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reus***

Growth inhibitory effect of ethanolic neem leaves extract on *Klebsiella*, *Salmonella* and *Staphylococcus aureus*

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Abstract

Neem (leaves, flowers, seeds etc.) have been used traditionally against many diseases. We, thus, investigated the antimicrobial effect of ethanolic neem leave extract on *Klebsiella*, *Salmonella* and *Staphylococcus aureus* and found its growth inhibitory effect on *Klebsiella* and *Salmonella* at the concentration of 12.5 and 6.2 mg/mL respectively with average diameter of zone of inhibition 18 and 20 mm respectively. It failed to inhibit the growth of *Staphylococcus aureus* at the highest concentration used (50 mg/mL). Findings of this preliminary experimental study suggest that, neem can be tried against *Klebsiella* and *Salmonella* showing limited efficacy.

Introduction

Since prehistoric times, neem (*Azadirachta indica*) has been used by humankind for medicinal purpose. It has been extensively used in Ayurveda, Unani and Homeopathic medicine (Rajasekaran et al., 2008; Girish and Bhat, 2008). Besides versatile medicinal uses it is as well used in agriculture (Mishra et al., 2013; Maragathavalli et al., 2012). Different parts of neem contain more than 135 compounds having vast arrays of biological activity (Yerima et al., 2012). Neem (leaves, flowers, seeds, fruits, roots, bark) found its utility since ancient days and have been used to treat infections, inflammation, fever, skin diseases and dental disorders (Helmy et al., 2007; Mosaddek and Rashid, 2008). Neem leaf has been the mostly used part of the tree and possesses immunomodulatory, anti-inflammatory, anti-hyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, anti-oxidant, antimutagenic and anticarcinogenic properties (Reddy et al., 2013; Girish and Bhat, 2008). It also possesses a wide spectrum of antibacterial action against Gram-positive and Gram-negative microorganisms (Mamman et al., 2013; Sarmiento et al., 2011; Jahan et al., 2007).

Although a number of antibiotics are available for the

treatment of infectious diseases, they have certain limitations like adverse effects, high cost and development of antimicrobial resistance (Kumar et al., 2013). Indiscriminate use of antibiotics is very common in Bangladesh and other developing countries being a major cause for development of antimicrobial resistance (Faiz and Basher, 2011; Rahman and Huda, 2014; Shamsuzzaman et al., 2007).

Considering all these, we tried to find an effective antimicrobial agent from plant source against a few common human pathogens like- *Klebsiella sp.*, *Salmonella sp.* and *Staphylococcus aureus*. We conducted this study to determine the antimicrobial effect of ethanolic neem leave extract on these commonly occurring bacteria to determine if it has any prospect as future antibiotic.

Materials and Methods

This experimental study was conducted from January 2010 to December 2010.

Preparation of neem extract: Fresh, mature deep green leaves of neem tree were collected from the medicinal plant garden of BCSIR, Dhaka. After identification and authentication by Dhaka Laboratories, BCSIR and these



were cleaned and washed with plain tap water and air dried in shade at room temperature by spreading in large stainless steel trays for 24 hours. The dried leaves were crushed well. Five hundred grams of crushed leaves were then suspended in 3 L of petroleum ether and kept in refrigerator overnight for removing all the fatty substances. After this, the supernatant was discarded and the residue was dried at room temperature. Then the residue material were suspended into 5 liters of 95% ethanol in sterile conical flask and kept at 4°C for 72 hours. The supernatant was filtered through filter paper (Whatman No. 1) and the filtrate was put into rotary vacuum evaporator to get concentrated neem extract. The filtrate thus obtained was further purified by filtration through Whatman No. 1 filter paper. This stock solution of extract was sterilized by filtration through Millipore membrane filter of 0.45 mm pore size. Then the concentrated extract was Freeze dried and stored in sterile capped conical flask and refrigerated at 4°C. Suitable amount of ethanol was mixed to dilute the extract to get a solution of different strength. The extract was tested for sterility by introducing 2 mL of extract into 10 mL of sterile nutrient broth. This was then incubated at 37°C for 24 hours.

Collection of microorganism

Klebsiella sp. ATCC strain, *Salmonella sp.* ATCC strain and *Staphylococcus aureus* ATCC 25923 was obtained from the Department of Microbiology, BSMMU, Dhaka.

Preparation of culture media, McFarland standard and bacterial cell suspension

Nutrient agar and McFarland standard was prepared by standard method (Hudzicki, 2009). For preparation and standardization of bacterial cell suspension 5 colonies each of *Klebsiella sp.*, *Salmonella sp.* and *S aureus* were picked and put into separate sterile test tube containing nutrient broth then incubated at 37°C for 24 hours. The turbidity produced by the organism was adjusted and used to match with the turbidity of 0.5 McFarland standard. If the suspension was too light more organisms was added or if it was too heavy it was diluted by sterile saline (Hudzicki, 2009).

Determination of minimum inhibitory concentration (MIC) on the test organisms

The MIC of the extract was determined by broth dilution method.

Preparation of different concentrations of extract

For preparation of different concentrations of extract, 10 g of freeze dried extract was taken and was diluted in 100 mL of ethanol. Thus, the initial concentration was 100 mg/mL. Then it was further diluted using double fold serial dilution to obtain 50, 25, 12.5, 6.25 and 3.125 mg/mL concentrations of extract.

Inoculation of bacteria into extract

In 3 sets of test tubes containing 5 test tubes each, 0.1 mL of *Klebsiella sp.*, *Salmonella sp.* and *S. aureus* inoculums were added. A negative control was set up by 5 mL sterile extract and 5 mL sterile nutrient broth and positive control by 5 mL of sterile nutrient broth and 0.1 mL of bacterial inoculum.

Examination of growth after overnight incubation

Test tubes were incubated at 37°C for 24 hours. The growth of the test organism in each concentration of extract was examined and compared against the controls by matching their turbidity. The growth of the bacterial inoculum in the broth was indicated by turbidity or cloudiness of the broth. The test tube containing the lowest concentration of extract and showing no visible sign of growth or turbidity was considered as the MIC. The mean MICs were recorded.

Detection of bacterial susceptibility

Bacterial susceptibility was determined by disc diffusion method in nutrient agar medium. The nutrient agar plate was inoculated by streaking a swab stick dipped into an inoculum tube containing standardized bacterial cell suspension three times over the entire agar surface. Then it was kept at room temperature for 3 to 5 min for drying. Whatman No. 1 filter paper discs of 6 mm diameter were made with the help of a punching machine and these discs were sterilized by hot air oven. Then 50 µL of different concentrations of extract were applied to soak the sterile discs and were placed on the inoculated agar plate. A disc was soaked by 50 µL of ethanol and was also placed on the agar plate which served as control. The plates were allowed to stand for one hour for pre-diffusion of the extracts then incubated at 37°C for 24 hours. Diameters of zone of inhibition were measured in millimeter.

Results

The lowest concentration of extract showing an inhibitory effect on the growth of *Klebsiella* and *Salmonella* was 12.5 and 6.25 mg/mL respectively. Growth of *S aureus* was failed to be inhibited with the highest concentration used (50 mg/mL). So, the MIC of extract against *Klebsiella sp.* and *Salmonella sp.* was 12.5 and 6.25 mg/mL respectively. *S. aureus* being resistant to the effects of extract (Table I).

Antibacterial susceptibility of *Klebsiella*, *Salmonella* and *S aureus* subculture on to nutrient agar was performed on MHA and diameters of zone of inhibition for extract was measured after overnight incubation at 37°C, aerobically. Average diameter of zone of inhibition against *Klebsiella* was 18 mm at 12.5 mg/mL and *Salmonella* 20 mm at 6.25 mg/mL. *S. aureus* did not show any zone of inhibition with the highest concentration (50.0 mg/mL) of extract (Table II).

Table I

Inhibitory effect of neem extract on *Klebsiella* sp., *Salmonella* sp. and *S. aureus*

Serial of test tubes	Concentration of extract (mg/mL)	<i>Klebsiella</i> sp.	<i>Salmonella</i> sp.	<i>S. aureus</i>
1	50	Growth completely inhibited	Growth completely inhibited	Growth not inhibited
2	25	Growth inhibited	Growth inhibited	Growth not inhibited
3	12.5	Growth inhibited	Growth inhibited	Growth not inhibited
4	6.25	Growth not inhibited	Growth inhibited	Growth not inhibited
5	3.125	Growth not inhibited	Growth not inhibited	Growth not inhibited
Positive control	-	Huge growth	Huge growth	Huge growth
Negative control	5	Growth inhibited	Growth inhibited	Growth not inhibited

Table II

Diameter of zone of inhibition of neem extract and ciprofloxacin disk against *E. coli*

	Name of bacteria with average diameter of zone of inhibition					
	<i>Klebsiella</i> sp.		<i>Salmonella</i> sp.		<i>Staphylococcus aureus</i>	
	Hole potency	Zone of inhibition	Hole potency	Zone of inhibition	Hole potency	Zone of inhibition
Neem extract	12.5 mg/mL	18 mm	6.25 mg/mL	20 mm	50.0 mg/mL	No zone of inhibition

Discussion

The World Health Organizations (WHO) has recently pronounced a warning saying that the world is entering a 'post-antibiotic era' when most of the commonly available antibiotics will become ineffective (Reardon, 2014). Antimicrobial resistance is a threat to all branches of medical and public health practice (Kumar et al., 2013). It jeopardizes progress in health sectors by increasing morbidity and mortality and imposes huge economic burden (WHO, 2014; Karmakar and Sattar, 2012; Cosgrove and Carmeli, 2003). Moreover, most of the new antibiotics are costly and possesses threat of adverse drug reaction. Quest is going on for safe and effective alternatives of modern antibiotics from plant sources (Victor and Igeleke, 2012; Huttner et al., 2013). Studies suggest that plants can be source of drugs against infective organisms (Rajasekaran et al., 2008; Reddy et al., 2013; Rozarina, 2013).

In the present study the extract was subjected to a preliminary screening for antimicrobial activity against *Klebsiella*, *Salmonella* and *S. aureus*. The MIC of extract inhibiting the growth of *Klebsiella* was 12.5 and 6.25 mg/mL for *Salmonella*. Growth of *S. aureus* was not inhibited even with the highest concentration used (50 mg/mL). This suggests that extract possesses antimicrobial activity against *Klebsiella* and *Salmonella*. Mamman et al. (2013) in their study found aqueous and methanolic neem leave extract inhibiting *Salmonella* spp. The finding was not consistent with that of Helmy et al. (2007) where native extracts from the neem leaves (20 µg/disk) were inhibitory to *S. aureus*, *E. coli* and some

fungi.

To see the bacterial susceptibility of extract, the test organisms were subcultured. The results obtained in the agar diffusion plates followed the same trend with what was obtained in the MIC tests. With extract, the size of zone of inhibition was 18 mm for *Klebsiella*, 20 mm for *Salmonella* with a disk potency of 12.5 and 6.25 mg/mL respectively. This finding is similar to findings of previous studies done by Odunbaku and Ilusanya (2008) where ethanolic extract of neem showed significant antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *Proteus mirabilis* and few other bacteria. Similar observations were also made by other investigators (Chaturvedi et al., 2011; Irshad et al., 2011). Jahan et al. (2007) in her study found growth inhibitory effect of neem oil on *Salmonella typhi*, *S. aureus* and *E. coli*. Mamman et al. (2013) in their study showed that crude methanolic and aqueous extract of neem leaves was inhibitory to *S. aureus*, *Salmonella* spp and *E. coli*.

In our study even at 50 mg/mL concentration of extract failed to suppress the growth of *S. aureus*. This finding is not supported by most of the previous studies where neem leaf extract at different concentrations inhibited the growth of *S. aureus* (Reddy et al., 2013; Mamman et al., 2013; Sarmiento et al., 2011; Mishra et al., 2013; Prashar et al., 2012). Khan et al. (2010) in their study found that chloroform extract of neem leaves inhibited the growth of *S. aureus*, which was just comparable to streptomycin. In another study Maragathavalli et al. (2012) found comparative inhibitory effect of extract

and gentamicin against *S. aureus*. Here an interesting finding was that methanolic extract of neem leaves inhibitory effect was much better than that of gentamicin.

In this context we can conclude by saying that the ethanolic neem leaves extract shows variable antibacterial effect on *Salmonella spp* and *Klebsiella spp*, and failed to inhibit *S aureus* which maybe due to the lower concentration used.

References

- Chaturvedi P, Bag A, Rawat V, Jyala NS, Satyavali V, Jha PK. Antibacterial effects of *Azadirachta indica* leaf and bark extracts in clinical isolates of diabetic patients. NJIRM. 2011; 2: 5-9.
- Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. Clinical Inf Dis. 2003; 36: 1433-37.
- Faiz MA, Basher A. Antimicrobial resistance: Bangladesh experience. Regional Health Forum. 2011; 15: 1-8.
- Girish K, Shankara Bhat S. Neem: A green treasure. Electronic J of Biol. 2008; 4: 102-11.
- Mamman PH, Mshelia WP, Susbatrus SC, Sambo KW. Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus*. Int J Med Med Sci. 2013; 5: 14-18.
- Helmy WA, Amer H, EL-Shayeb NMA. Biological and antimicrobial activities of aqueous extracts from neem tree (*Azadirachta indica* A Juss, Meliaceae). J Appl Sci Res. 2007; 3: 1050-55.
- Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. MicrobeLibrary.org. 2009.
- Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, Jarlier V, Voss A, Pittet D. Antimicrobial resistance: A global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrob Res Inf Cont. 2013; 2: 31.
- Irshad S, Butt M, Younus H. *In vitro* antibacterial activity of two medicinal plants neem (*Azadirachta indica*) and peppermint. Int R J Pharmaceut. 2011; 1: 9-14.
- Jahan T, Begum ZA, Sultana S. Effect of neem oil on some pathogenic bacteria. Bangladesh J Pharmacol. 2007; 2: 71-72.
- Karmakar P, Sattar MM. Antibiotic prescribing pattern in Bangladesh. B J Prog Sci Tech. 2012; 10: 13-16.
- Khan I, Srikakolupu SR, Darsipudi S, Gotteti SD, Amaranadh CH. Phytochemical studies and screening of leaf extracts of *Azadirachta indica* for its anti-microbial activity against dental pathogens. Archives of App Sci Res. 2010; 2: 246-50.
- Kumar BU, Bhubaneswari A, Tejasri MVV, Radhakrishna P and Amritha KDK. Comparative antimicrobial activities of the combined crude leaf extract of *Bixa Orellana*, *Azadirachta indica* and *Ocimumscantum*. Int Res J Pharm. 2013; 4: 189-93.
- Maragathavalli S, Brindha S, Kaviyarasi NS, B. Annadurai B, Gangwar SK. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). IJSN. 2012; 3: 110-13.
- Mosaddek ASM, Rshid MMU. A comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. Bangladesh J Pharmacol. 2008; 3: 44-47.
- Mishra A, Mamta, Neema, Niketa, Poonam, Pranjul, Priyanka. Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli* and *Staphylococcus aureus*. Int J Sci Environ Tech. 2013; 2: 989-93.
- Odunbaku OA, Ilusanya OA. Antibacterial activity of the ethanolic and methanolic leaf extracts of some tropical plants on some human pathogenic microbes. Res J Agric Biol Sci. 2008; 4: 373-76.
- Prashar P, Pruthi H, Akhlaq A. *In vitro* antimicrobial activity of *Azadirachta indica* against pathogenic bacteria. J Pharm Res. 2012; 5: 363-64.
- Rahman MS, Huda S. Antimicrobial resistance and related issues: An overview of Bangladesh situation. Bangladesh J Pharmacol. 2014; 9: 218-24.
- Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R, Jayakumararaj R. Investigations on antibacterial activity of leaf extracts of *Azadirachta indica* A. Juss (Meliaceae): A traditional medicinal plant of India. Ethnobotanical Leaflets. 2008; 12: 1213-17.
- Reardon S. Antibiotic resistance sweeping developing world. Nature 2014; 509: 141-42.
- Reddy YRR, Kumari CK, Lokanatha O, Mamatha S, Reddy CD. Antimicrobial activity of *Azadirachta indica* (neem) leaf, bark and seed extracts. Int J Phytochem Pharmacol. 2013; 3: 1-4.
- Rozarina NJA. Antimicrobial potentials of the methanolic extracts of plants. IJSRR. 2013; 2: 89-95.
- Sarmiento WC, Maramba CC, Gonzales MLM. An *in vitro* study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. PIDSP J. 2011; 12: 40-45.
- Shamsuzzaman AKM, Paul SK, Mahmud MC, Musa AKM, Hossain MA. Emerging antimicrobial resistance amongst common bacterial pathogens in Mymensingh Medical College Hospital. Bangladesh J Med Microbiol 2007; 1: 4-9.
- Victor IU, Igeleke CL. Antimicrobial properties of the extracts of locally sold garlic and neem leaf in Benin City, Nigeria. Int J Biosci. 2012; 2: 21-27.
- WHO. Antimicrobial resistance: Global report on surveillance 2014.
- Yerima MB, Jodi SM, Oyinbo K, Maishanu HM, Farouq AA, Junaidu AU, Al-Mustapha MN, Shinkafi AL. Effect of neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth. Nigerian J Basic App Sci. 2012; 20: 64-67.

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