Studies on Antibacterial Properties of Neem (Azadirachta indica) against Pathogenic Bacteria

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***Abstract*- Population rise, prohibitive cost of treatment, side effects of several allopathic drugs and development of resistance to drugs have led to increased emphasis on the use of plant material as a source of medicine for a wide variety of human ailments. The present study was carried out to evaluate antibacterial activity of Neem (Azadirachta indica) tree leaves against pathogenic bacteria. Different solvent extracts of Neem tree leaves were prepared for determination of antibacterial activity. From the results of Zone of Inhibition (ZOI) and Minimum inhibitory concentration (MIC) it can be seen that Neem possessed good antibacterial activity.**

***Index terms*- Neem (Azadirachta indica), Pathogenic bacteria, Solvent extracts, Antibacterial activity, Zone of Inhibition.**

INTRODUCTION

In Sanskrit, Ayurveda means "The science of life." Ayurvedic knowledge originated in India more than 5000 years ago and it is often called the "Mother of healing." Ayurveda was considered as one of the best ways to cure diseases in ancient India so we started using the principles of Ayurveda in our modern world too. Use of natural substances and herbs lead to a healthy, happy and diseases free life. Ayurveda was officially recognized by World Health Organization (WHO) in 1976.

The Neem (Azadirachta indica) is recognized as a powerful medicinal tree possessing health promoting properties. It's use originated in ancient India and neighbouring countries. Neem tree belongs to family Meliaceae. Neem tree is fast growing that can reach 15 – 30 meters height with compound leaves. The Neem tree is used in Chinese, unani and traditional medicine in many countries. Almost every part of Neem tree i.e. leaves, stem, bark, root and fruits are useful because of having medicinal properties. The Neem tree exhibits anti-inflammatory, antihyperglycaeric, antiulcer, antibacterial, antifungal, antimalarial, anticarcinogenic, antiviral and antioxidant properties. The Neem tree also has pesticidal and insecticidal properties.

Neem tree possess therapeutic role in disease management because of various types of chemical constituents. The most important constituent is azadirachtin and others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n - hexacosanol and amino acid. 7 - desacetyl - 7 - benzoylazadiradione, 7-desacetyl- 7 benzoylgedunin, 17-hydroxyazadiradione, and nimbiol, Quercetin and ß-sitosterol, polyphenolic flavonoids were purified from fresh Neem leaves and were known to have antibacterial and antifungal properties, seeds hold valuable constituents including gedunin and azadirachtin.

The purpose of present study was to investigate the antibacterial activity of Neem tree leaves extract against various pathogenic bacteria.

MATERIALS AND METHODS

1. Collection of plant material

The leaves required Neem (Azadirachta indica) were collected from Botanical garden of Yashwantrao Chavan College of Science, Karad. The leaves were shade dried then cut into small pieces and coarsely powdered. This coarse powder is used for extraction with solvents.

2. Preparation of solvent extract

100 gm of powdered material was suspended in 300 ml of petroleum ether then kept this mixture in refrigerator for overnight. Next day supernatant was discarded and residue dried at 28 ± 2ºc. Dried residue further divided into three parts for another solvent extraction (Natrajan etal).

2.1 Preparation of Hexane extract

30 gm of dried extract was suspended in 250 ml of Hexane. This mixture was incubated overnight at 40ºc. After incubation supernatant is filtered through Whatmann's filter paper no.1 and then filtrate was dried at 28 ± 2ºc to evaporate the solvent. After drying the sediment extract was weighed and it was reconstituted in 5% of Dimethyl Sulfoxide (DMSO).

2.2 Preparation of Ethyl acetate extract

30 gm of dried extract was suspended in 250 ml of Ethyl acetate. This mixture was incubated overnight at 40ºc. After incubation supernatant is filtered through Whatmann's filter paper no.1 and then filtrate was dried at 28 ± 2ºc to evaporate the solvent. After drying the sediment extract was weighed and it was reconstituted in 5% of Dimethyl Sulfoxide (DMSO).

2.3 Preparation of Ethanol extract

30 gm of dried extract was suspended in 250 ml of Ethanol. This mixture was incubated overnight at 40ºc. After incubation supernatant is filtered through Whatmann's filter paper no.1 and then filtrate was dried at 28 ± 2ºc to evaporate the solvent. After drying the sediment extract was weighed and it was reconstituted in 5% of Dimethyl Sulfoxide (DMSO).

Table 1: Protocol for reconstitution of plant extracts

Sr. No	Name of extract	Extract Obtained (gm)	Extracts Dissolved (mg)	Amount of 5% aq. DMSO (ml)	Amount of extract available in solvent (mg)
1	Hexane	0.779	500	5	100
2	Ethyl acetate	0.851	500	5	100
3	Ethanol	0.619	500	5	100

3. Preparation of standard bacterial culture

For studying antibacterial properties of Neem (Azadirachta indica) the isolates required were collected from Dept of Microbiology, Yashwantrao Chavan College of Science, Karad. Following bacterial cultures were used.

1 Bacillus subtilis
2 Escherichia coli
3 Klebsiella pneumoniae
4 Pseudomonas aeruginosa
5 Sthaphylococcus aureus

For determination of antibacterial activity exponentially growing cultures were suspended in sterile saline water to get enough density.

4. Medium used

For determination of antibacterial activity sterile nutrient agar plates were used.

5. Determination of antibacterial activity of crude plant extract

Fresh plant material was washed with sterile distilled water. Then surface sterilization was carried out by 95% ethyl alcohol and washed again by sterile distilled water to remove ethyl alcohol traces. The washed plant leaves were crushed in mortar pestle. Agar well diffusion method was used for observing antibacterial activity. The test bacterial cultures were spread inoculated on sterile nutrient agar plates and on those plates wells were prepared by using sterile cork borer. Crushed plant material was inoculated in that wells and finally all plates were incubated at 37º c for 24 hrs. After incubation plates were observed for zone of inhibition.

6. Determination of antibacterial activity of plant solvent extracts

For determination of antibacterial activity of plant solvent extracts the agar well diffusion method was used. Here test bacterial cultures were spread inoculated on sterile nutrient agar plates and on those plates wells were prepared by using sterile cork borer. 100 µl (0.1ml) of reconstituted each solvent extracts were added in that wells and control was prepared by using 5% DMSO. All the plates were kept in refrigerator for 30 min and then all plates were incubated at 37ºc for 24 hrs. After incubation all the plates were observed for zone of inhibition and results were recorded.

7.Determination of Minimum Inhibition Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by broth dilution method (Patel.R.J). The MIC was defined as the lowest concentration of the compound to inhibit the growth of organisms. Varying concentrations of extracts (10 mg/ml to 100

mg/ml) were prepared. 0.1 ml of test organism was inoculated into tubes containing different concentrations of extract. Control tube was equally set by using solvent and test organism. Then all the tubes were incubated at 37ºc for 24 hrs. Next day all tubes were observed for growth inhibition. The tube with least concentration of extract without any bacterial growth was taken and recorded as Minimum Inhibitory Concentration. Followed this procedure for all three extracts and recorded the results.

RESULT AND DISCUSSION

1. Determination of antibacterial activity

Table 2: Result of antibacterial activity of solvent extracts

Sr.No	Name of organism	Zone of Inhibition (mm)			control
		Hexane extract	Ethyl acetate extract	Ethanol extract	
1	Bacillus subtilis	28	25	25	-
2	Escherichia coli	19	32	29	-
3	Klebsiella pneumoniae	22	12	14	-
4	Pseudomonas aeruginosa	24	13	22	-
5	Staphylococcus aureus	26	26	23	-

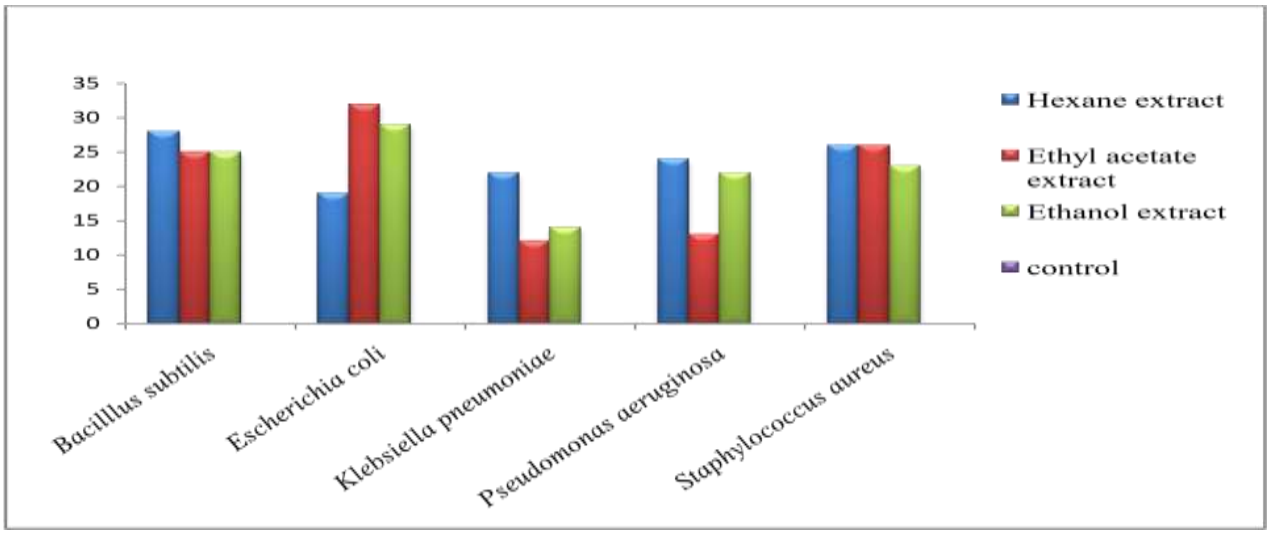

Figure 1: Result of antibacterial activity of solvent extracts

The results of antibacterial activity of solvent extracts of Neem (Azadirachta indica) were showed in table no. 2 and fig no. 1. From table no. 2 and fig no. 1 it can be seen that zone of inhibition were ranging from minimum 12 mm to maximum 32 mm for 100 mg of solvent extracts.

The Hexane extract showed zone of inhibition ranging from minimum 19 mm in case of Escherichia coli to maximum 28 mm for Bacillus subtilis . The zone of inhibition of other test bacterial cultures were 22 mm, 24 mm and 26 mm for Klebsiella pneumoniae, Pesudomonas aeruginosa and Staphlyococcus aureus respectively.

The Ethyl acetate extract showed zone of inhibition ranging from minimum 12 mm in case of Klebsiella pneumoniae to maximum 32 mm for Escherichia coli. The zone of inhibition of other test bacterial cultures were 25 mm, 13 mm and 26 mm for Bacillus subtitles, Pseudomonas aeruginosa and Staphylococcus aureus.

The Ethanol extract showed zone of inhibition ranging from minimum 14 mm in case of Klebsiella pneumoniae to maximum 29 mm for Escherichia coli. The zone of inhibition of other test bacterial cultures were 25 mm, 22 mm and 23 mm for Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus.

2.Determination of Minimum Inhibitory Concentration (MIC)

The results of Minimum Inhibitory Concentration (MIC) of test bacterial cultures for three solvent extracts are as followed

Table 3: Growth of bacterial isolates at various concentrations of Hexane

Sr.No	Concentration mg/ml	B. subtilis	E. coli	K. pneumoniae	P. aeruginosa	S. aureus
1	10	+	+	+	+	+
2	20	+	+	+	+	+
3	30	+	+	+	+	+
4	40	+	+	+	+	+
5	50	+	+	+	+	+
6	60	+	-	+	+	+
7	70	+	-	+	+	-
8	80	-	-	-	-	-
9	90	-	-	-	-	-
10	100	-	-	-	-	-
Control	Solvent	+	+	+	+	+

+ = Growth - = No growth

From table no. 3 it can be seen that the Hexane extract showed growth inhibition of Escherichia coli at and above 60 mg/ml so MIC of Escherichia coli for Hexane extract was 60 mg/ml. The MIC of other bacterial cultures were 80 mg/ml, 80 mg/ml, 80 mg/ml and 70 mg/ml of Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphlyococcus aureus respectively.

Table 4: Growth of bacterial isolates at various concentrations of Ethyl acetate

Sr.No	Concentration mg/ml	B. subtilis	E. coli	K. pneumoniae	P. aeruginosa	S. aureus
1	10	+	+	+	+	+
2	20	+	+	+	+	+
3	30	+	+	+	+	+
4	40	+	+	+	+	+
5	50	+	+	+	+	+
6	60	+	-	+	+	+
7	70	+	-	+	+	-
8	80	-	-	+	+	-
9	90	-	-	-	-	-
10	100	-	-	-	-	-
Control	Solvent	+	+	+	+	+

+ = Growth - = No growth

From table no. 4 it can be seen that the Ethyl acetate extract showed growth inhibition Escherichia coli at and above 60 mg/ml so MIC of Escherichia coli for Hexane extract was 60 mg/ml. The MIC of other bacterial cultures were 80 mg/ml, 90 mg/ml, 90 mg/ml and 70 mg/ml of Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphlyococcus aureus respectively.

Table no 5: Growth of bacterial isolates at various concentrations of Ethanol

Sr.No	Concentration mg/ml	B. subtilis	E. coli	K. pneumoniae	P. aeruginosa	S. aureus
1	10	+	+	+	+	+
2	20	+	+	+	+	+
3	30	+	+	+	+	+

4	40	+	+	+	+	+
5	50	+	+	+	+	+
6	60	+	+	+	+	+
7	70	+	-	+	+	+
8	80	-	-	+	-	-
9	90	-	-	-	-	-
10	100	-	-	-	-	-
Control	Solvent	+	+	+	+	+

+ = Growth - = No growth

From table no. 5 it can be seen that the Ethanol extract showed growth inhibition of Escherichia coli at and above 70 mg/ml so MIC of Escherichia coli for Ethanol extract was 70 mg/ml. The MIC of other bacterial cultures were 80 mg/ml, 90 mg/ml, 80 mg/ml and 80 mg/ml of Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphlyococcus aureus respectively.

CONCLUSION

The present study was carried out for evaluation of antibacterial activity of Neem (Azadirachta indica) leaves. Based on results it can be seen that secondary compounds of this plant showed antibacterial activity which can be used for the treatment of diseases caused by organisms. This study has helped us to understand the importance of traditional medicine. The solvent extracts of Neem (Azadirachta indica) leaves possessed good antibacterial activity which has great potential as a bioactive compound and it can be useful for primary health care treatment.

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