg/disc)** |
| **Gram positive** | | |
| *Bacillus megaterium* | 30 ± 0.12 | NA |
| *Bacillus subtilis* | 23 ± 0.02 | NA |
| *Staphylococcus aureus* | 26 ± 0.10 | 12± 0.12 |
| *Sarcina lutea* | 24 ± 0.13 | 13± 0.13 |
| *Bacillus anthracis* | 23± 0.23 | NA |
| *Bacillus cereus* | 24 ± 0.03 | NA |
| **Gram negative** | | |
| *Escherichia coli* | 22 ± 0.22 | 14± 0.21 |
| *Pseudomonas aeruginosa* | 25 ± 0.13 | 9± 0.21 |
| *Salmonella paratyphi* | 25 ± 0.02 | 8± 0.15 |
| *Salmonella typhi* | 25 ± 0.15 | 7± 0.11 |
| *Shigella boydii* | 25 ± 0.17 | 12± 0.12 |
| *Shigella dysenteriae* | 25 ± 0.11 | 13± ± 0.09 |
| *Vibrio mimicus* | 28 ± 0.02 | 10± 0.14 |
| *Vibrio parahemolyticus* | 26 ± 0.19 | 10± 0.23 |

[a]Values of the observed diameter inhibition zone (mm) excluding cap diameter. Incubation conditions for bacteria – 24 hours at 37°C. Assay was performed in triplicate and results are the mean of three values±Standard Deviation. [b]Reference standard; anamycin. NA- Zone of inhibition ≤ 5 mm is considered as no activity.

**Table 2. Brine Shrimp Lethality Bioassay of MEHS barks**

| Test Sample | Concentration C (µg/ml) | log C | No. of dead Shrimps (out of 10) | % of mortality | $LC_{50}$ of test sample (µg/ml) | $LC_{50}$ of vincristine sulphate (µg/ml) |
|---|---|---|---|---|---|---|
| MEHS | 2.5 | 0.398 | 3 | 30 | 9.605±0.21 | 0.21±0.19 |
| | 5 | 0.699 | 4 | 40 | | |
| | 10 | 1 | 5 | 50 | | |
| | 20 | 1.301 | 6 | 60 | | |
| | 40 | 1.602 | 7 | 70 | | |
| | 60 | 1.778 | 8 | 80 | | |
| | 80 | 1.903 | 8 | 80 | | |
| | 100 | 2 | 9 | 90 | | |
| | 120 | 2.079 | 10 | 100 | | |

**Fig 1. Insecticidal effects of MEHS barks on *Tribolium castaneum***




## CONCLUSION

In conclusion, the results of the present study are in agreement with the previous study and indicated that the extract exhibited more cytotoxic, insecticidal and antimicrobial properties than previous study. As a result of strong cytotoxicity, insecticidal and antibacterial activity, MEHS barks would be considered as anticancer, insecticidal and antibacterial agent but further studies will be carried out for toxic and safe dose of the extract.

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**REFERENCES**


1. Stepanovic S, Antic N, Dakic I, Svabic VM. *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microb. Res.*, 158(4), 2003, 353-357.
2. Carballo LJ, Hernandez-inda LZ, Perzer P, Gravalos MD. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *Biol. Med. Central.,* 2(1), 2002, 17.
3. Coolins PJ. Resistance to grain protectants and fumigants in insect pests of stored products in Australia. Presented in Australian Postharvest Technical conference, 1998, 55-57.
4. Islam MS, Zahan R, Nahar L, Alam MB, Naznin M, Sarkar GC, Mosaddik MA and Haque ME. Antibacterial, Insecticidal and *In Vivo* Cytotoxicity activities of *Salix Tetrasperma. IJPSR*, 2(8), 2011, 2103-2108.
5. Joshi B, Lekhak S and Sharma A. Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana. Kathmandu University Journal of Science, Engineering and Technology*, 5, 2009, 143-150.
6. Ghani A. Medicinal Plants of Bangladesh: Chemical constituents and uses; Asiatic Society of Bangladesh, 1998, 84-85.
7. Umerchuruba CI. An annotated list of plant diseases in Nigeria. Pen and Paper Publication, Owerri, Nigeria, 1997.
8. Khatun MH, Islam MR, Mamun A, Nahar L, Luthfunnesa, Islam MAU. In Vivo Evaluation of CNS Depressant and Antinociceptive Activities of Methanol Extract of *Hibiscus sabdariffa* Fruits. *J. Appl. Sci. Res.*, 7(6), 2011, 798-804.
9. Mahadevan N, Shivali and Kamboj P. *Hibiscus sabdariffa* L.-An Overview. *Natural Product Radiance*, 8(1), 2009, 77-83.
10. Chen CC, Hsu JD, Wang SF, Chiang HC, Yang MY, Kao ES, Ho YC and Wang CJ. *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterolfed rabbits. *J. Agri. Food Chem.*, 51, 2003, 5472-5477.
11. Gosain S, Ircchiaya R, Sharma PC, Thareja S, Kalra A, Deep A and Bhardwaj TR. Hypolipidemic effect of ethanolic extract from the leaves Of *Hibiscus sabdariffa* L. in hyperlipidemic rats. Acta Poloniae Pharmaceutica-Drug Research, 67(2), 2010, 179-184.
12. El-saadany SS, and Sitohy MZ. Biochemical dynamics and hypocholesteremic action of Hibiscus sabdariffa (Karteade). *Nahrung*, 35, 1991, 567–576.
13. Tseng TH, Kae ES and Chu CY. Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. *Food Chem Toxicol*., 35, 1997, 1159–1169.
14. Dignan CA, Burlingame BA, Arthur JM, Quiley RJ and Milligan GM. The Pacific Islands Food Composition Tables. South Pacific Commission, Noumea, New Caledonia, New Zealand, 1994, 1-4.
15. Rios JL, Recio MC and Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. *Journal of Ethnopharmacol*, 23, 1988, 127- 149.
16. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE and Mclaughlin JL. "Brine shrimp; a convenient general bioassay for active plant constituents. *Planta Medica*, 45, 1982, 31-34.
17. Bousquet Y. Beetles associated with stored products in Canada. Canadian Government Publishing Centre, Ottawa, 1990, 189-192.
18. Abott WS. A method of computing effectiveness of an insecticide. *J. Econ. Entomol.*, 18, 1925, 265-267
19. Doss A, Mubarack HM and Dhanabalam R. Pharmacological importance of *Solanum trilobatum*. *Ind. J. Sci. Tech*., 2(2), 2009, 41-43.
20. Ho KY, Tasi CC, Huang JS, Chen CP, Lin TC and Lin CC. Antimicrobial activity of tannin components from *Vaccinium vitis- idaea* L. *J. Pharm. Pharmacol.*, 53(2), 2001, 187-191.
21. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ and Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a by product of the essential oil industry. *J. App. Micro.*, 103(6), 2007, 2056-2064.
22. Pepeljnjak S, Kalodera Z and Zovko M. Antimicrobial activity of flavonoids from *Pelergonium radula* (Cav.) L' Herit. *Acta Pharma.*, 55, 2005, 431-435.
23. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bially Z and Jurzysta M. Antimicrobial activity of saponins from *Medicago sp*.: structure activity relationship. *Phytother. Res.*, 20(6), 2006, 454-457.
24. Baharaminejad S, Asenstorfer RE, Riley IT and Schultz CJ. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of Oats (*Avena sativa* L.). *J. Phytopathol*., 156(1), 2007, 1-7.
25. Fnatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H and Hirari Y. Antimicrobial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori. Microbiol. Immunol.*, 48(4), 2004, 251-261.
26. Navarro V and Delgado G. Two antimicrobial alkaloids from *Bocconia arborea*. *J. Ethnopharmacol.*, 66(2), 1999, 223-226.
27. Faizi S and Ahmed RAT. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. *pendula. Planta Med.*, 69(4), 2003, 350-355.
28. Olaleye and Tolulope M. Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus sabdariffa. J. Med. Plant Res*, 1(1), 2007, 009-013.
29. Mungole A and Chaturvedi A. *Hibiscus sabdariffa* L a rich source of secondary metabolites. *International Journal of Pharmaceutical Sciences Review and Research*, 6(1), 2011, 83-87.
30. Mazid M, Datta BK, Nahar L, Sarker SD. Assessment of anti-bacterial activity and brine shrimp toxicity of two Polygonum species. *Ars Pharm*, 49(2), 2008, 127-134.

31. Krishnaraju AV, Tayi Rao VN, Sundararaju D, Vanisree M, Tsay HS and Subbaraju GV. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). *International Journal of Applied Science and Engineering*, 2, 2006, 115-25.
32. De Rosa S, De Giulio A, Iodice C. Biological effects of prenylated hydroquinones: structure–activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. *Journal of Natural Products*, 57, 1994, 1711-1716.
33. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. *Drug Information Journal*, 32, 1998, 513-524.
34. Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C. Cytotoxic Potentials of Red Alga, Laurencia brandenii Collected from the Indian Coast. *Global Journal of Pharmacology*, 3(2), 2009, 90-94.
35. Haque M, Ullah MO, Nahar K. *In vitro* antibacterial and cytotoxic activities of different parts of plant *Swietenia mahagony*. *Pakistan Journal of Biological Sciences*, 12(7), 2009, 599-602.