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Screening of Antibacterial Activity of *Azadirachta Indica* (Neem) by Using Leaf Extract against *Escherichia Coli* and *Bacillus Subtilis*

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Abstract: *Azadirachta Indica* (Neem) is the member of the Meliaceae family. It is rich source of Antibacterial properties and Antioxidants. They have consist 65 biochemically active compounds. In ancient time plant and plant product are used for medicine. Neem (*Azadirachta indica*) is the most useful and traditional medicine used in India. They are also used in cosmetics and beauty aid. The demand of neem products are increasing day by day. In agricultural sector is now becoming a major consumer of the neem products. They also have use agricultural, Pesticides made from neem are product if natural plant origin. They are biodegradable and non-toxic. They do not have any side effects. It is widely used in Ayurvedic, Unani and Homeopathic. All the Parts of neem plants like Neem leaves, Fruits, Seeds, Organic Product, Roots and Bark are utilized to make various medicine. But Neem leaves and its constituents have been shown to display many properties like- Antiulcer, Antimalarial, Antifungal, Antibacterial, Antiviral, Antimutagenic and Anticarcinogenic. The present study was evaluate Antimicrobial activity of Neem extract against some pathogens. The Antibacterial effect of *Azadirachta Indica* against Gram Negative Bacteria (*Escherichia coli*) and Gram Positive Bacteria (*Bacillus Subtilis*) was determined using the MIC (Minimum Inhibitory Concentration) on Agar Media by Disk Diffusion Method. The inhibition zone were measured in millimetre with the help of Zone Reader. The data show that the inhibition zone of Neem Extract were greater than each of Phytoconstituents 80mg/ml concentration was the more effective than 40 mg/ml and 20mg/ml was more effective than 10mg/ml.

Keywords: Anti bacterial activity, Neem, *E.coli*, *Bacillus Subtilis*, Pesticides, Antimutagenic.

I. INTRODUCTION

Neem is a fast growing tree usually reaches a height of 15-20 m, and under very favourable condition up to approximately 30-35 m. It is a evergreen plant, which is cultivated in various parts of countries. Every parts of the tree has been used in traditional medicine. It is used in Ayurveda, Unani and Homeopathic medicine.

The Sanskrit name of the neem tree is **Arishtha**, meaning reliever of sickness (Aslam *et al.*, 2009). It is belonging the family of Meliaceae. *Azadirachta indica* is commonly know as Neem. In India, Neem is knows as “the village pharmacy” because of healing versatility (Prashanth *et al.*, 2014).

They also have contain mainly biologically active compounds which have tendency for the development as medicinal plant. The medicinal plants are also used for human beings (Abalaka *et al.*, 2012). Many Ayurveda plants will continue to be a valuable source of new molecules which may, after possible many chemicals manipulation to provide or improved new drugs (Shah *et al.*, 2006).

Neem involved a vast assay of biologically active compounds, they have consist many chemical diverse and structurally complex (Talwar *et al.*, 1997). All the parts of neem plants are used for various activities. Like tree- leaves, flowers, seeds, fruits, roots, and bark have been used traditionally for the treatment of many inflammation, fever, infection, skin diseases and dental disorder (Biswas *et al.*, 2002). Neem tree has adaptability to grow in wide range of climatic, topographic and particular factor.

They are grow in dry, shallow soils and hard soil. Mostly they require a some water or plenty of sunlight (Francine *et al.*, 2015). It is an excellent wound healer.

It is also work as Antiseptic and make good for minor cuts and abrasions .They also work in skin burns (Krishna *et al.*, 2015). There are many constituents in Neem. The most important of these are Alkaloids, Glycosides, Saponins and Tannic acids (Aslam *et al.*, 2009)).

II. MATERIALS AND METHODS

A. Collection of Plant Material

The plant Neem was used for the Practical. The Leaves were collected from Shiv Ganga Apartment, Vasundhara.

The plant extract was prepared of Neem Leaves. Mature neem leaves was collected and wash with distilled water. The dried leaves and straw were removed from it. The leaves were completely dried at room temperature without any contamination. It take 6 weeks for dried the leaves.

B. Preparation of Neem Powder

After 6 weeks, when the Neem leaves were completely dried. We take Mixer grinder to grind the Neem leaves into powder and then sieved to get fine powder and get stored in air tight container until needed for use.

C. Laboratory Equipment's

Materials and reagents used in this study listed in appendices. The research was mainly carried out in Institute Of Applied Medicine And Research (IAMR), Biology laboratory.

D. Preparation Of Aqueous Extract From Dried Neem Leaves

Azadirachta indica 50 gram of powered leaves were mixed with 200ml of solvent (Methanol) in a beaker. Mix the extract completely, After then we filtered the extract with Whatman Filter paper No.1. The supernatant was collected and solvent was evaporated. The crude extract was diluted with 5% Dimethyle sulfoxide (DMSO) and stored for further use .

E. Preparation Of Different Concentration In Vial From Neem Extract

Four different concentration are (800 milligram, 400 milligram, 200 milligram and 100 milligram) are prepared in four different glass vial and put some disk (which is made by sterilized Whatman filter paper) in each of them and soaked for 24 hours.

F. Microorganism

The pathogenic strains of *E.coli* and *Bacillus Subtilis* for antibacterial test were used. These strain were obtained from bacterial stocks, Department Of Microbiology, Institute Of Applied Medicine Research, Duhai.

For bacterial sensitivity test Nutrient Agar Media (NAM) was prepared.

G. Antibacterial Property was determined by Disk Diffusion Method

The method Disk Diffusion was described by Kirby-Bauer. Normally, this method is used for testing the effect of chemical drugs on bacteria, therefore the same method was used in order to compare the effectiveness of different Neem extract concentration from leaves.

The plant extract were prepared by taking 1 organic solvent (Methanol) for 2 days. For this experiment Nutrient Agar Media (NAM) were prepared and Autoclave at 121°C for 121psi. After Autoclaving media was poured in sterilized petri plates and allowed solidify. After few minutes take a sterilized cotton swabs dip into the bacterial culture (*Escherichia coli*), and spreading on the few plates and then dip the new sterilized cotton swabs into other bacterial culture (*Bacillus Subtilis*) and spreading on the few plate.

Now put the Disk of different concentration (800 milligram, 400 milligram, 200 milligram, 100 milligram) on different petri plate aseptically on the Agar surface of the Nutrient Agar Medium Plates.

After then, the plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones were measured in cm.

III. RESULTS

As per my present study neem have a antibacterial activity. It is used in pharmaceutical company as used in medicine. The neem leaf extract was prepared in methanol extract. Neem leaf extract inhibited the zone of inhibition by disk diffusion method of both bacteria, (*Escherichia coli*) and (*Bacillus Subtilis*).

The result conclude that the greater the concentration more wider the zone of inhibition. In this paper show that, (*Escherichia coli*) have wider zone of inhibition as compared to (*Bacillus Subtilis*). Neem is used since ancients time as antibacterial. It is also used in Ayurvedic, Unani and Homeopathic.

They also have many properties like Antiulcer, Antimalarial, Antifungal, Antibacterial, Antiviral, Antimutagenic and Anticarcinogenic. They do not have any side effect. Hence, it is used in antibacterial.

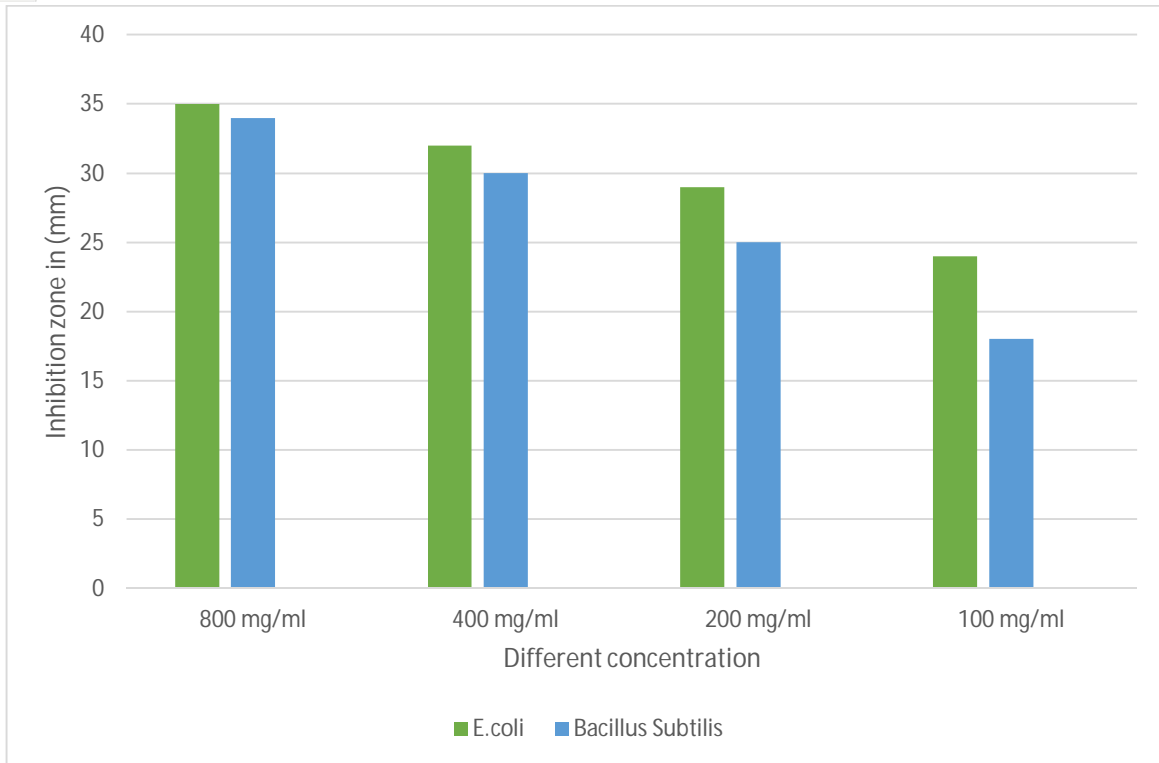


Fig: Antibacterial activity of leaves extract of *Azadirachta Indica* by Disk Diffusion Method

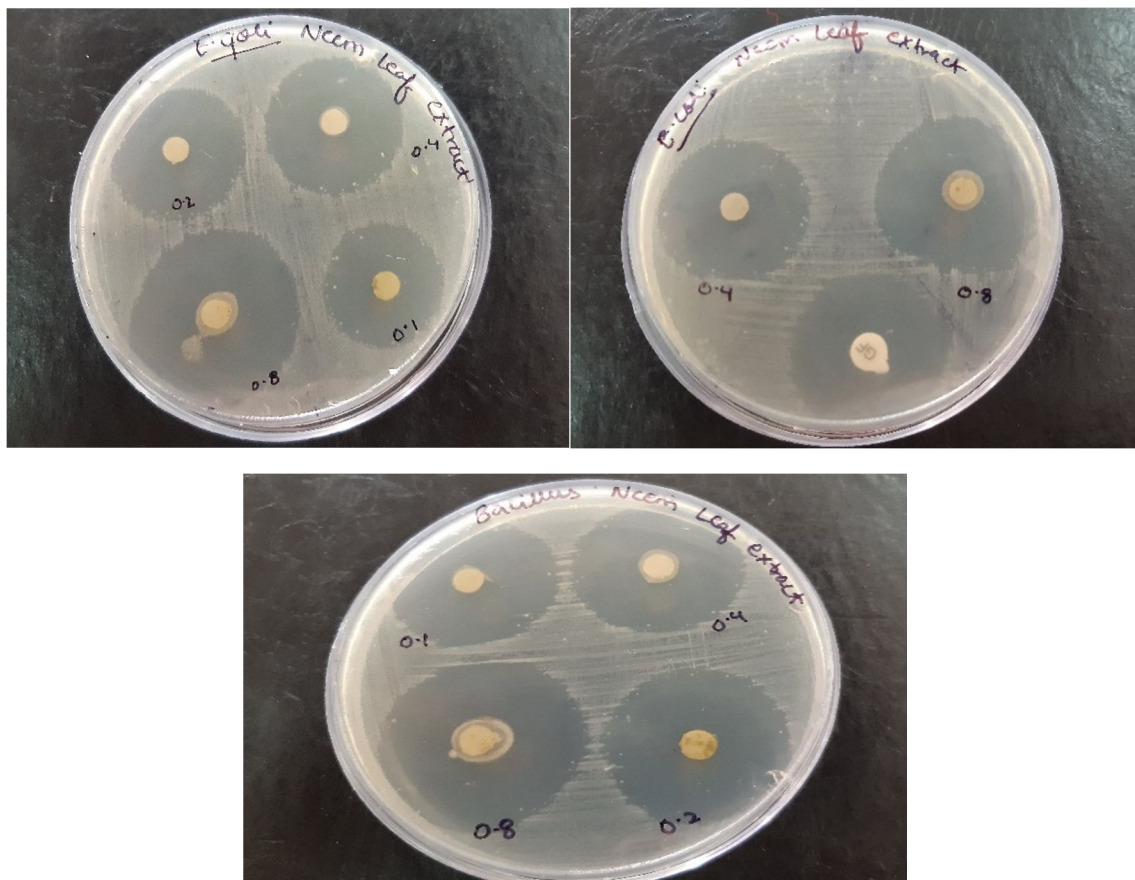


Fig: Antibacterial activity of leaves extract of *Azadirachta Indica* by Disk Diffusion Method

Table:1 Antibacterial Activity of *Azadirachta Indica* Powder

Sample	Solvent	Zone Of Inhibition (mm)									
		<i>Bacillus Subtilis</i>					<i>Escherichia Coli</i>				
<i>Azadirachta Indica</i> Leaves Extract	Methanol	100 mg/ml	200 mg/ml	400 mg/ml	800 mg/ml	Positive Control (GF)* ¹	100 mg/ml	200 mg/ml	400 mg/ml	800 mg/ml	Positive Control (GF)* ¹
		18 mm	25 mm	30 mm	34 mm	36 mm	24 Mm	29 mm	32 mm	35 mm	36 mm

*¹GF- Gatifloxacin

IV. DISCUSSION

Azadirachta Indica extracts used in this study had shown an antibacterial effects as prepared in different tables and graphs used in result, and the effect was shown on both *Escherichia coli* and *Bacillus Subtilis* of the used extract. In general, Gram Negative and Gram Positive bacteria show the resistance to antibiotics because of their cell wall (Francine *et al.*, 2015). More the concentration greater the zone of inhibition . when the concentration decrease zone of inhibition also decreases (Mehrotra *et al.*, 2015). The leaves of neem plant have vast variety Medicinal property as they are used from ancient times, now a days they are also used in Pharmaceutical company as a medicine or they also used in cosmetics company because they regenerative properties of cellular growth (Mamman *et al.*, 2013)

V. CONCLUSION

The result of this study suggest that the neem Extract of *Azadirachta Indica* can be used as an antibacterial agent against growth caused by *Escherichia Coli* and *Bacillus Subtilis*. Methanol is more effective for neem powder extract. This information provided by this study on antibacterial effect of *Azadirachta Indica*, will make its easier for different concentration of the extract if they were to be proceed into drugs. Lastly, based on the information got from the study of many research paper, the antibacterial of *Azadirachta Indica* (Neem) can change the zone of inhibition, depending on neem parts used, the solvent used, even the state of materials used whether it is dry or fresh because each of the extract has its has different inhibition zone of different concentration of Neem extract we used. Hence, this tree was found to be useful in medicine, it is used commonly found in every area. Neem is used for ancient time as herbs. So, that we are familiar with.

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