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"Assessment of Antimicrobial Potency of *A. indica* against *S. aureus* with the Formulation and Evaluation of Neem Oil"

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ABSTRACT

Plant have been a source of herbal remedies throughout the history of mankind. Various medicinal plant have been used for years in daily life to treat diseases all over the world. The present study was conducted to isolate and identify the bacteria *S.aureus* from various clinical samples & to detect the phytochemical constituents of Neem in various solvents (Aqueous, Ethanol, Methanol). Ethanolic neem extract shows strong antibacterial activity as compared to such as Methanol and Aqueous solvents. Crude neem oil was prepared in the lab condition & its antimicrobial potential was also checked against the *Staphylococcus aureus*. Neem oil was found to be effective in controlling *S. aureus*.

KEY WORDS:- *Azadirachta indica* (Neem), *Staphylococcus aureus*, Neem oil, Antimicrobial activity.

INTRODUCTION

Medicinal plants have started to consider an essential source in treating/preventing a various kind of disease (Rakotoarivelo NH *et a*l., 2015). Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, and herbivorous mammals. (Gershenzon J et al.,2022).

Neem (*Azadirachta indica L.*) commonly called 'India Lilac' or 'Margosa", belongs to the family Meliaceae, subfamily Meloideae and tribe Melicae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India (Girish K et al.,2008).

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) whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years.

Medicinal properties of the plant *Azadirachta indica* L.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).

Further *Staphylococcus aureus* was characterized into Methicillin sensitive *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) by using cefoxitin disc diffusion method. *Staphylococcus aureus* has long been recognized as one of the most important bacteria that cause disease in humans.

Neem oil has been used for centuries in traditional Indian medicine for its medicinal properties. It is known to have anti-inflammatory, antimicrobial, and antifungal properties, which makes it beneficial in treating a variety of skin and hair conditions. It is also known to be an effective insect repellent, which is why it is often used in organic farming. In addition, its taste and smell can be used to naturally flavor food, which has become increasingly popular in recent years.

The extraction of Neem oil is a complex process and there are several methods used to extract the oil from the leaves. These include mechanical pressing, which is the most common method, solvent extraction, and steam pressure extraction.

Solvent There are 3 types of solvents commonly used in the extraction of Neem namely methanol, ethanol and water. Each solvent produces different extracts in terms of quantity and quality of the final product. However, there is controversy over the use of solvents as it may cause a cytotoxic effect on the user. Another common type of solvent is water. Although water is a cheap solvent and relatively safe, aqueous extracts have more impurities that make isolating the desired compound difficult. After the extraction process, the crude extract was fractionated into the desired compounds and this technique is widely applied, especially in the whole process of extraction of the Neem.

Plants produce an extensive range of phytochemical components which are secondary metabolites (M. E. Ojewumi *et al*., 2017) These secondary metabolites work uniquely and are used directly or indirectly in the pharmaceutical industry. Phytochemicals have the ability to act as antioxidants by preventing cell damages which is usually caused by free radicals such as those associated with heart disease and cancer. Examples of phytochemicals with antioxidant activity are allyl sulphides, flavonoids, polyphenols and caternoids (E. M. Yahia, 2017; E. M.Yahia *et al,.* 2017).

MATERIALS AND METHODS

Collection of clinical samples

Total 20 clinical samples including pus, skin infection and wound infection were collected from the various pathology laboratories and Hospital at Khamgaon city. Clinical samples were also collected from outpatient visiting the clinic. The sample was collected form patients includes male and female having age of above 18 years. The pus sample collected aseptically with the help of sterile cotton swab and by a clinician wearing gloves and mask.

Isolation and Identification of *S. aureus* from clinical samples

The collected samples were further processed in Microbiology lab by inoculating the sample on various selective media such as Nutrient agar, Mannitol salt agar, Baired parker agar. Further the isolates were processed for confirmation on the basis of Cultural and Morphological characteristics. The isolates were identified on the basis of biochemical characteristics and were confirmed on the basis of Bergey's manual of Determinative bacteriology.

Collection of Plant Material

Neem (*Azardirachta indica* L.) leaves collected from area of Jatharpeth, Akola in Augest 2023. The plant material collected was healthy and free from any deformalities. Then the leaves was cleaned by tap water and also cleaned in sterile distilled water, dried in the shade at room temperature, they were break into small pieces and the 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. And were used for extraction. (Ranjit R, Raut *et al*., 2014)

Phytochemical Analysis

The following methods were used for qualitative phytochemical analysis extracts from Neem leaves (B.Q. Guevara 2005)(A. J. Harborne 1998).

1. **Test for Carbohydrates:** 1 ml of Molisch's reagent was added to 2 ml of the Leaves extract after which a few drops of concentrated sulphuric acid was added. A purple colouration depicts the presence of carbohydrates.

2. **Test for Tannins:** 2 ml of 5% ferric chloride was added to 1 ml of Leaves extract. A greenish black colouration depicts that tannins are present.

3. **Test for Saponins:** 2 ml of distilled water was added to 2 ml of Leaves extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.

4. **Test for Flavonoids:** 5 ml of dilute NH3 solution was added to 1 ml of Leaves extract prior to the addition of concentrated sulphuric acid. A yellow colouration depicts that flavonoids are present.

5. **Test for Alkaloids:** 2 ml of concentrated HCI was added to 2 ml of Leaves extract before a few drops of Mayer's reagent were added. A greenish colouration depicts that alkaloids are present.

6. **Test for Terpenoids:** 2 ml of chloroform and concentrated H_2SO_4 was added to 0.5 ml of Leaves extract. A red brown colouration at the interface depicts that terpenoids are present.

7. **Test for Phenols:** 2 ml of distilled water and a few drops of 10% ferric chloride was added to 1 ml of Leaves extract. A green colouration depicts that phenols are present.

8. **Test for Glycosides:** 3 ml of chloroform and 10% NH_3 solution was added to 2 ml of Leaves extract. A pink colouration depicts that glycosides are present.

9. **Test for steroids:** 1ml of extract was dissolved in 10 ml of chloroform and equal volume of sulphuric acid was added by the sides of the test tube. If the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence then the steriods are present.

10. **Test for Reducing sugar:** The plant extract was treated with Fehling's solution (A and B) In the test tube. The colour change from deep blue to brick red indicates the presence of reducing sugar (Harborne 1998).

Preparation of Plant Extract (Ranjit R, Raut *et al*., 2014)

The plant extract was brought to the laboratory for further processing. 5 gm of powder is accurately weighed and transferred to the conical flask containing 50 ml(Water, Ethanol, Methanol) and shaken well and powder mixed properly in solvent. The flask containing the mixture of powder and solvent was kept at room tempereture under aseptic condition for 3 days. The Neem extract was filtered using whatsman filter paper. This obtained pure extract was stored at 4^0 c for further work.

Antimicrobial activity of Neem in different solvent (Ugwu Celestina Chibuzo 2019)

Agar well diffusion method was used to test the antibacterial activity of Aqueous, Ethanol, and Methanol extract of Neem leaves. A suspension of *S. aureus* bacteria was applied in duplicate to the surface of a sterile Muller hinton agar plate. 0.1 ml of each plant extract was added to a hole that was made in the Muller Hinton Agar plate using a sterile, 6 mm cork borer. For 24 hours, the plates were incubated at 37° C. The zone of inhibition in millimetres was used to measure the antibacterial activity. .

Preparation of Neem Oil

Grinded Neem leaves measuring 25 gm were soaked into 40 ml coconut oil and heated at 120°C for 1 hrs. After they have cooled to room temperature, the mixture was then filtered by using filter paper to remove the leaves residue. The filter oil was kept for further experiment.

Antimicrobial activity of Neem oil (Ugwu Celestina Chibuzo in 2019)

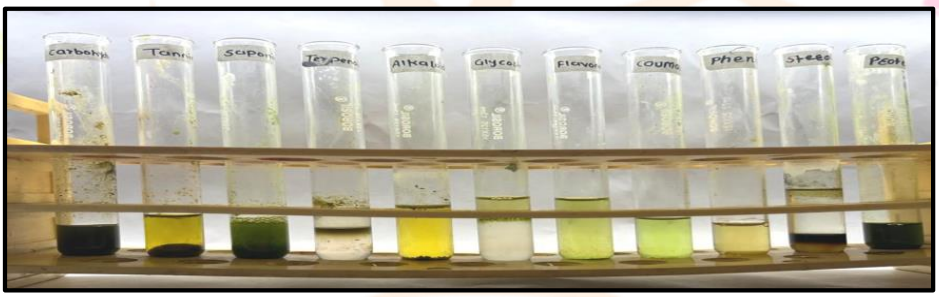
The antimicrobial activity of formulated Neem oil was performed by Agar Well Diffusion Method. *S. aureus* bacteria suspension was spread on the surface of sterile Muller Hinton agar plate in duplicate.A sterile 6 mm core borer was use to make a hole on the Muller Hinton Agar plate in which 0.1 ml of the Neem oil extract were added . The plate were incubated at 37^0 c for 24 hours. The antimicrobial activity was detected by measuring zone of inhibition in mm.

RESULT AND DISCUSSION

Phytochemical analysis of *A. indica* Neem leaves

Sr no.	Test	*Azadirachta indica* A	*Azadirachta indica* B	*Azadirachta indica* C	Observation (Positive result)
1	Alkaloid	Positive	Positive	Positive	Greenish coloured depict.
2	Glycosides	Positive	Positive	Positive	Pink colouration
3	Flavonoid	Negative	Negative	Negative	Yellow coloured depicts
4	Saponins	Negative	Negative	Positive	Foam formation
5	Terpenoids	Negative	Negative	Positive	Greenish black colouration depict
6	Phenol	Negative	Negative	Positive	Green colouration depicts
7	Carbohydrate	Positive	Positive	Positive	Purpal colouration depicts
8	Acid	Negative	Negative	Positive	Formation of effervescence
9	Steroid	Negative	Negative	Positive	Violet in colour
10	Reducing Sugar	Positive	Positive	Positive	Deep blue and red indicated

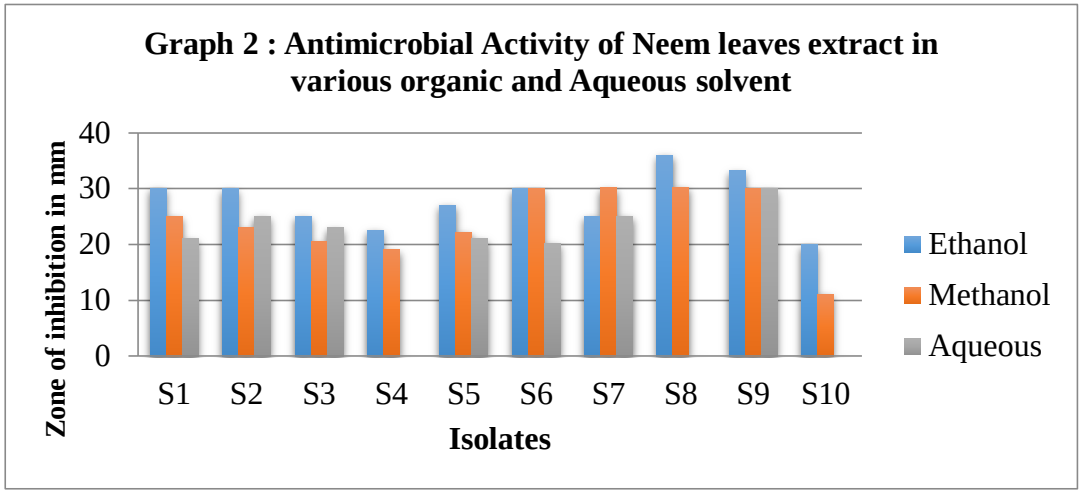
(A=Hurb B= Shrub, C=Tree)



Phytochemical Test

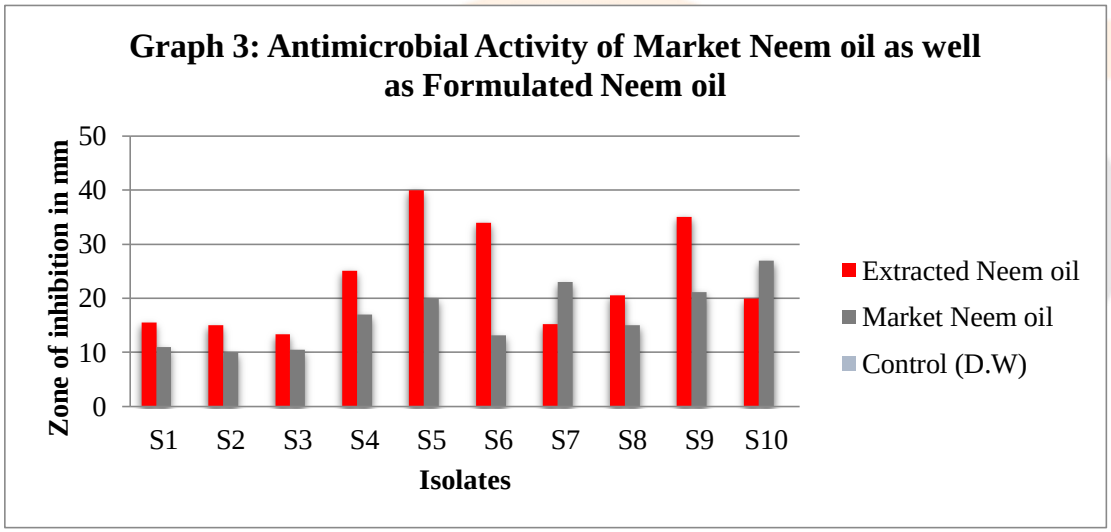
Antimicrobial Activity of Neem leaves extract in various organic and Aqueous solvent

Sr. No	Sample	Organic solvent used Zone of inhibition in mm			D.W (Control)
		Ethanol	Methanol	Aqueous	
1	S1	30.1	25.0	21.0	Control
2	S2	30.0	23.0	25.0	Control
3	S3	25.0	20.5	23.0	Control
4	S4	22.5	19.1	R	Control
5	S5	27.0	22.2	21.0	Control
6	S6	30.0	30.0	20.2	Control
7	S7	25.0	30.2	25.0	Control
8	S8	36.0	30.3	R	Control
9	S9	33.2	30.0	30.0	Control
10	S10	20.0	11.1	R	Control

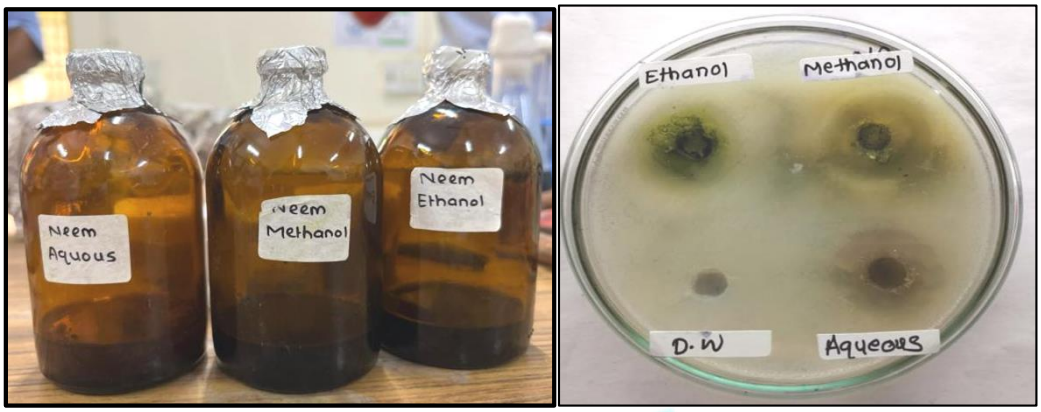


Antimicrobial Activity of Market Neem oil as well as formulated Neem oil

Sr. No	Sample No	Zone of Inhibition in mm		
		Extracted Neem oil	Market Neem oil	Control (D.W)
1	S1	15.5	11.0	-
2	S2	15.0	10.0	-
3	S3	13.3	10.5	-
4	S4	25.1	17.0	-
5	S5	40.0	20.0	-
6	S6	34.0	13.2	-
7	S7	15.2	23.0	-
8	S8	20.5	15.0	-
9	S9	35.0	21.1	-
10	S10	20.0	27.0	-



Antimicrobial Activity of Neem Extracts in different solvent against *S. aureus*



Antimicrobial Activity of Formulated Neem Oil against *S. aureus*

DISCUSSION

Neem (*A. indica*) plant have many biological active compound. Neem show great properties like Antiallergic, Antifungal, Antibiotic, Antidermatic, Antibacterial, Antiulcer, Food preservation, cancer, Insecticides, pesticides and other biological activity.

In the current study the Hurb, Shurb and Tree of *Azadirachta indica* is selected for the study as per the age of the plant different phytochemicals was observed. The phytochemical analysis was done for the Neem plant extract. It was noticed that according to the age of plant, the phytochemical differs. During the study various phytoconstituents were checked, showing positive result for Alaloid, Glucosides, Carbohydrate, Reducing sugar and the observation was made on the basis of color obtained Flavonoid was found to be negative whereas saponins, Terpenoids, Phenol, Acid, Steroid were negative for Hurb and Shrub, but was found in Tree of Neem.

Antimcrobial activity of Neem leaves extract in various organic solvent and aqueous solvent against *S. aureus* isolated from cilincal sample was done and show in (Table 9). It was observed that the Ethanolic extract of Neem had shown antibacterial activity against S8, S9, S1, S2 isolate the highest zone of inhibition S8 was shown for 36.0 mm. S8 was followed by S9 shows 33.2 mm, S1 shows 30.2 mm and S2 show 30.0 mm. The lowest zone of inhibition by the

Ethanolic extract was shown 20.0 mm. Methanol extract against S8 isolate showed highest zone of inhibition i.e 30.3 mm while lowest zone of inhibition is 11.1 mm. However the *S. aureus* bacteria was found to resistant for the Aqueous extract showing zone of only 10.0 mm only.

Crude Neem oil was prepared in lab condition and it's antimicrobial potential was also checked against the *S. aureus*. It was observed that Neem oil was found to be effective in controlling *S. aureus* bacteria as compared to market oil and present in Table 10. Formulated Neem oil had antimicrobial activity against S5, S9 ,S6 isolate with excellent zone of inhibition. S5 was shown 40.0 mm followed by S9 showed 35.0 mm and S6 showed 34.0 mm. Lowest zone of inhibition by crude Neem oil was shown in S3 was 13.3 mm and Marketed Neem oil against S10, S7, S5 isolate showed highest zone of inhibition S10 was shown 27.1 mm followed by S7 showed 23.0mm and S5 showed 20.0 mm while lowest zone of inhibition was shown in S2 was 10.0 mm. Distilled water were used as control.

CONCLUSION

Neem leaves extract in ethanolic solvent have significant antimicrobial activity against *S. aureus* isolated from pus, wound and skin infection. Crude Neem oil was prepared by using powdered from of Neem leaves. The extracted Neem oil shows relatively high activity against *S. aureus* isolated from different clinical samples. Further research to be done toward isolating purifying and standarizing the active antimicrobial ingredients of *A. indica. A. indica* plant served as effective tool in hand of clinician in management of multidrug resistant infection.

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