

Azadirachta Indica: Indian Neem, A Natural Killer of Microorganisms**Nazam Khan¹, Amir Khan², Umar Farooq^{2*}**¹College of Applied Medical Sciences, Shaqra University, Shaqra, KSA^{2,2*}Department of Oral Medicine and Applied Dentistry, College of Dentistry, Taif University, Taif, Kingdom of Saudi Arabia (KSA)^{*}*Email: ufarooq8@gmail.com**DOI 10.51129/ujpah-2020-28-1(1)*

Abstract-Medicinal Plants are always of immense vital component for pharmaceutical word. *Azadirachta indica*, commonly known as neem, has attracted worldwide prominence in recent years owing to its wide range of medicinal properties. Neem is a medicinal plant which is always at top from ancient world to modern world. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem- a tree for solving global problems'. Various parts of neem are sources for mankind to treat many severe diseases. 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. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic

properties. Keeping these points in view the present study is aimed to study antimicrobial activity of plant extracts prepared from leaves of neem plant against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. There is a significant antimicrobial activity observed by neem leave extract against these microbial strains.

Keywords: *Azadirachta indica*, Antibacterial activity, Immunomodulatory activity.

Introduction

Plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations obtained from commonly used herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. Thousands of natural products obtained from plant sources have been used by mankind for alleviating diseases and/or their symptoms.

The evergreen tree neem has been used as a traditional medicine for many centuries in

India. Various preparations of neem obtained from its different parts have been found to exert antibacterial, antimalarial, contraceptive and antiulcer activities (Biswas, et al, 2002).

Neem is perhaps the most commonly used traditional medicinal plant in India. Almost all parts of the plant are endowed with medicinal property. During the past few decades, apart from the studies in the chemistry of neem compounds, considerable progress has been made in evaluating biological activity of phytochemical compounds for medicinal applications. In the modern era, Neem is a component of Ayurvedic medicine, which has been practised in India since post-vedic period. The Neem plant possesses antibacterial, antifungal, antiviral and antiseptic properties (Subapriya, 2005).

First of all the three bioactive compounds namely nimbin, nimbinin, nimbidin were derived from Neem tree in 1942 (Siddiqui et al., 1986). These compounds have anthelmintic, antifungal, antibacterial and antiviral activities. Nimbin is a sulphur free crystalline product with melting point 205°C. Nimbidin have potential antibacterial ingredient and highest yielding bitter compound. These compounds are stable and found in substantial quantities in the Neem plant.

Neem leaves have antibacterial properties and could be used for controlling airborne

bacterial contamination in the residential premises. Administration of alcoholic extract of neem 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 aqueous extract of neem have powerful chemotherapeutic and antiviral agent (Hassan et al., 2010). Aqueous leaf extract of neem has good therapeutic potential as an anti-hyperglycemic agent (Sonia and Srinivasan, 1999). More than 135 compounds have been isolated from different parts of neem and the chemical properties have been studied and structural diversity of these compounds has been elucidated (Khan and Wassilar, 1987).

In view of the medicinal value of Neem tree, the present work was intended to study the antibacterial activity of aqueous and methanolic extracts of the leaves of *Azadirachta indica*.

Material and Methods

Collection of Leaves

Leaves of *Azadirachta indica* (neem) were collected from Yashwant Singh Parmar, University of Horticulture and Forestry, Nauni, Solan, India.

Bacterial culture

The bacterial strains i.e., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and

Escherichia coli for evaluating antibacterial activity were obtained from the Department of Microbiology, SILB, Solan. These strains were then sub-cultured and maintained in Molecular and Immuno-parasitology Laboratory over Nutrient Agar medium.

Preparation of Methanolic and Aqueous extracts of Neem leaves

For preparing methanolic extract, the dried and crushed neem leaves were used. Twenty grams of this powder was weighed and loaded to the Soxhlet apparatus. 200 ml of methanol was used as solvent for preparing the alcoholic extract. Extraction was carried out for 16 hours. After extraction the solvent containing the crude extract of neem was dried in a china dish under air current. The dried extract was scrapped from the china dish after 4-5 days with the help of surgical blade. This final product was stored at 4°C until use. For preparing aqueous extract of neem leaves, the same procedure as that of methanolic extract was involved. Only the solvent used here was 200 ml distilled water for 20 grams of neem leaves (Syarifah *et al.*, 2014).

Antibacterial activity

Antibacterial activity of the methanolic and aqueous extracts was studied by agar-well

diffusion method on Mueller Hinton Agar (MHA) medium using five different species of bacteria namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* as described by NCCLS methods. Methanolic extract was dissolved in Dimethyl Sulphoxide (DMSO) and concentrations of 10 mg/ml i.e., 500 µg crude extract per well were used for the study. 50 µl of aqueous extract was poured in one of the wells without dissolving in DMSO. Ciprofloxacin was used as a standard antibiotic and was applied in the fourth well with a concentration of (1 mg/ml i.e., 50 µg per well). DMSO alone was also applied in one of the four wells to check whether it has any activity or not. These Petri plates were then incubated at 37 °C for two days. After two days zone of inhibitions around the wells were measured using an antibiotic zone scale.

Results and Discussion

The results of antibacterial assay have shown that *E.coli* has the maximum zone of inhibition for both methanolic and aqueous extracts, whereas *Bacillus subtilis* has shown minimum zone of inhibition for both methanolic as well as aqueous extracts (Table-1, Fig. 1-5) Ciprofloxacin was used as a control.

Table -1 Antibacterial activity of aqueous and methanolic extracts of leaves of *Azadirachta indica*

S. No.	Bacterial species	Gram+ or Gram-Bacteria	Zone of inhibition (mm)		
			Methanolic	Aqueous	Ciprofloxacin
1	<i>Staphylococcus aureus</i>	G+	32	22	38
2	<i>Bacillus subtilis</i>	G+	29	17	35
3	<i>Escherichia coli</i>	G-	34	19	36
4	<i>Pseudomonas aeruginosa</i>	G-	-	-	39
5	<i>Klebsiella pneumoniae</i>	G-	-	-	33

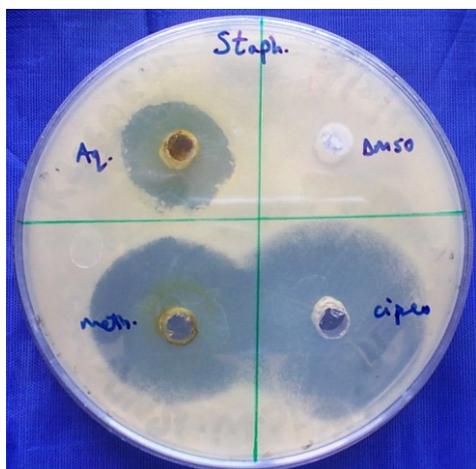


Fig.-1 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Staphylococcus aureus*.

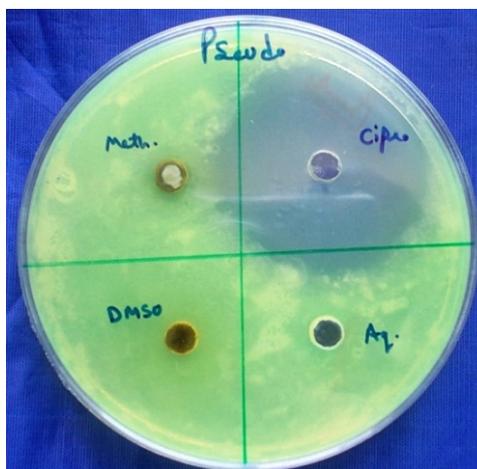


Fig.-2 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Pseudomonas aeruginosa*.

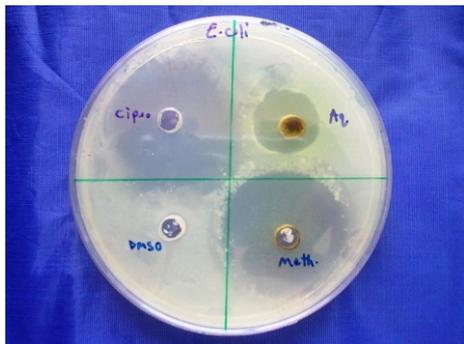


Fig.-3 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Escherichia coli*

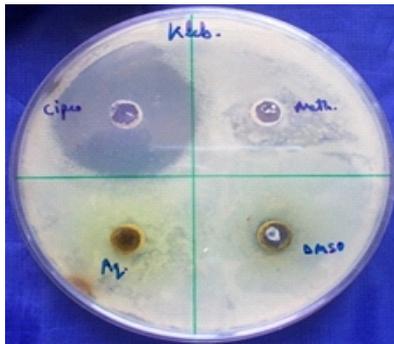


Fig.-4 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Klebsiella pneumoniae*

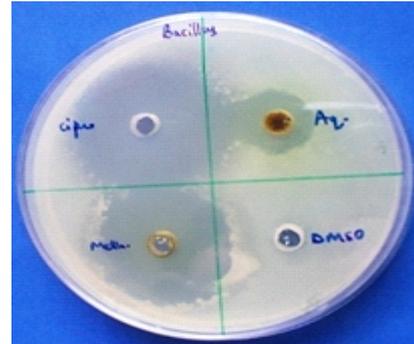


Fig.-5 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Bacillus subtilis*

Azadirachta indica (Neem) is perhaps the most commonly used traditional medicinal plant in India. Neem is one of the major components in Ayurvedic medicine, which has been practised in India since many centuries due to its antibacterial, antifungal, antiviral and antiseptic properties. It is also reported to have antiplaque and many other pharmacological properties. Microorganisms are concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as on other living organisms which may be fatal. The agents which have the capacity to kill the microbes or arrest the multiplication are called the antimicrobial agents or drugs. Keeping these points in view, we planned to study the antimicrobial activity of Neem leaves. The extracts of plant leaves were prepared in aqueous and methanol solution. The bacteria selected for the study were *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *B. Subtilis*. These are the most commonly found micropathogens

causing number of infections in human in day to day life.

Methanolic and aqueous extracts prepared from leaves were then subjected for antimicrobial activity by using five bacterial species on Muller Hinton Agar plates by agar well diffusion method. Our findings suggest that methanolic extract of neem is highly efficient in inhibiting *E. coli* followed by *S. aureus* and *B. subtilis*. Jahan *et al.*, (2007) has also reported that the neem leaves are active against these three bacteria with similar zones of inhibition. Neem extract has not shown any activity against *P. aeruginosa* and *K. pneumoniae*. A study carried out by Maragathavalli *et al.*, (2012) have shown considerable activities of neem extract against *P. aeruginosa* and *K. pneumoniae* which is contrary to the results obtained in the present study. This might be due to the different bacterial strains used in the antibacterial assay. Similarly in another study antibacterial activity of neem extract was reported against *Mycobacterium*

tuberculosis which is a potential human pathogen (Chakraborty *et al.*, 2001). Alam sher, (2015) has reported anti-dermatophytic activity of the ethanolic and aqueous extracts of the neem leaves. Neem extracts have also been reported to have inhibitory action against food borne pathogens which include *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Pseudomonas putida* etc. (Mahfuzul *et al.*, 2007). The present work and the literature reports suggest that the neem tree has broad spectrum of inhibition of microbial pathogens and this may be further evaluated along with the standard antibiotics to determine the synergistic effect of neem extract against the pathogenic microflora to serve the mankind.

Conclusion

Neem is used as a traditional medicine and as a source of many therapeutic agents in India. It grows well in the tropical country like India. In the present study, antimicrobial activity of neem leaves was studied against different pathogenic bacteria. Methanolic extract of Neem possesses a good antibacterial activity and is comparable to the standard antibiotic.

We found potent antimicrobial activity of neem leaves extract against different bacteria. It has been observed that the leaf extract have shown strong antimicrobial activity against *E. coli*, whereas weak antimicrobial activity was observed against *B. subtilis* as zone of inhibition was smallest. No zone of inhibition in the case of *P. aeruginosa* and *K. pneumoniae* was observed revealing the inefficiency of neem extracts in inhibiting these bacteria. We observed high efficacy of methanolic extracts of neem leaves and moderate activity of aqueous extract against

three out of five bacterial species involved in the study.

To conclude, the study that *Azadirachta indica* leaf extract has potent antibacterial activity against different pathogenic bacteria. Further studies are required in order to understand its mechanism of action which may limit the use or replace the antibiotics which are now no longer safe and efficient to full extent.

References

- Alam, M.; ATM.; Rakib, F.K.; Al-Hasan, M.A.; Hasan, M.S. and Ali, M.A. Effects of neem leave powder as a growth promoter in broilers. *International Journal of Natural and Social Sciences*, 2015, 2: 22-26 .
- Biswas, K.; Chattopadhyay, I.; Banerjee, R.K., and Bandyopadhyay, U. "Biological activities and medicinal properties of neem (*Azadirachta indica*)," *Current Science*, 2002, 82(11):1336-1345.
- Chakraborty, M.S.K.; Maiti, A.P. and Kundu. Antibacterial activity of neem extract against *Mycobacterium tuberculosis*. *Journal of Ethnopharmacology*. 2001, 77(1): 49-55 .
- Gbotolorum, S.C.; Osinubi, A.A.; Noronha, C.C. and Okanlawon, A.O. Antifertility potential of neem flower extract on adult female Sprague-Dawley rats. *African health science*, 2008, 8(3): 168-173.
- Hassan, A.; Helmy, Walaa and Taie, Hanan. In vitro antitumor and antiviral activities of seeds and leaves neem (*Azadirachta indica*) extracts. *International Journal of Academic Research*, 2010, 2(2).
- Jahan, T.; Begum, Z.A. and Sultana, S. Effect of neem oil on some pathogenic bacteria. *Bangladesh J. Pharmacol.*, 2007, 2: 71-72.

Khan, M. and Wassilew, S.W. The effect of raw materials from the neem tree in: Natural pesticides from the neem tree and other tropical plants. Schmutterer, H. and K.R.S. Ascher(eds)GIZ, Eschborn, Germany,1987, Pp 645-650.

Mahfuzul Hoque, M.D.; Bari, M.L.; Inatsu, Y.; Juneja, V.K. and Kawamoto, S. Extracts against food borne pathogens and spoilage bacteria. Food Borne Pathogens dis.Winter. 2007, 4(4):481.

Maragathavalli, S.; Brindha,S.; Kaviyarasi, N.S.; B. Annadurai, B. and Gangwar, S.K. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). 2012, 3: 110-113.

Siddiqui, S.; Siddiqui, H.E. and Shaheen F.D. Studies on the Chemical Constituents of *Azadirachta indica*. *Naturforsch*,1986, 41b(1): 922-924.

Sonia, B. and Srinivasan B.P. Investigation into the anti-diabetic activity of *azadirachta indica*. *Indian Journal of Pharmacology*, 1999, 31:138-141.

Subapriya, R. and Nagini, S. Medicinal properties of Neem leaves. A Review Source, Current Medicinal Chemistry - Anti-Cancer Agents, 2005, 5:149-156.

Syarifah, M.H.D and Izham Bin, A.R. Antimicrobial Activity of Methanolic neem extract on Wound Infection Bacteria. International Conference on Biological, Chemical and Environmental Sciences, 2014, 14(15):72-16.