

RESEARCH ARTICLE

Antimicrobial activity of *Azadirachta indica* (Neem) against Pathogenic Microorganisms

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Abstract

Plants have been a source of herbal remedies throughout the history of mankind. Various medicinal plants have been used for years in daily life to treat diseases all over the world. In the present study, antimicrobial activity of *Azadirachta indica* was evaluated against gram negative pathogenic bacteria (*Escherichia coli*, *Salmonella typhi* and *Vibrio cholerae*) and gram positive bacteria (*Bacillus subtilis*). *Azadirachta indica* leaves and barks were collected from the fields of Botanical Garden in Padmashri Vikhe Patil College, Pravaranagar and pure cultures of the test organisms used for antimicrobial study were obtained from the Dept. of Microbiology, Pravara Institute of Medical Sciences, Loni. All the test organisms were screened for their antibacterial activity against leaf and bark extract of *A. indica* by agar well diffusion method. Leaf and bark extract of *Azadirachta indica* showed more inhibition zone against *Vibrio cholerae* and *Bacillus subtilis*, while *E. coli* and *S. typhi* are less susceptible to neem extract.

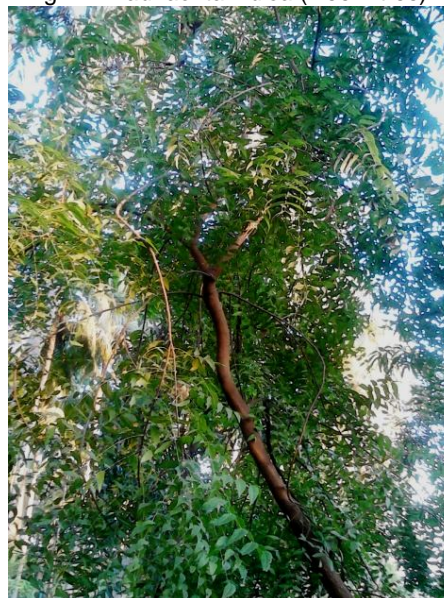
Keywords: Antimicrobial activity, *Azadirachta indica*, pathogenic bacteria, leaf and bark extract.

Introduction

Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent. Earlier studies on neem have showed that it contains active substances with multiple medicinal properties (Maragatharavlli *et al.*, 2012). Aqueous extract of neem leaf has a good therapeutic potential as antihyperglycemic agent in IDDM and NIDDM (Mossadek and Rashid, 2008; Patil *et al.*, 2013). Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed and Aslam, 2008; El-Mahmood *et al.*, 2010). Administration of alcoholic extract of neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent (Gbotolorun *et al.*, 2008). The popularity of the plant products is increasing because of their biodegradability, least persistence and least toxic to non-target organisms, economic and easy availability. Today about 200 plants with insecticidal activities are known. Among the natural products, one of the most promising natural compounds is Azadirachtin, an active compound extracted from the *Azadirachta indica* A. Juss (neem) tree (Meliaceae) (Fig. 1) whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years. Chemical investigation on the products of the neem tree was extensively undertaken in the middle of the isolation of nimbin, the first bitter compound isolated from neem oil, more than 135 compounds have been isolated from different parts

of neem (Ganguli, 2002). Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. Medicinal properties of the plant *Azadirachta indica* were studied by several workers. They were anti-pyretic (Okpanyi and Ezeukwk, 1981; Khattak *et al.*, 1985), anti-malarial and anti-tumour effect (Fujiwara *et al.*, 1982), anti-ulcer effect (Pillai and Santhakumari, 1984), anti-diabetic effect (Patil *et al.*, 2013), anti-fertility effect (Sinha *et al.*, 1984), effect on central nervous system and antioxidant activity (Bandyopadhyay *et al.*, 2002).

Fig. 1. *Azadirachta indica* (Neem tree).



Boiled neem leaf water makes an excellent antiseptic to clean wounds, soothes, swellings and eases skin problems (Bonjar and Holland, 2004). Keeping the above facts in view, this study was planned to find out the antibacterial activity of neem parts against human pathogenic bacterial strains.

Materials and methods

Collection of plant material and extract preparation: *Azadirachta indica* leaves and barks were collected from the fields of Botanical Garden in Padmashri Vikhe Patil College, Pravaranagar. The plant material collected was healthy and free from any deformalities. The collected plant material was brought to the laboratory for further processing. The plant materials were break into small pieces and then blended into powder by mixture blender. The powder is then passed from the sieve to get the equal size particles. The powder should be aseptically kept in air tight container at the moisture free place. For the extraction, the selection of solvents is done with care to meet extractability and regulatory criteria. About 25 g of powder is accurately weighed and transferred to the conical flask containing 200 mL distilled water and shaken well and powder mixed properly in water. The flask containing the mixture of powder and water is put on room temperature on aseptic condition for 7 to 8 d, extracted and filtered using muslin cloth and Whatman filter paper. The filtered liquid material is centrifuged at 4,000 rpm for 5 min and the pure extract was obtained in form of supernatant. This obtained pure extract was stored at 4°C for further work.

Isolation of test organisms: Pure cultures of the test organisms used for antimicrobial study were obtained from the Dept. of Microbiology, Pravara Institute of Medical Sciences, Loni. All the test organisms were cultured on nutrient agar slant. The cultures were maintained by sub-culturing periodically and preserved at 4°C prior to use. The gram negative bacterial strain includes: *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae* and gram positive bacterial strain included *Bacillus subtilis*.

Antibacterial activity: All the test organisms were screened for their antibacterial activity against leaf and bark extract of *Azadirachta indica* by agar well diffusion method. With the introduction of variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test. For this, the antimicrobial agent was allowed to diffuse out into the medium and interact in a plate freshly spreaded with the test organism. Antibacterial activity was performed using Muller-Hinton agar.

Preparation of stock solution: Stock solution of the extract was prepared to carry out the antimicrobial activities against selected cultures. For the preparation of the stock solution, 1 g of the extract in which 0.5 g Leaf extract and 0.5 g bark extract was accurately weighed

and dissolved in 10 mL DMSO; giving concentration of the stock solution as 100 mg/mL. This solution was then centrifuged and supernatant liquid was collected in a separate test tube, covered with paraffin wax and stored at 4°C for further use.

Agar well diffusion method: The Muller-Hinton agar plates were prepared for the antibacterial activity. About 0.1 mL of the fresh 18 h old broth culture was spread on the respective media. After spreading the culture, wells of 6 mm in dia was made at the center of the plate by using sterile cork borer. The wells were open with the help of sterile forceps. Then 100 µL of original stock solution was added by using micropipette in each well. The final concentration in the well was 1 mg/mL. The extract was allowed to diffuse; hence the prepared plates were left at room temperature for 30 min and then stored in incubator at 37°C for 24 h.

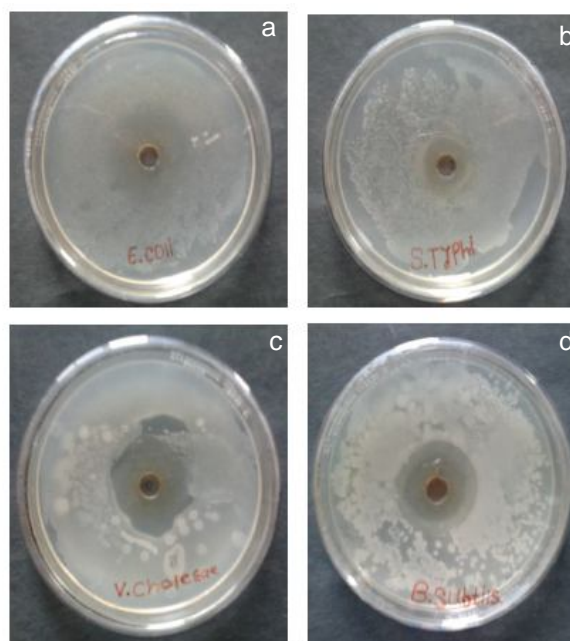
Results and discussion

As per the observations on cultured nutrient agar plates, antibacterial activity of leaf and bark extract of neem was evaluated against both gram positive and negative bacteria. Leaf and bark extract of *Azadirachta indica* showed more inhibition zone against *Vibrio cholerae* and *Bacillus subtilis*, while *E. coli* and *S. typhi* are less susceptible to neem extract (Table 1; Fig. 2a-d).

Table 1. Antimicrobial activity of leaf and bark extract of *Azadirachta indica*.

Test organism	Zone of inhibition
<i>Salmonella typhi</i>	10 mm
<i>Escherichia coli</i>	11 mm
<i>Vibrio cholerae</i>	17 mm
<i>Bacillus subtilis</i>	17 mm

Fig. 2a-d. Antimicrobial activity of leaf and bark extract of *A. indica* by agar well diffusion method.



The findings of this study coincide with the observations of several researchers. Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial activity against gram-negative and gram-positive microorganisms. Koonna and Budida (2011) reported the antimicrobial activity of the seed oil against a variety of pathogens. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus* spp. (Mehrotra *et al.*, 2010).

Conclusion

Azadirachta indica extract is an important source of compounds having anti-microbial, anti-oxidant, anti-tumor, anti-malarial, anti-fungal, anti-inflammatory and anti-viral properties. The results indicated that using plant parts of neem had beneficial effect in controlling the pathogenic microbial organisms and thus can be used in therapeutic formulations in near future.

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References

1. Maragatharavlli, S., Brinda, S., Kaviyaarshi, N.S. and Gangwar, S.R. 2012. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). *Int. J. Sci. Nature*. 3(1): 110-113.
2. Mosaddek, A.S.M. and Rashid, M.M.U.A. 2008. Comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. *Bangl. J. Pharmacol.* 3: 44-47.
3. Patil, P.R., Patil, S.P., Mane, A. and Verma, S. 2013. Antidiabetic activity of alcoholic extract of Neem (*Azadirachta indica*) root bark. *Natl. J. Physiol. Pharm. Pharmacol.* 3: 142-146.
4. Saseed, A.K. and Aslam, J. 2008. Study on the effect of neem (*Azadirachta indica*) leaves smoke in controlling airborne bacteria in residential premises. *Curr. Res. Bacteriol.* 1(2): 64-66.
5. El-Mahmood, A.M., Ogbonna, O.B. and Raji, M. 2010. The antibacterial activity *Azadirachta indica* (Neem) associated with eye and ear infections. *J. Med. Pl. Res.* 4(14): 1414-1421.
6. Gbotolorun, S.C., Osinubi, A.A., Noronha, C.C. and Okanlawon, A.O. 2008. Antifertility potential of Neem flower extract on adult female Sprague Dawley rats. *Afri. Health Sci.* 8(3): 168-173.
7. Ganguli, S. 2002. Neem: A therapeutic for all seasons. *Curr. Sci.* 82: 1304.
8. Okpanyi, S.N. and Ezeukwk, G.C. 1981. Anti-inflammatory and anti-pyretic activities of *Azadirachta indica*. *Planta Medica.* 41: 34-49.
9. Khattak, S.G., Gilani, S.N. and Ikram, M. 1985. Antipyretic studies on some indigenous Pakistani Medicinal Plants. *J. Ethno Pharmacol.* 14: 45-51.
10. Fujiwara, T., Takeka, T.O., Gihara, Y., Shimizu, M., Nomura, T. and Tomuta, Y. 1982. Studies on the structure of polysaccharides from the bark of *Medica Azardichta*. *Chem. Pharmaceut. Bull.* 30: 4025-4030.
11. Pillai, N.R. and Santhakumari, G. 1981. Hypoglycemic activity of *Melia azadirachta*. *Ind. J. Med. Res.* 74: 931-933.
12. Sinha, K.C., Riar, S.S., Tiwary, R.S., Dhawan, A.K., Bhadhan, J., Thomas, P., Kain, A.K. and Jain, R.K. 1984. Neem oil as a vaginal contraceptive. *Ind. J. Med. Res.* 79: 131-136.
13. Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C.K., Chakraborty, T., Bhattacharya, K. and Banerjee, R.K. 2002. Gastroprotective effect of neem (*Azadirachta indica*) bark extract: Possible involvement of H⁺-K⁺-ATPase inhibition and scavenging of hydroxyl radical. *Life Sci.* 71: 2845-2865.
14. Bojar, R.A. and Holland, K.T. 2004. Acne and Propionibacterium acnes. *Clin. Dermatol.* 22: 375-379.
15. Koonna, S.J. and Budida, S. 2011. Antimicrobial potential of the extracts of the leaves of *Azadirachta indica* Linn. *Nat. Sci. Biol.* 3(1): 65-69.
16. Mehrotra, S., Srivastava, A.K. and Nandi, S.P. 2010. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J. Med. Pl. Res.* 4: 2473-2478.
17. Arya, M. and Tiwari, R. 2013. Efficacy of plant and animal origin bio-products against lesser grain borer, rhyzoperthadominica (fab.) in stored wheat. *Int. J. Rec. Sci. Res.* 4(5): 649- 653.