International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Preliminary Phytochemical and Antibacterial Activities of *AzadirachtaIndica* (Bark) against Some Bacteria

Mrityunjoy Das[1], Md. AbulKalam Azad[2], Md. Belal Uddin[3], Monira Khatun[4], Md. Ferozur Rahaman[5]

[1, 5]Institute of Environmental Science (IES), University of Rajshahi, Bangladesh

[2]Institute of Environmental Science (IES), University of Rajshahi, Bangladesh

[3]Department of Biochemistry & Molecular Biology, University of Rajshahi, Bangladesh

[4]Department of Botany, University of Rajshahi, Bangladesh

Abstract: ***The antibacterial effect of Azadirachtaindica against Pseudomonas aeruginosa, Klebsiellaozanae, Staphy-lococcusaureus and Escherichia coli was determined using the agar cup plate technique. The phytochemical components of A. indica showed the presence of saponin and phlobatanin and the absence of alkaloids, tannins, phenolics, glycosides, flavonoids and triterpenes. The result showed that the test organisms were susceptible to 100mg/ml, 50mg/ml and 5mg/ml of the plant extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The result showed that the MIC and MBC were 5mg and 50mg respectively for Pseudomonas aeruginosa, Klebsiellaozanae, Staphylococcus aureus and Escherichia coli. The result of the study suggests that extracts of A. indica could be suitable for the treatment of various infections caused by P. aeruginosa, K. ozanae, S. aureusand E. coli.***

Keywords: Antibacterial, *AzadirachtaIndica*, Phytochemical, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

1. Introduction

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (De and Ifeoma, 2002; El-Mahmood *et al*., 2010). Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties (Kayode and Kayode, 2011). It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs (Shah *et al*., 2006). Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases (Natarajan *et al*., 2003). *Azadirachtaindica* is one of such medicinal plants belonging to the Meliaceae family and is indigenous to southern Asia (Akula *et a*l., 2003). *Azadirachtaindica*, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyper-glycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Talwar *et al.*, 1997; Biswas *et al*., 2002; Subapriya and Nagini, 2005). The objectives of this study therefore are to determine the phytochemical components of the leaf extracts of *A. indica*, to determine the minimum inhibitory concentration (MIC) of the extract on *Pseudomonas aeruginosa, Klebsiellaozanae, Staphylococcus aureus* and *Escherichia coli* and determine the minimum bactericidal concentration (MBC) of the extract on *Pseudomonas aeruginosa, Klebsiellaozanae, Staphylococcus aureus* and *Escherichia coli.*

2. Materials and methods

2.1 Collection of plant

The leaves of the plant *A.nilotica* were collected from Rajshahi University campus, Bangladesh. It was identified and authenticated in the Department of Botany, Rajshahi University, Bangladesh.

2.2 Test microorganisms

The pure culture microorganisms were collected from the Institute of Biological Science (IBSc), Department of Pharmacy, University of Rajshahi, and Env. Microbiology Lab, ICDDR, B Mahakhali, Dhaka, Bangladesh. The bacteria were used for the study of antibacterial activity as follows *Pseudomonas aeruginosa, Klebsiellaozanae, Staphylococcus aureus* and *Escherichia coli*

2.3 Preparation of plant extract

Fresh leaves of the plant were washed under running tap water and air dried for about one week and then homogenized to fine powder and stored in airtight bottle. The powder of leaves (100gm) was extracted with 100 ml ethanol using conical flask in a shaking incubator at 28°C for two days. The extract was filtered and evaporated until dryness. The extract was stored at 4°C.

Phytochemical Screeningof Extracts of *A. indica*

The phytochemicalcomponents of extracts of *A. indica*was determined using methods describedby Odebiyi and Sofowora (1978) and Trease and Evans (1989). The phytochemical components analysed for were alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, steroids, phlobatanins and triterpenes.

Antimicrobial susceptibility test

Susceptibility test of the test organismsto extracts of *A. indica*at concentrations of 100mg/ml, 50mg/ml and 5mg/ml was carried out using agar cup plate technique as described by Silver *et al*. (1997). Nutrient agar was sterilized using autoclave at 121oC for 15 minutes. It was then poured on to plates and allowed to solidify. Standardized inoculum of each test organisms was spread on to agar plates so as to achieve a confluent growth. The impregnated discs with different concentrations of the extract were placed on the surface of the medium at three points equidistant from one another. The plates were then incubated at 37oC for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the test organisms was determined using the tube dilution technique. Nine millilitre (9ml) of the nutrient broth was pippeted into various test tubes containing concentrations of 100mg/ml, 50mg/ml and 5mg/ml of the extract. The overnight culture of the test organisms diluted at 106cfu/ml was added to the test tubes and then incubated at 37oC for 24 hours. The least concentration of the extract that did not indicate any visible growth of the incubated organisms in broth culture was taken as the minimum inhibitory concentration (MIC) (Hugo and Russel, 1983).

3. Result

Phytochemical screening of the extracts

Table 1 shows the phytochemical screening of the extract of the *A. indica*. The phytochemical components of *A. indica*showed the presence of saponin and phlobatanin and the absence of alkaloids, tannins, phenolics, glycosides, flavon-oids and triterpenes.

Table 1: Phytochemical screening of extract

Compounds	*A.indica*
Alkaloid	-
Tannins	-
Phenols	-
Glycoside	-
Saponin	+
Flavonoid	-
Phlobtanin	+
Triterpenes	-

Antimicrobial activities of the extracts

Table 2 shows the zones of inhibitions (mm) of plant extracts at different concentrations (mg/ml). *P. aeruginosa* showed the highest susceptibility of 14±2mm at 100mg/ml, followed by *S. aureus* (12±2mm), *K. ozanae*10±2mm and *E. coli* 8±2mm. At concentration of 50mg/ml, *P. aeruginosa* had the highest zone of inhibition (10±2mm), followed by *S. aureus* (8±2mm). The least was *E. coli* with zone of inhibition of 5±1mm. At concentration of 5.0mg/ml, both *P. aeruginosa* and *K. ozanae* had the same zone of inhibition of 8±2mm followed by *S. aureus* (5±1mm) and the least zone of inhibition of 4±1mm was recorded in *E. coli*.

Table 2: Antibacterial activities of the extract

Organisms	Concentration of extract of *A. indica*		
	100mg/ml	50mg/ml	5mg/ml
P.aeruginosa	14±2	10±2	8±2
K.ozanae	10±2	8±2	8±2
S.aureus	12±	8±	5±1
E.coli	8±2	5±1	4±1

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extract

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the test organisms on the extract of *A. indica*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 5mg/ml and 50mg/ml respectively for *P. aeruginosa, Kl. ozanae, S. aureus*and *E. coli*.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extract

Organisms	MIC(mg/ml)	MBC(mg/ml)
P.aeruginosa	5	50
K.ozanae	5	50
S.aureus	5	50
E.coli	5	50

Susceptibility testing using standard antibiotics (Positive control)

Table 4 shows the susceptibility test results of some selected standard antibiotics. *P. aeruginosa* and *S. aureus* were susceptible to Amoxacillin (AMX) but *K. Ozanae*and *S. aureus*were resistant. All the test organisms were susceptible to Rifampin (RF) and Gentamycin (GEN), *K. ozanae*, *S.aureus* and *E.coli* were susceptible to Augmentin (AU) but *P. aeruginosa* was resistant. *P. aeruginosa*, *K. ozanae*and *E.coli*were susceptible to Streptomycin (S) but *S.aureus*was resistant. None of the test organisms were susceptible to Ciptrofloxacin (CPX) and Ceporex (CEP).

Table 4: Susceptibility testing using standard antibiotics (Positive control)

Organisms	AMX	CPX	RD	AU	GEN	AU	S
P.aeruginosa	9±3.05	-	10±2	-	9±2.51	-	7.7±0.6
K.ozanae	-	-	12±8	12±1.5	10±2	7±1.5	6±2
S.aureus	12±8	-	10±2	8±1	13±2.6	12±1	-
E.coli	-	-	9±3.05	10±2	10±2	-	10±2

Susceptibility testing using standard antibiotics (negative control)

Table 5 shows the susceptibility of the test organisms to some commonly used antibiotics *K.ozanae* and *S. aureus* were susceptible to Gentamycin *P. aeruginosa* were resistant. *S.aureus* was susceptible to Ciprofloxacin (CPX) but *S. aureus, K ozanae* and *E.coli* were resistant. *P.aeruginosa, S. aureus* and *E.coli* are susceptible to Ampicillin (AMP) but *K. ozanae* was resistant. Only *P. aeruginosa* was susceptible to AU- Augmentin (AU) and Streptomycin (S).

Table 5: Susceptibility testing using standard antibiotics (Negative control)

Organisms	GEN	CPX	PN	AU	S
P.aeruginosa	-	-	8±2	-	-
K.ozanae	10±2	-	-	8±2	10±2
S.aureus	6±2	5±3.05	10±2	10±2	6±2
E.coli	-	-	8±2	6±1	10±2

4. Discussion

The photochemical screening of *A. indica* extract indicated the presence of saponin and phlobatanins (Table 1), the presence of these compounds may be responsible for the antibacterial activities of the extracts of *A. indica* on the test organisms. The phytochemical components of the *A. indica* have been established in previous studies and these include tannins, saponins, alkaloids, carbohydrates, phenols, flavonoids, anthraquinones, cardiac glycosides, sterols and resins (De and Ifeoma, 2002; Natarajan *et al*., 2003; Biswas *et al*., 2002, El-Mahmood*et al*,. 2010). Several studies have linked presence of these bioactive compounds in plant materials to antimicrobial activity. The presence of these secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by micro-organisms, predation by insects and herbivores, while some give plants their odors and or flavors and some still are responsible for their pigments (El-Mahmood*et al*,. 2008). In some cases, the activity has been associated with specific compounds or classes of compounds. These active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs (Ogbonnia*et al*., 2008; El-Mahmood*et al*., 2010).

The antibacterial effects of *A. indica*on the test organisms revealed that *P. aeruginosa* showed the highest zones of inhibition (mm) followed by *S. aureus* while *E. coli* had the least zone of inhibition (mm) at various extract concentrations of 100mg/ml, 50mg/ml and 5mg/ml (Table 2). Compared to commonly applied antibiotic (Tables 4 and Table 5), bark extracts of *A. indica* showed a higher value of zones of inhibition on the tested organisms. In a similar study hexane and aqueous extract of *Azadirachtaindica*, inhibited *Escherichia coli, P. aeruginosa, S. pyogenes* and *S.aureus* (El-Mahmood *et al.*,2010). The test organisms had the same minimum inhibitory concentration (MIC) value of 5mg/ml and minimum bactericidal concentration (MBC) value of 50mg/ml. This indicated that the bark of *A. indica* has similar potency on *P. aeruginosa, Kl. ozanae, S. aureus* and *E. coli*. This is similar to the findings of the National Library of Medicine at the National Institutes of Health (www.pubmed.com) who reported that in test tubes *A. indica* has been shown to have significant effects on both gram-positive and gram-negative organisms and other bacteria that cause a wide array of human and animal diseases.

5. Conclusions

The results of this study suggest that the bark of *A. indica* can be used as an antibacterial agent against infections caused by *P. aeruginosa, K. ozanae, S. aureus* and *E. coli.* The futureprospect for this study was to analyze the purified compound for drug validation. However furtherresearch is needed *in-vitro* as well as *in - vivo* and the extracts against other different types ofmicroorganism to reach a better conclusion. This is an ongoingstudy and further work is being carried to investigate its biological activities.

6. Acknowledgement

We are grateful to the Institute of Environmental Science (IES), University of Rajshahi, and Department of Biochemistry and Molecular Biology and Pharmacy, University of Rajshahi and BCSIR laboratory, Rajshahi, Bangladesh where all of the works were done.

References

[1] Akula, C., Akula, A. and Drew, R. 2003. Somatic Em-bryogenesis in colonial neem. *Azadirachtaindica*. A. juss. *J. Microbiol. Res.* 3:162-166
[2] Biswas, K., IshitaC.., Ranajit K.B. and Uday, B. 2002. Biological activities and medicinal properties of Neem (*Azadirachtaindica*). *Current Science* 82(11): 1336-1345.
[3] De, N. and Ifeoma, E. 2002. Antimicrobial effects of com-ponents of the bark extracts of neem (*Azadirachtaindica*A. juss). J. Technol. Dev., 8: 23-28.
[4] EL-Mahmood AM, Doughari JH, Ladan N 2008. Antimi-crobial screening of stem bark extracts of *Vitellariaparadoxa*against some enteric pathogenic microorganisms. *Afr. J. Pharm. Pharmacol*. 2(5):
[5] El-Mahmood, A. M., Ogbonna, O. B. and Raji, M. 2010. The antibacterial activity of *Azadarichtaindica*(neem) seeds extracts against bacterial pathogens associated with eye and ear infections, *Journal of Medicinal Plants Research* 4(14), pp. 1414-1421.
[6] Hugo, S.B. and Rusell, A.D. 1983. *Pharmaceutical Micro-biology* 3rd Edition. Blackwell Scientific Publication, Lon-don pp. 105-125.
[7] Kayode A.A.A. and Kayode, O.T. 2011. Some Medicinal Values of *Telfairiaoccidentalis:* A Review. *American Jour-nal of Biochemistry and Molecular Biology*, 1: 30-38.
[8] Natarajan, V., Veugopal, P.V. and Menon, T. 2003. Effect of *Azadirachtaindica*(neem) on the growth pattern of der-matophytes. *Indian J. Med. Microbiol*., 21: 98-101.
[9] National Library of Medicine at the National Institutes of Health, available at www.pubmed.com.

[10] Ogbonnia, S.O., Enwuru, N.V., Onyemenen, E.U., Oyedele, G.A. and Enwuru, C.A. 2008. Phytochemical evaluation and antibacterial profile of Treculia Africana Decne bark ex-tract on gastrointestinal bacterial pathogens. *Afr. J. Biotech-nol.*, 7(10): 1385-1389.
[11] Shah, J.S., Shah, M.B., Goswami, S.S. and Santani, D.D. 2006. Mechanism of action of antiulcer activity of bark ex-tracts of *Manikarahexandra* against experimentally induced gastric ulcers in rats. *Phcog. Mag*., 2: 40-45.
[12] Silver, O.A., Cabrita, T., Pimentel, M., Diniz, A. and Gomes, E. 1997. Antimicrobial activity of Guinea Bissau traditional remedies. *Journal of Ethnopharmacology*, 50: 55-59.
[13] Subapriya, R. and Nagini, S. 2005. Medicinal properties of neem leaves: a review, *Curr Med Chem Anticancer Agents* 5(2):149-156.
[14] Talwar, G.P., Raghuvanshi, P., Misra, R., Mukherjee, S. and Shah, S. 1997. Plant immunomodulators for termination of unwanted pregnancy and for contraception and reproductive health, *Immunol Cell Biol.*, 75(2):190-192.
[15] Trease, G.E. and Evans, W.C. 1989. *A textbook of phar-magnosy*13th edition. Baluiere, Tindali, London pp. 100-101.