Libyan Agriculture Research Center Journal International 2 (3): 144-149, 2011
ISSN 2219-4304

In vitro Evaluation of the Antibacterial, Cytotoxic and Insecticidal Activities of *Hibiscus sabdariffa* Fruits

[1]Al-Mamun, [2]Mst. Hajera Khatun, [3]Mst. Luthfun Nesa,
[4]Md. Rafikul Islam and [3]Mst. Shirajum Munira

[1]Department of Pharmacy, Jessore Science and Technology University, Jessore, Bangladesh
[2]Department of Pharmacy, Southeast University, Dhaka, Bangladesh
[3]Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh
[4]Department of Pharmacy, International Islamic University Chittagong, Bangladesh

Abstract: The present study was designed to investigate the antibacterial, cytotoxic and insecticidal activities of the methanol (85%) extract of fruits 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 *Sarcina lutea* with a zone of inhibition 13±0.21mm followed by *Shigella dysenteriae* (12±0.07mm), *Escherichia coli* (12±0.11mm) and *Shigella boydii (*12±0.13mm) and inactivity against *Bacillus subtilis, B. megaterium, B. anthracis, B. cereus* and *P. aeruginosa.* 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 was found to be 0.21±0.19µg/ml and 5.082±0.12µg/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 the activity was a dose dependent manner.

Key words: *Hibiscus sabdariffa* % Disc diffusion % Brine Shrimp Lethality % Insecticidal Activity

INTRODUCTION

Globalization interferes with infectious diseases 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]. 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 become 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 [3]. 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 grains and to maintain high quality food products by preventing them from attack of the most frequently invading organisms [4]. 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 problems 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 [5]. 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

Corresponding Author: Mst. Hajera Khatun, Lecturer, Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh. Phone: +88-01722502355.

traditional and synthetic medicines containing different types of organic compounds having therapeutic properties. In Bangladesh thousands of species 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 [6].

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 [7]. The plant is thought of native to tropical central or West Africa but well cultivated in tropical Asia, central America, northern Australia and many other tropical and sub-tropicat countries. It is mainly cultivated for its leaves, stem, seed and calyces [8]. Roselle is an herbaceous, annual or bi-annual plant, which grows up to 5 meters tall. The stem is robust; the leaves are dark green, oval, alternate, simple or divided into 3 lobes; the flowers grow solitary and axillary and are usually red. *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 [9-12]. *H. sabdariffa* fruit has been used in medicine for its anti-inflammatory, antioxidant and anticholesteremic properties [13, 14]. Tseng *et al*. [14] 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 fruits are healths and protective foods containing a number of essential nutrients such as vitamin A, vitamin C, minerals, carotene and dietary fibers [15]. Based on these reports our studies have been designed to examine the cytotoxicity and insecticidal activity of methanol extract of *H. sabdariffa* fruits.

MATERIALS AND METHODS

Plant Materials: The fruits of *H. sabdariffa* were collected from Mirpur, Dhaka, Bangladesh and identified by the experts of Bangladesh National Herbarium, Dhaka, where voucher specimen have been kept for further reference. The collected plant parts were dried under shade and pulverized 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 120 gm of powdered material of each individual plant was taken in a clean, flat bottomed glass container and soaked in 200 ml 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 Whatmann filter paper (Bibby RE200, Sterilin Ltd. UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. The semi-solid mass of the extract (MEHS) 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 [16] 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 the respective solvent (1 ml) to attain a concentration of 500µg/disc. The test microorganisms were inoculated onto the respective medium by pour plate method with 24 hrs cultured bacteria, grown in a nutrient agar medium. After solidification the filter paper disc (5 mm diameter) impregnated with sample. Standard antibiotic (Kanamycin-30µg/disc) and a blank disc impregnated with 10µl respective solvent were included as positive and negative controls.

The MEHS was tested against six Gram- positive (*Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Bacillus anthracis, Staphylococcus aureus, Sarcina lutea*) and eight Gram negative (*Salmonella paratyphi, Salmonella typhi, Vibrio parahaemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Shigella boydii*) bacteria. The inoculated plates were incubated at 37°C for 24 hrs. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm. The experiment was done in triplicate.

Screening of Cytotoxic Activity: The cytotoxic activity of the plant extract was evaluated using Brine Shrimp lethality bioassay method [17]. 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 48 hrs to mature shrimp called nauplii. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl DMSO in 5 ml solution to avoid toxicity of itself) plus sea water (3.8% NaCl in water) to attain concentrations - 2.5, 5, 10, 20, 40, 60, 80, 100 and 120 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml of what 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 hrs was counted. The median lethal concentration LC_{50} of the test sample after 24 hrs 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, 5, 10, 20, 40 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 [18]. 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. The mortality records of the *Tribolium castaneum* adults were corrected by the Abbott's formula [19]

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

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 fruits. The extract at a dose of 500µg/disc showed moderate antibacterial activity against *Salmonella typhi, Vibrio mimicus, Vibrio parahaemolyticus and Staphylococcus aureus* with the zones of inhibition range 8±0.21 to 11±0.04 mm. The highest zone of inhibition was found against *Sarcina lutea* (13± 0.21mm) followed by *Shigella dysenteriae* (12±0.07mm), *Escherichia coli* (12±0.11mm) and *Shigella boydii (*12±0.13mm) where as the lowest activity was shown against *Salmonella paratyphi* (7±0.10mm). On the other hand MEHS fruits showed no activity against *Bacillus subtilis, B. megaterium, B. anthracis, B. cereus* and *P. aeruginosa.*

Plant extracts having the compounds tannins [20, 21], flavonoids [22, 23], saponins [24, 25], terpenoids [26] and alkaloids [27, 28] have been documented for antimicrobial activities. Previous Phytochemical studies of this plant revealed that cardiac glycosides, alkaloids, saponins, flavonoids [29] as well as steroids [30] are the main chemical constituents of fruits of *Hibiscus sabdariffa*. So, the high antimicrobial activity showed by the extract of *H. sabdariffa* may be due to presence of such type of phyto constituents.

The results of Brine Shrimp lethality bioassay are given in Table 2. The MEHS fruits were found to be potent against brine shrimps with LC_{50} value of 5.082±0.12 µg/ml. The Brine Shrimps lethality was found to be concentration-dependent.

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, pesticidal effects and various pharmacologic actions [31]. Previos results indicated the ability of the plant extract to kill cancer cells in cell cultures, kill pests and exert a wide range of pharmacologic effects [17, 31]. The earlier report of Morton [32] stated that the extract could be used to treat cancer. The presence of saponins, alkaloids and cardiac glycosides may be responsible for the observed brine shrimps lethality activities of the extracts.

For insecticidal activity MEHS fruits have shown 100% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml in 12 hrs. Since the extract have strong

Table 1: *In vitro* antimicrobial activity of methanol extracts of *Hibiscus sabdariffa* (MEHS) fruits.

	Diameter of zone of inhibition (mm)	
Bacterial Strain	Kanamycin (30µg/disc)	MEHS (500µg/disc)
Gram positive		
Bacillus megaterium	30 ± 0.12	NA
Bacillus subtilis	23 ± 0.02	NA
Staphylococcus aureus	26 ± 0.10	11± 0.04
Sarcina lutea	24 ± 0.13	13± 0.21
Bacillus anthracis	23± 0.23	NA
Bacillus cereus	24 ± 0.03	NA
Gram negative		
Escherichia coli	22 ± 0.22	12± 0.11
Pseudomonas aeruginosa	25 ± 0.13	NA
Salmonella paratyphi	25 ± 0.02	7± 0.10
Salmonella typhi	25 ± 0.15	8± 0.21
Shigella boydii	25 ± 0.17	12± 0.13
Shigella dysenteriae	25 ± 0.11	12± ± 0.07
Vibrio mimicus	28 ± 0.02	10± 0.15
Vibrio parahaemolyticus	26 ± 0.19	10± 0.32

NA= Zone of inhibition = 5 mm was considered as no activity.

Table 2: Brine Shrimp lethality bioassay of methanol extracts of *Hibiscus sabdariffa* (MEHS) fruits.

					LC_{50} (µg/ml)	
Test Sample	Concentration (µg/ml)	Log C	No. of dead shrimps (out of 10)	% of Mortality	MEHS	Vincristine sulphate
MEHS	2.5	0.398	4	40	5.082±0.12	0.21±0.19
	5	0.699	5	50		
	10	1	6	60		
	20	1.301	7	70		
	40	1.602	8	80		
	60	1.778	8	80		
	80	1.903	9	90		
	100	2	9	90		
	120	2.079	10	100		

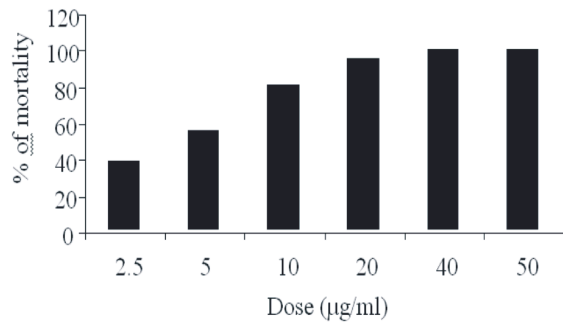

Fig. 1: Insecticidal effects of methanol extract of *Hibiscus sabdariffa* (MEHS) fruits on *Tribolium c*

insecticidal activity, dose dependent activity was done, where 6 graded doses were used and the percentage of mortality were 100, 100, 95.33, 80.53, 55.66 and 38.33% at the dose of 50, 40, 20, 10, 5 and 2.5 µg/ml, respectively (Fig. 1).

As a result of the extract strong cytotoxicity, insecticidal and antibacterial activity, MEHS fruits would be considered as anticancer, insecticidal and antibacterial agent but further studies have to be carried out for determining the toxic and safe dose of the extract.

REFERENCES

1. Stepanovic, S., N. Antic, I. Dakic and V.M. Svabic, 2003. *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol. Res., 158: 353-357.
2. Carballo, L.J., L.Z. Hernandez-inda, P. Perzer and M.D. Gravalos, 2002. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. Biol. Med. Central., 2(1): 17.

3. Lopez, A., J.B. Hudson and G.H.N. Towers, 2001. Antiviral and antimicrobial activities of Colombian medicinal paints. J. of Ethnopharmacol., 77: 189-196.
4. Coolins, P.J., 1998. Resistance to grain protectants and fumigants in insect pests of stored products in Australia. Presented in Australian Postharvest Technical Conference. pp: 55-57.
5. Islam, M.S., R. Zahan, L. Nahar, M.B. Alam, M. Naznin, G.C. Sarkar, M.A. Mosaddik and M.E. Haque, 2011. Antibacterial, Insecticidal and *In Vivo* Cytotoxicity activities of *Salix Tetrasperma*. IJPSR. 2(8): 2103-2108.
6. Joshi, B., S. Lekhak and A. Sharma, 2009. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kathmandu University J. Science, Engineering and Technol., 5: 143-150.
7. Ghani, A., 1998. Medicinal Plants of Bangladesh: Chemical constituents and uses; Asiatic Society of Bangladesh. pp: 84-85.
8. Umerchuruba, C.I., 1997. An annotated list of plant diseases in Nigeria. Pen and Paper Publication, Owerri, Nigeria.
9. Khatun, M.H., M.R. Islam, A. Mamun, L. Nahar, Luthfunnesa and M.A.U. Islam, 2011. In Vivo Evaluation of CNS Depressant and Antinociceptive Activities of Methanol Extract of Hibiscus sabdariffa Fruits. J. Appl. Sci. Res.m 7(6): 798-804.
10. Mahadevan, N. Shivali and P. Kamboj, 2009. *Hibiscus sabdariffa* L.-An Overview. Natural Product Radiance. 8(1): 77-83.
11. Chen, C.C., J.D. Hsu, S.F. Wang, H.C. Chiang, M.Y. Yang, E.S. Kao, Y.C. Ho and C.J. Wang, 2003. *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterolfed rabbits. J. Agri. Food Chem., 51: 5472-5477.
12. Gosain, S., R. Ircchiaya, P.C. Sharma, S. Thareja, A. Kalra, A. Deep and T.R. Bhardwaj, 2010. Hypolipidemic effect of ethanolic extract from the leaves Of *Hibiscus sabdariffa* L. in hyperlipidemic rats. Acta Poloniae Pharmaceutica-Drug Res., 67(2): 179-184.
13. El-saadany, S.S. and M.Z. Sitohy, 1991. Biochemical dynamics and hypocholesteremic action of Hibiscus sabdariffa (Karteade). Nahrung. 35: 567-576.
14. Tseng, T.H., E.S. Kae and C.Y. Chu, 1997. Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food Chem Toxicol., 35: 1159-1169.
15. Dignan, C.A., B.A. Burlingame, J.M. Arthur, R.J. Quiley and G.M. Milligan, 1994. The Pacific Islands Food Composition Tables. South Pacific Commission, Noumea, New Caledonia, New Zealand. pp: 1-4.
16. Rios, J.L., M.C. Recio and A. Villar, 1988. "Screening methods for natural products with antimicrobial activity: A review of the literature."J. of Ethnopharmacol. ,23: 127-149.
17. Meyer, B.N., N.R. Ferrigni, J.E. Putnam, J.B. Jacobsen, D.E. Nicholsand and J.L. Mclaughlin, 1982. "Brine shrimp; a convenient general bioassay for activeplant constituents". Planta Medica. 45: 31-34.
18. Bousquet, Y., 1990. Beetles associated with stored products in Canada. Canadian Government Publishing Centre, Ottawa, pp: 189-192.
19. Abott, W.S., 1925. A method of computing effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
20. Doss, A., H.M. Mubarack and R. Dhanabalam, 2009. Pharmacological importance of *Solanum trilobatum.* Ind. J. Sci. Tech., 2(2): 41-43.
21. Ho, K.Y., C.C Tasi, J.S. Huang, C.P. Chen, T.C. Lin and C.C. Lin, 2001. Antimicrobial activity of tannin components from *Vaccinium vitis- idaea* L. J. Pharm. Pharmacol., 53(2): 187-191.
22. Mandalari, G., R.N. Bennett, G. Bisignano, D. Trombetta, A. Saija, C.B. Faulds, M.J. Gasson and A. Narbad, 2007. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a by product of the essential oil industry. J. App. Micro., 103(6): 2056-2064.
23. Pepeljnjak, S., Z. Kalodera and M. Zovko, 2005. Antimicrobial activity of flavonoids from *Pelergonium radula* (Cav.) L' Herit. Acta Pharma. 55: 431-435.
24. Avato, P., R. Bucci, A. Tava, C. Vitali, A. Rosato, Z. Bially and M. Jurzysta, 2006. Antimicrobial activity of saponins from *Medicago sp.*: structure activity relationship. Phytother. Res., 20(6): 454-457.

25. Baharaminejad, S., R.E. Asenstorfer, I.T. Riley and C.J. Schultz, 2007. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of Oats (*Avena sativa* L.). J. Phytopathol. 156(1): 1-7.
26. Fnato gawa, K., S. Hayashi, H. Shimomura, T. Yoshida, T. Hatano, H. Ito and Y. Hirari, 2004. Antimicrobial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. Microbiol. Immunol., 48(4): 251-261.
27. Navarro, V. and G. Delgado, 1999. Two antimicrobial alkaloids from *Bocconia arborea*. J. of Ethnopharmacol., 66(2): 223-6.
28. Faizi, S. and R.A.T. Ahmed, 2003. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. *pendula*. Planta Med., 69(4): 350-355.
29. Olaleye and M. Tolulope, 2007. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. J. Med. Plant Res., 1: 009-013.
30. Mungole, A. and A. Chaturvedi, 2011. *Hibiscus sabdariffa* L a rich source of secondary metabolites. International Journal of Pharmaceutical Sciences Review and Res., 6(1): 83-87.
31. MacLaughin, J.L., C.J. Chnag and D.L. Smith, 1991. "Bench-Top" Bioassays for the discovery of Bioactive Natural Product: An update In: Studies in natural product Chemistry, Ed. Ur-Rahman, A. Elsevier Science Publisher B.V. Amsterdam, pp: 101-103.
32. Morton, J.F, 1987. Roselle. In: fruits of warm climate, Ed. Dowling, C.F. Media, Inc. Greenboro, NCP. pp: 281-286.