IJPSR (2014), Vol. 5, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 April, 2013; received in revised form, 12 March, 2013; accepted, 20 May, 2013; published 01 June, 2014

ANTIBACTERIAL ACTIVITY OF SIX MEDICINAL PLANTS USED IN TRADITIONAL MEDICINE GROWING IN BANGLADESH

M. Mostafa*[1], Hemayet Hossain [1], M. Mahmudul Hasan [1], M. Anawer Hossain [1] and Sahana Parveen [2]

BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR)[1], Dhaka- 1205, Bangladesh
Institute of Food Science and Technology (IFST) [2], BCSIR, Dhaka-1205, Bangladesh

Keywords:

Acacia nilotica, anticancer activity, Phytochemicals, docking, Cheaper drug

Correspondence to Author:

Dr. Mohammad Mostafa

Principal Scientific Officer, Analytical Research Division, BCSIR Laboratories, BCSIR, Dhanmondi, Dhaka-1205, Bangladesh

E-mail: drmostafabcsir@yahoo.com

ABSTRACT: The antibacterial activity of the hexane, methanol, and water extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalyptus camaldulensis*, *Switenia mahagoni*, and *Psidium guajava* extensively used in traditional medicine were investigated. The disc diffusion assay method was used for the evaluation of the antibacterial activity of these extracts against 11 bacteria species. Kanamycin (30 µg /disc) was used as a standard antibacterial agent. The results indicated that all the six plant species (not all extracts) showed a moderate antibacterial activity against a wide variety of gram positive and gram negative bacteria at a concentration of 500 µg/disc. The hexane extract of *Eucalyptus camaldulensis* and *Switenia mahagoni* revealed the moderate antibacterial activity against 10 and 6 bacteria respectively with zone of inhibition of 10-19 mm. The methanol extract of *Eucalyptus camaldulensis, Psidium guajava*, and *Switenia mahagoni* also exhibited antibacterial activity against 6, 4 and 5 bacteria respectively with a zone of inhibition of 9-15 mm whereas the water extract of *Azadirachta indica* and *Cucumis sativus* was only active against only two bacterial strains with zone of inhibition of 9-12 mm at the concentration of 500 µg/disc. The results of this study support the traditional uses of these medicinal plants as antibacterial agents.

INTRODUCTION: For centuries, a significant percentage of the populations in Bangladesh have relied on a system of traditional medicines, which consist of either empirico-rational and magico-religious elements or at times a combination of both.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(6).2210-15

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(6).2210-15

Infectious diseases, caused by exposure to bacterial, fungal, viral and other microbial agents, constitute one of the main problems that modern medicine have faced over the last 30 years. Despite the high proportion of effective antibiotics available today, the emergence of resistant microorganisms has lowered their potency [1]. In addition, certain antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem [2]. This has led scientists to search for new bioactive substances from various sources including medicinal plants.

The discovery of modern drugs such as quinine, vincristine, digoxin, emetine, artemisine, taxol etc., from medicinal plants signify the huge potential that still exists for the production of many more novel pharmaceuticals [3]. Thus, there has recently been a resurgence of interest in the development of drugs from plants, especially from those of the developing countries that have a rich heritage of botanical ethnopharmacopoeia. In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics. So, it is important to find out newer, safer and more effective natural or synthetic antibacterial drug molecules.

Considering the high cost of the synthetic drugs and their side effects, wide varieties of natural plants can be considered as a vital source for anti-microbial agents [4]. Therefore, the demand for new and effective anti-microbial agents with broad-spectrum of activity from natural sources is increasing day by day day [5]. Therefore, the purpose of our present investigation was to evaluate the antibacterial activity of six Bangladeshi indigenous medicinal plants for the discovery of potential antibacterial agents that might be used for the management of bacterial infectious diseases. The folkloric activity and preliminary reports of pharmacological screening of the selected plants are summarized in **Table 1**.

TABLE 1: FOLKLORIC USES AND REPORTED PHARMACOLOGICAL ACTIVITIES OF SIX MEDICINAL PLANTS

SI. No.	Plant name (Local name, Family)	Folkloric uses	Reported pharmacological activities
1	*Tamarindus indica* Linn (Tentul, Caesalpiniaceae)	Tender leaves and flowers are cooling and antibilious. Leaves and seeds are astringent [6].	The fruit and seed extracts have been reported to possess various pharmacological activities such as antioxidant, anti-inflammatory, antifungal, anti-virul, antidiabetic, and cytotoxic activities [7-10].
2	*Azadirachta indica* A. Juss (Neem, Meliaceae)	Varius parts of the plant are used in inflammation of gums, gingivitis, fever, tumours, smallpox, diarrhea and cholera. The leaves, bark, gum and seed are used as antiviral, antineoplastic and antifungal agents [6].	The plant extract has been reported to show anti-inflammatory, anti-ulcer, antimalarial, antifungal, antiplasmodial, antioxidant, and anti-carcinogenic activity [11-16].
3	*Cucumis sativus* Linn (Shasha, Cucurbitaceae)	Infusion of the leaves is used in throat affections. Seeds are cooling, tonic, diuretic and antihelmintic [6].	The plant extract has been reported to exhibit hypoglycemic, hypolipid emic, antidiabetic, hepatoprotec tive effects[17].
4	*Eucalyptus camaldulensis* (Eucalyptus, Myrtaceae)	Eucalyptus is used in the treatment of bronchial catarrh, fevers, croup, diphtheria, whooping cough, wounds and ulcers [6].	Essential oils from this plant have been reported to have activities like, pulmonary disorders, antimicrobial, antifungal, analgesic and anti-inflammatory activities [18-20].
5	*Switenia mahagoni* (Mahogony, Meliaceae)	Bark is used as antipyretic, tonic and astringent [21].	The seed extract has been reported to have medicinal value for the treatment of hypertension, diabetes, cough, chest pains, and malaria [22-23].
6	*Psidium guajava* Linn (Goam, Myrtaceae)	Leaves are used as astringents for bowels, wounds and ulcers. Decoction of leaves is used in cholera and diarrhea[6].	The leaf, bark, and fruit extracts have been reported for the treatment of plaque, diabetes, pain, cough, acne, hypertension, vaginal disorders, inflammation, malaria, diarrhoea, and rheumatism [24-31].

MATERIALS AND METHODS:

Plant materials: The *Tamarindus indica* leaves (Tentul), *Azadirachta indica* leaves (Neem), *Cucumis sativus* leaves (Shasha) and *Lens culinaris* (Masur) were collected from the Norshindi district, Bangladesh. The *Eucalyptus camaldulensis* (Eucalyptus), *Switenia mahagoni* (Mahogoni) were collected from the BCSIR campus, Dhaka and the *Psidium guajava* leaves (Goam) was collected from the BCSIR Laboratories, Rajshahi campus.

The leaves were dried under shade and finally dried in an oven at 45^{o}C for 48 hours before grinding. The dried plant materials were ground into powder with an electrical blender.

Extraction of plant materials: The plant powders (100 g each plant sample) were separately extracted in hexane, methanol and water for 24 h on an orbital shaker. The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The hexane and methanol extracts were evaporated to dryness under reduced pressure at 40^{o}C using a vacuum rotary evaporator, while the water extract was freeze-dried with Savant Refrigerated Vapor Trap. Each extracts were kept in freeze for further work in future.

Test organisms: Eleven bacterial species used in this study were laboratory isolates from the food samples in the Food Microbiology Division, IFST, Dhaka. The bacterial species consisted of four Gram-positive bacteria, *Staphylococcus aereus (SA, BTCC-43), Bacillus cereus* (BC, BTCC-19), *Bacillus subtitis (BS,* BTCC-17), *Bacillus megaterium* (BM, BTCC-18) and seven Gram-negative bacteria, *Escherichia coli* (EC, BTCC-172), *Shigella dysenteriae (SD,* BTCC-142*), Klebsella pneumonia (KP,* BTCC-144*), Salmoella typhi (*ST, BTCC-173), *Pseudomonas aeruginosa* (PA, BTCC-1252), *Vibrio cholera (*VC, BTCC), *Vibrio parahemolyticus (*VP, BTCC-1255).

Preparation of extract: Each extract was re-constituted in their respective solvents to give 1000 µg/disc. This was then diluted to the required concentrations of 500 µg/disc before being used for the antibacterial assay.

Antibacterial assay: The antibacterial activity of the extractives was determined against the test organisms (Table 1) by the disc diffusion method[32]. Solutions of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipettes and the residual solvents were completely evaporated. Discs containing the test materials were placed onto nutrient agar medium uniformly seeded with the test microorganisms. Standard discs of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively.

These plates were kept at low temperature (4ºC) for 24 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37ºC for 24 hours to allow maximum growth of the organisms. The test material having antibacterial activity will show a clear, distinct zone of inhibition was visualized surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean values were taken.

Statistical analysis: In case of each extract, three samples were prepared for the bioassay. The zones of inhibition were calculated as mean ±S.D. (n=3).

RESULTS AND DISCUSSION: The three different extracts of six indigenous medicinal plants of Bangladesh have been tested for antibacterial activity against four gram positive and seven gram negative bacteria, and the results have been summarized in Table 2. Standard antibiotic disk of Kanamycin was used for comparison purposes. The results revealed that the hexane extracts of five plants namely *Tamarindus indica*, *Lens culinaris*, *Eucalyptus camaldulensis*, *Switenia mahagoni* and *Psidium guajav* demonstrated mild to moderate broad-spectrum antibacterial activity against ten microorganisms.

Among these plants, the hexane extract of *Eucalyptus camaldulensis* showed promising antibacterial activity against five gram negative bacteria specially *Escherichia coli* (18 mm), *Shigella dysenteriae* (17 mm), *Salmoella typhi* (19 mm), *Vibrio cholerae* (14 mm) and *Vibrio parahemolyticus* (22 mm) compared to standard antibiotic Kanamycin. This extract also showed mild antibacterial activity against gram positive bacteria *Bacillus cereus* (11 mm).

The hexane extract of *Switenia mahagoni* showed also strong antibacterial activity against only one gram negative bacteria *Vibrio parahemolyticus* (21 mm) and mild antibacterial activity against one gram positive bacteria *Bacillus cereus* (12 mm. Whereas the hexane extract of *Cucumis sativus* didn't showed any activity against eleven microorganism.

The results of antibacterial activity also revealed that the methanol extracts of three plants *Eucalyptus camaldulensis*, *Psidium guajava* and *Switenia mahagoni* showed mild antibacterial activity against eight micro-organisms.

The methanol extract of *Eucalyptus camaldulensis* also exhibited moderate antibacterial activity against two gram positive bacteria *B. cereus* (11 mm), *Bacillus megaterium* (15 mm) and four gram negative bacteria *Shigella dysenteriae* (15 mm), *Vibrio cholerae* (11 mm), *Salmoella typhi* (14 mm), *Vibrio cholerae* (15 mm) and *Vibrio parahemolyticus* (14 mm), whereas the methanol extracts of *Psidium guajava* exhibited moderate antibacterial activity against two gram positive bacteria bacteria *B. cereus* (15 mm), *Bacillus megaterium* (14 mm), and two gram negative bacteria *Pseudomonas aeruginosa* (15 mm) and *Vibrio cholerae* (13 mm). On the other hand, the methanol extract of *Switenia mahagoni* showed moderate activity against two gram positive bacteria *B. cereus* (14 mm) and *Bacillus megaterium* (15 mm).

TABLE 2: ANTIBACTERIAL ACTIVITY OF SIX BANGLADESHI MEDICINAL PLANTS AGAINST ELEVEN MICRO- ORGANISM

Extracts (500µg/disc)	Plants	Zone of Inhibition (mm)										
		Bacteria										
		SA	BC	BS	BM	EC	SD	KP	ST	PA	VC	VP
Hexane extract	AI	---	12	---	---	---	---	---	---	---	---	---
	CS	---	---	---	---	---	---	---	---	---	---	---
	EC	12	11	10	08	18	17	10	19	---	14	22
	PG	---	9	---	08	---	---	---	---	---	---	---
	SM	10	12	9	---	---	---	09	---	---	11	21
	TI	08	---	---	---	---	---	---	---	---	---	---
Methanol extract	AI	---	---	---	---	---	---	---	---	---	---	---
	CS	---	---	---	---	---	---	---	---	---	---	---
	EC	---	11	---	15	---	15	---	14	---	15	14
	PG	---	15	---	14	---	---	---	---	15	13	---
	SM	---	14	8	15	---	11	---	---	---	10	---
	TI	---	---	---	---	---	---	---	---	---	---	---
Water extract	AI	---	9	10	---	---	---	---	---	---	---	---
	CS	---	9	9	---	---	---	---	---	---	---	---
	EC	---	10	---	---	---	---	---	---	---	---	---
	PG	---	12	---	---	---	---	---	---	---	---	---
	SM	---	---	---	---	---	---	---	---	---	---	---
	TI	---	---	---	14	---	---	---	---	---	---	14
KM (30µg/disc)		30	21	30	26	24	25	22	26	32	22	23

SA= *Staphylococcus aureus*, BC= *Bacillus cereus*, BS= *Bacillus subtitis*, BM= *Bacillus megaterium*, EC= *Escherichia coli*, SD= *Shigella dysenteriae*, KP= *Klebsiela pneumonia*, ST= *Salmoella typhi*, PA= *Pseudomonas aeruginosa*, VC= *Vibrio cholerae*, VP= *Vibrio parahemolyticus*, AI= *Azadirachta indica*, CS= *Cucumis sativus*, EC= *Eucalyptus camaldulensis*, PG= *Psidium guajava*, SM= *Switenia mahagoni*, TI= *Tamarindus indica*, and KM= Kanamycin (Standard). "---" Indicates no sensitivity.

The results also demonstrated that the water extracts of five plants *Azadirachta indica, Tamarindus indica*, *Lens culinaris*, *Eucalyptus camaldulensis*, and *Psidium guajava* showed poor antibacterial activity against only three micro-organisms.

Among the plants, the extracts of *Eucalyptus camaldulensis, Psidium guajava* and *Switenia mahagoni* gave strong antibacterial activity against tested microorganisms.

The similar antibacterial activity of the *Eucalyptus camaldulensis, Psidium guajava* and *Switenia mahagoni* were reported earlier by other workers [33-35] and further supported the results. The demonstration of antibacterial activity of these plants against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. The results obtained from the preliminary evaluation of antibacterial activity in this experiment have provided a scientific basis on the uses of these

plants in traditional medicine and can be a vital source of promising antibacterial agents and thus can be considered as leads for the discovery of new antibacterial agents. Further investigation is also needed for proper utilization of these plants as antibacterial agents either in traditional medicines directly or as sources for active antibacterial principle(s).

ACKNOWLEDGEMENT: The authors are grateful to Shahidul Islam, Jr Technician, CRD, BCSIR Laboratories, Dhaka for assisting the research. The work was supported by a Special Research Grant from the Ministry of Science and Information & Communication Technology (MOSICT/Sha-9/PRC-24/2009-2010/(Part-1)/BS-72/192, date-03/12/2009), Government of Bangladesh.

REFERENCES:

1. Bacq-Calberg CM, Coyotte J, Hoet P and Nguyem-Disteche M: Microbiologie. Partie V Technologie de I'ADN et genomique. De Boeck & Larcier, Bruxelle 1999; 338.
2. Marchese A and Shito GC: Resistance patterns of lower respiratory tract pathogens in Europe. International Journal of Antimicrobial Agents 2001; 16: 25-29.
3. Plokin NJ: Conservation, ethnobotany and the search for jungle medicine: Pharmacology comes of age. Pharmacotherapy 1998; 8:257-262.
4. Afolayan AJ: Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. Pharmaceutical Biology 2003; 41:22-25.
5. Rahman MS, Rahman MZ, Wahab,MA, Cowdhury R and Rashid MA: Antimicrobial activity of some Indigenous Plants of Bangladesh. Dhaka University Journal of Pharmaceutical Science 2008; 7: 23-27.
6. Ghani A: Medicinal plants of Bangladesh, Asiatic society of Bangladesh, Dhaka. 1998.
7. Soong YY and Barlow PJ: Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry 2004; 88: 411-417.
8. Krishnaveni M, Durairaj S, Madhiyan P, Amsavalli L and Chandrasekar R: *In vitro* free radical scavenging activity of aqueous leaf extract of plants near thermal power plant, Mettur, Salem. International Journal of Pharmaceutical Sciences and Research 2013; 4(9):3659-3662.
9. Goyal B, Shashi Alok, Jain SK, and Verma A: Evaluation of analgesic activity of ethanolic extract of *Tamarindus indica* leaves on experimental animal model. International Journal of Pharmaceutical Sciences and Research 2013; 4(5):1994-1997.
10. Al-Fatimi M, Wurster M, Schröder G and Lindequist U: Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacology 2007; 111: 657-666.
11. Okapanyi SN and Ezeukwu GC: Anti-inflammatory and anti-pyretic Activities of *Azadirachta indica*. Planta Medica 1981; 41:34-39.
12. Febry W, Okema P and Ansorg R: Activity of East African medicinal plants against *Helicobacter pylori*. Chemotherapy 1996; 42:315-317.
13. Irondi AE, ObohG and Akintunde JK: Comparative and synergistic antioxidant properties of *Carica papaya* and *Azadarichta indica* leaves. International Journal of Pharmaceutical Sciences and Research 2012; 3(12):4773-4779.
14. Khan M and Wassilew SW: Natural pesticides from the neem tree and other tropical Plants, GTZ, Eschborn, Germany 1987.
15. L.A. Oseni and G.M. Akwetey: An in-vivo evaluation of antiplasmodial activity of aqueous and ethanolic leaf extracts of *Azadirachta indica* in plasmodium berghei infected balb/c mice. International Journal of Pharmaceutical Sciences and Research 2012; 3(5): 1406-1410.
16. Sarkar K, Bose A, Haque E, Chakraborty K, Chakraborty T, Goswami S, Ghosh D and Baral R: Induction of type 1 cytokines during neem leaf glycoprotein assisted carcinoembryonic antigen vaccination is associated with nitric oxide production 2009.
17. Sharmin R, Khan MRI, Akhter MA, Alim A, Islam MA, Anisuzzaman ASM and Ahmed M: Hypoglycemic and hypolipidimic effect of cucumber, white pumpkin and ridge gourd in Alloxan induced diabetic rats. Journal of Scientific Research 2013; 5 (1): 161-170.
18. Nagpal N, Shah G, Arora MN, Shri R, and Arya Y: Phytochemical and pharmacological aspects of Eucalyptus genus. International Journal of Pharmaceutical Sciences and Research 2010; 1(12): 28-36.
19. 2 Ramezani H, Singh HP, Batish DRO and Kohli RK: Antifungal activity of volatile oil of *Eucalyptus citriodora*. Fitoterapia 2002; 73: 261–262.
20. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA: Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. Journal of Ethnopharmacology 2003; 89: 277–283
21. Wealth of India: Raw materials: Publication & Information Directorate, CSIR, New Delhi 1976; 84-87.
22. Panda SP, Bera S, Naskar S, Adhikary S, Kandar CC and Haldar PK: Depressant and anticonvulsant effect of methanol extract of *Swietenia mahagoni* in mice. Indian Journal of Pharmaceutical Education and Research 2010; 44(3): 283-287.
23. Nagalakshmi MAH, Thangadurai D, Muralidara D and Pullaiah RT: Phytochemical and antimicrobial study of *Chukrasia tabularis* leaves. Fitoterapia 2001; 72*:* 62–64.
24. Rishika D and Sharma R: An update of pharmacological activity of *Psidium guajava* in the management of various disorders. International Journal of Pharmaceutical Sciences and Research 2012; 3(10): 3577-3584.
25. Mukhtar HM, Ansari SH, Bhat ZA, Naved T and Singh P: Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae). Pharmazie 2006; 61(8): 725-727.
26. Ojewole JA, Awe EO and Chiwororo WD: Antidiarrhoeal activity of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract in rodents. Journal of Smooth Muscle Research 2008; 44(6): 195-207.
27. Prabu GR, Gnanamani A and Sadulla S: Guaijaverin-a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. Journal of Applied Microbiology 2006; 101(2): 487-495.
28. Ojewole JA: Anti-inflammatory and analgesic effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract in

rats and mice. Methods and Findings in Experimental and Clinical Pharmacology 2006; 28(7): 441-446.
29. Shaheen HM, Ali BH, Alqarawi AA and Bashir AK: Effect of *Psidium guajava* leaves on some aspects of the central nervous system in mice. Phytotherapy Research 2000; 14(2): 107-111.
30. Nundkumar N and Ojewole JA: Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine. Methods and Findings in Experimental and Clinical Pharmacology 2002; 24(7):397-401.
31. Payal M, Vikas G, Gurpreet K, Ashish KG and Amarjeet S: Phytochemistry and pharmacological activities of *Psidium guajava*: a review. International Journal of Pharmaceutical Sciences and Research 2010; 1(9): 9-19.
32. Bauer AW, Kirby WMM, Sheriss JC and Truck M: Antibiotic susceptibility testing by standardized single method. American Journal of Clinical Pathology 1966; 45: 493-496.
33. Ghalem BR and Mohamed B: Antibacterial activity of leaf essential oil of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. African Journal of Pharmacy and pharmacology 2008; 2 (10): 211-215.
34. Sushmit C, Sharan L and Sinha MP: Phytochemical and antimicrobial screening of *Psidium guajava* L leaf extracts against clinically important gastrointestinal pathogens. Journal of Natural Product Plant Resource 2012; 2 (4): 524-529.
35. Mollik J and Banik RK: In vitro studies on antimicrobial and thrombolytic activity of *Swietenia macrophylla* King. Journal of Pharmaceutical Research and Opinion 2012; 2 (5): 45-48.

How to cite this article:
Mostafa M, Hossain H, Hasan MM, Hossain MA and Parveen S: Antibacterial activity of six medicinal plants used in traditional medicine growing in Bangladesh. Int J Pharm Sci Res 2014; 5(6): 2210-15.doi: 10.13040/IJPSR.0975-8232.5(6).2210-15