Asian Journal of Multidisciplinary Studies

ISSN: 2321-8819 (Online)
2348-7186 (Print)
Impact Factor: 1.498
Vol. 4, Issue 3, February 2016

# Evaluation of antibacterial and antifungal activity of various concentrations of *Azadirachta indica* (Neem) against human pathogenic bacterial and fungal strains

***Ashwini .R.Tambe , *R.M.Pawar, and ** S.B.Deokar**
*****Department of Zoology, Nowrosjee Wadia College, Pune, India
**Department of Biotechnology, Nowrosjee Wadia College, Pune, India

***ABSTRACT –*** *Drugs from natural sources are used for treating various diseases since ancient times. From the literature it is clear that various type of pharmacological and biological activities are associated with Azadirachta indica. The objective of study is to evaluate antibacterial and antifungal activity of Neem (Azadirachta indica) leaves extracts (Ethanol and Aqueous) against bacterial strain Escherichia coli(Gram negative), Staphylococcus aureus (Gram positive) and fungal strain Aspergillus niger. Ethanol and aqueous extracts of varying concentrations such as 0% , 3% , 6% , 9% were prepared and tested against test microorganisms using agar well diffusion method.The values of Zone of inhibition were tabulated according to the concentration of the tested agent and data was statistically analyzed.. The Zone of inhibition showed efficiency of plant extract. Neem's apparent ability to control certain strains of this bacterium is been of great importance to dairying in the nations where Neem is used. The results showed that E.coli showed highest antibacterial activity of 24mm Zone of inhibition at 9% as compaired with activity against S.aureus of 22mm Zone of inhibition at 9%. Antifungal activity against A.niger showed highest Zone of inhibition of 19mm and 15mm at 9% concentration of both aqueous and ethanol extracts.The antibiotics such as gentamicin, penicillin and antifungicides such as clotrimazole were tested against human pathogens as positive control. The antibiotic and antifungal activity of leaves of Neem tree and their utility in diseases have been confirmed experimentally. The results therefore confirm the traditional use of Neem for its antimicrobial properties.*

**KEYWORDS**–*Azadirachta indica, Escherichia coli , Staphylococcus aureus , Aspergillus niger,* Zone of inhibition (ZOI) , Antibacterial activity , Antifungal activity

**INTRODUCTION**- In human society from time immemorial medicinal plants have played an important role in prevention and control of diseases[1]. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise supports the use of the Neem in traditional medicine to treat infections[2].*Azadirachta indica* (Neem) is perhaps the most useful traditional medicinal plant. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. Most of the parts of the plant such as fruits , seeds , leaves , bark and roots contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory , antiulcer and antifungal properties[3]. Neem tree (*Azadirachta indica*) is a tree in the mahogany family Meliaceae, is an evergreen tree. It is a fast growing tree, average height 15-20 m but rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. It is also used as an household pesticide. Ayurvedic uses of Neem include the treatment of fever, leprosy, malaria and tuberculosis. Various folk remedies use as an anthelmintic, antifeedant, antiseptic, diuretic, contraceptive, parasiticide and insecticide. The extract or oil of Neem is effective against inflammatory, analgesic, antipyretic activities, andimmuno modulatory activities. It also shown an immune – stimulant activity, antidiabetic, antiulcer effect. Oral administration of aqueous extract of Neem leaf also shows antifertility effect in mice[4]. Neem aqueous extract has powerful chemotherapeutic and viral agent. *A.indica* is used for the treatment of diabetes like Neem biscuits and shows the potential role of anti-diabetic activity[5].Neem leaves are used in India for curing diarrhea and cholera[6]. Millions of people in India and Africa use twigs as "tooth brush" everyday[7].

## MATERIALS AND METHODS -

**SAMPLE COLLECTION-** The leaves of Neem were collected from Botanical garden of Nowrosjee wadia college, Pune, India. The samples were washed thrice using tap water followed by distilled water and were dried under shade in hygiene conditions for 10-12 days. All the materials was ground in an electric grinder to produce fine powder. Powdered material was stored at 4°C in an air tight bottle.

## COLLECTION OF TEST ORGANISMS-

The test organisms used in this study consisted of *E.coli* NCIM 5010 (Gram negative)and *S.aureus* NCIM 2079 (Gram positive) bacteria and *A.niger* NCIM 501 fungal strain. Cultures were obtained

from NCIM, Pune.The test organisms were cultured on agar slants and stored at 4°C in refrigerator.

**PREPARATION OF WATER EXTRACTS-**

Fine grounded powder was measured with electronic weighing balance.Various concentrations were made such as 0%,3%,6% and 9%. For 0% ,3%,6%,9% solutions 0gm,3gm,6gm,9gm fine powder was suspended in 100ml each of distilled water .These were soaked for 72 hours ,kept on rotary shaker for constant stirring. The solution was carefully filtered with help of Whatmann filter paper no.1 into a sterilized test tubes and filterates were obtained. Filterates were covered with aluminium foil and stored in refrigerator at 4°C until required.

**PREPARATION OF ETHANOL EXTRACTS**-

Fine grounded powder was measured with electronic weighing balance.Various concentrations were made such as 0%,3%,6% and 9%. For 0% ,3%,6%,9% solutions 0gm,3gm,6gm,9gm fine powder was suspended in 100ml each of ethanol solution .These were soaked for 72 hours,kept on rotary shaker for constant stirring.The solution was carefully filtered with help of Whatmann filter paper no.1 into a sterilized test tubes and filterates were obtained.Filterates were covered with aluminium foil and stored in refrigerator at 4°C until required.

**Agar diffusion method**- The method is suitable for organisms that grows rapidly overnight at 35-37 °C .The well of 6mm is made in medium with sterile cork borer after inoculation with microorganisms. When well is loaded with antibiotics, it diffuses in the medium and inhibits the growth of organism. There is logarithmic reduction in antibiotic concentration. The zone of inhibition of bacterial growth around each well is measured and the susceptibility is determined. Nutrient Agar (2.8 gm/100 ml of distilled water HiMedia) and Potato dextrose agar (3.9gm in 100ml distilled water HiMedia) was prepared, autoclaved at 121°C for 15 minutes at 15lbs and poured in sterile petri plates up to a uniform thickness of approximately 5-6mm and the agar was allowed to set at ambient temperature and used. Inoculums- The bacterial cultures were inoculated in Nutrient broth and incubated at 37°C and were used as inoculums. For fungal inoculum fungal culture was grown on Czapek Dox agar slants a sporulating medium. Slants was incubated at ambient temperature for 2-3 days. Spore suspension was prepared in sterile 0.01% Tween-20 and used as inoculum.

**METHODS-**

**Anti-bacterial activity assay**- Using a micropippete added 30µl of bacterial suspension on nutrient agar plates.With help of sterile glass spreader, spreaded these suspension of *E.coli* and *S.aureus* respectively throughout the plate.Wells were punched of 6mm diameter into plates. Loaded 20-30µl of the plant extract of various concentrations .Allowed to stand for 30mins for agar diffusion.Plates were incubated at 37°C for 24hrs. Observed the bacterial activity by measuring the zone of inhibition against the test organism by clear measuring scale. Antibiotics such as Penicillin, gentamicin also showed zone of inhibition when used in different concentrations against bacterial strain ,used as a positive control .0% extract was used as negative control, which did not showed zone of inhibition against bacterial strains.

**Anti-fungal activity assay**- Using a micropippete added 30µl of fungal spore suspension of *A.niger* on potato dextrose agar plates. With help of sterile glass spreader, spreaded the suspension throughout the plate.Wells were punched of 6mm diameter into plates. Loaded 20-30µl of the extract. Allowed to stand for 30mins for agar diffusion. Plates were incubated at 22°C for 48-72 hrs. Observed the antifungal activity by measuring the zone of inhibition against the test organism by measuring scale. Antifungicide such as clotrimazole showed zone of inhibition when used in different concentrations against fungal strain ,used as a positive control .0% extract was used as negative control, which did not showed zone of inhibition against fungal strains.

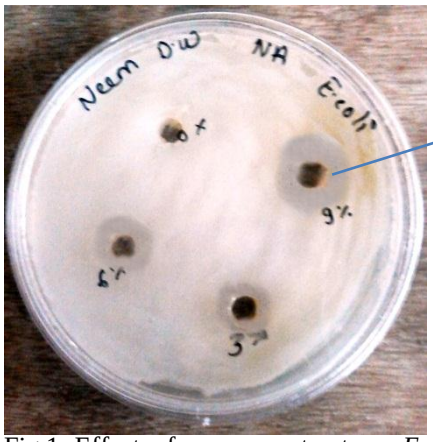
**RESULTS-** The current study showed that plant extract of Neem exerted antibacterial and antifungal activity against selected human pathogens.The effect of different concentrations of Neem on *E.coli* is tabulated in Table.1. It is interested to know that even at the lowest concentration ,test plant extract showed significant antimicrobial activity. The results showed that the extract possessed antimicrobial activity against test organisms, depending upon their capacity for diffusion into agar medium. Aqueous extract showed maximum zone of inhibition of 24mm at 9% and minimum zone of inhibition of 15mm at 3% concentration. Ethanol extract showed maximum zone of inhibition of 19mm at 9% and minimum zone of inhibition of 8mm at 3%. Aqueous extract was found to be more efficient when compaired with that of ethanol extract against *E.coli*. Figure 1 and figure 3 shows effect of Neem extract against *E.coli*.

Table 2 showed effect of different concentrations of Neem extract against *S.aureus*.Aqueous extract showed maximum ZOI of 22mm at 9% and minimum ZOI of 10mm at 3% which is comparatively less as compared with ZOI against *E.coli*. Ethanol extract showed maximum ZOI of 20mm at 9% and minimum ZOI of 15mm at 3% which is comparatively greater than ZOI of *E.coli*.

Figure 2 and figure 4 shows effect of Neem extract against *S.aureus.*

Table 3 showed antifungal activity of Neem against *A.niger*. figure 5 and figure 6 showed antifungal activity which is tabulated in table 3.maximum ZOI was about 19mm at 9% and minimum ZOI was about 8mm at 3% by aqueous extract. Ethanol extract showed maximum ZOI of 15mm at 9% and minimum ZOI of 7mm at 3% which is comparatively less as compaired with antibacterial activity.Extract of Neem leaves showed highest zone of inhibition against each bacterial and fungal strain according to its concentrations.The higher the concentration higher efficiency was found against human pathogens. Hence, plant extracts of Neem can be used for treating various diseases of mankind and even other mammals.

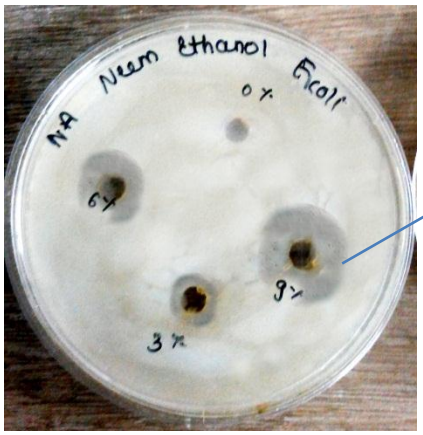
**1.Figures indicate antibacterial activity of Neem against *E.coli* and *S.aureus***




Zone of inhibition


Fig.1- Effect of aqueous extract on *E.coli*

Fig.2- Effect of aqueous extract on *S.aureus*




Zone of inhibition

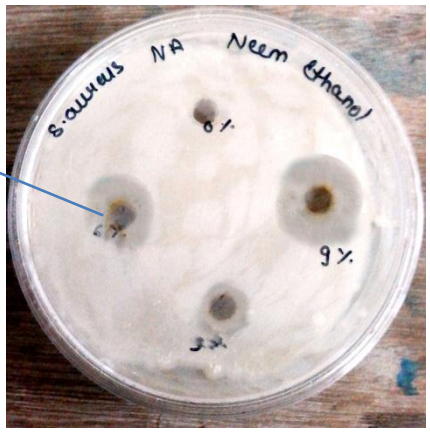
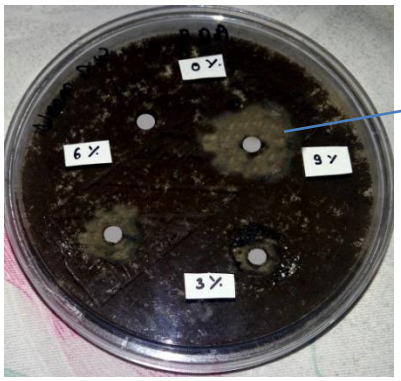



Fig.3-Efffect of ethanol extract on *E.coli*

Fig.4- Effect of ethanol extract on *S.aureus*

**2. Figures indicate antifungal activity of Neem against *A.niger***




Zone of inhibition

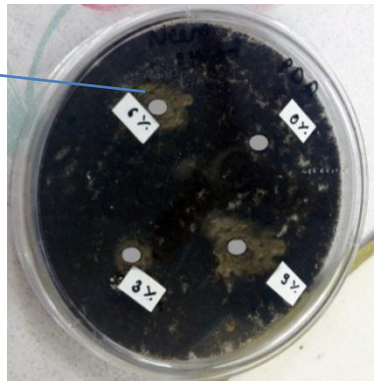
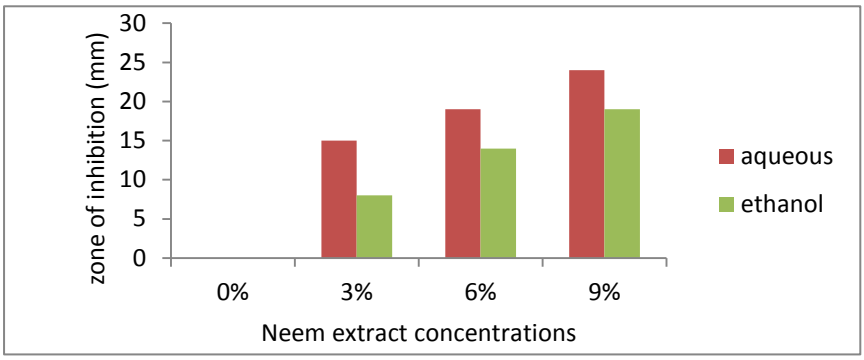



Fig.5- Effect of aqueous extract on *A.niger*

Fig.6- Effect of ethanol extract on *A.niger*

| solvent | Concentration | *E.coli* (ZOI in mm) |
|---|---|---|
| Aqueous | 0% | 0 |
| | 3% | 15 |
| | 6% | 19 |
| | 9% | 24 |
| Ethanol | 0% | 0 |
| | 3% | 8 |
| | 6% | 14 |
| | 9% | 19 |

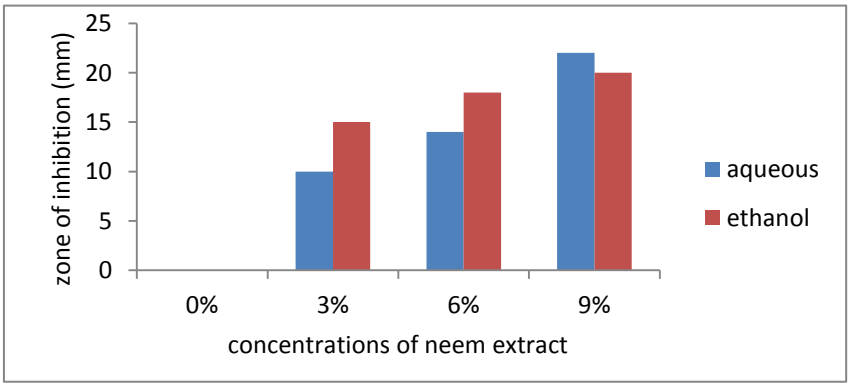
**Table.1- Antibacterial activity of Neem extract against *E.coli***




**Graph.1- Antibacterial activity of Neem extract against *E.coli***

| solvent | Concentration | *S.aureus*(ZOI in mm) |
|---|---|---|
| Aqueous | 0% | 0 |
| | 3% | 10 |
| | 6% | 14 |
| | 9% | 22 |
| Ethanol | 0% | 0 |
| | 3% | 15 |
| | 6% | 18 |
| | 9% | 20 |

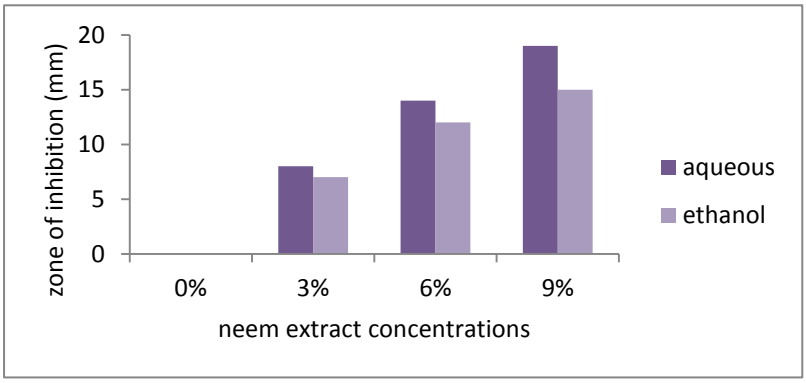
**Table .2- Antibacterial activity of Neem extract against *S.aureus***




**Graph .2- Antibacterial activity of Neem extract against *S.aureus***

| | Concentration | *A.niger*(ZOI in mm) |
|---|---|---|
| Aqueous | 0% | 0 |
| | 3% | 8 |
| | 6% | 14 |
| | 9% | 19 |
| Ethanol | 0% | 0 |
| | 3% | 7 |
| | 6% | 12 |
| | 9% | 15 |

**Table .3- Antifungal activity of Neem extract against *A.niger***




**Graph .3- Antifungal activity of Neem extract against *A.niger***

**DISCUSSION-** Medicinal plants continue to be an important therapeutic aid for alevating the ailments of humankind. The green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse effects. Neem extracts were screened for potential antibacterial activity against medically important bacterial strains namely *E.coli* and *S.aureus*. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs. Natural products are known to play an important role in both drug discovery and chemical biology. These antibiotic and antifungal principles are actually the defensive mechanism of the plants against different pathogens.

**ACKNOWLEDGEMNT**- I am grateful to our Principal Dr. K. S .Venkataraghavan**,** and Head of the Department of Zoology Dr. Veena Rambal for giving me the opportunity and necessary infrastructure. I am thankfull to the Department of Zoology, Nowrosjee Wadia College, Pune for providing me the laboratory facilities during the study work.

**REFERENCES-**

1. Ad hoc panel.Neem: A Tree for solving global problems. National academy press.Washington,1992,23-75
2. Cappucino .J. G,Sherman N.Microbiology A Laboratory Manual. Pearson. 7th edition.2005.13-16.
3. Chibber S.,Sharma N.Medicinal and therapeutical potential of Neem (*Azadirachta indica*) :A Review.International Journal of Scientific and Research Publications.2014,4(5):1-5
4. Deshmukh. A. M.Media,Stains and reagents in Microbiology.PAMA Publication.1997:39-139
5. Jain.S.K. Medicinal plants.National book trust, India.1994.31-33
6. Magargathavalli s.,et al.,Antimicrobial activity in leaf extract of Neem (*Azadirachtaindica*). International Journal of Science and Nature.2012,3(1):110-113
7. Mistry K.S.et al.,The antimicrobial activity of *Azadirachta indica,Mimusops elengi,Tinospora cardifolia, Oscimum sanctum* and 2% chlorhexidine gluconateon common endodontic pathogens:An in vitro study.European Journal of Dentistry.2014;3:172-177
8. Mohammad Asif .Antimicrobial Potential of *Azadirachta indica* against pathogenic bacteria and fungi.Journal of Pharmacognosy and Phytochemistry.2012,1(4):78-83
9. Nair L. N, Methods of microbial and plant biotechnology. New central book agency ltd. London.27-82

10. Panchal P,Bajaj H,et al.,*Azadirachta indica* (Neem) antibacterial effects against *Escherichia coli*and Salmonella.Guru Drone Journal of Pharmacy and Research.2013,1(1):18-21
11. Ravva S.V and Korn A.Effect of Neem (*Azadirachta indica*) on the survival of *Escherichia coli* O157:H7 in dairy manure.International Journal of Environmental Research and Public health.2015,12:7794-7803
12. Yerima M.B.,et al.,Effect of Neem extracts (*Azadirachta indica*) on bacteria isolated from adult moth.Nigerian Journal of Basic and Applied Science.2012,20(1):64-67