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Extraction of Neem *(Azadirachta indica)* Seed Oil and Its Applications

Amar Gaje [1], Jaishree Dange[1], Pavan Golwal[1], Shubham Tathe[1] and Kalyani Pawar*

P.G. Student[1], Assistant Professor*, Department of Biotechnology, Shivchhatrapati College, N-3 Cidco, Aurangabad – 431001, Maharashtra, India.

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***Corresponding Author Email:** kalyanipawar104@gmail.com

Abstract

Neem seed kernel represents the most important source of active ingredients found in neem tree, such as properties of extracted neem oil was also determined. In this experiment, Neem oil is extracted from kernels of Neem seeds. A soxhlet extraction was used to extract the oil. The extract was concentrated by using rotary evaporator. Results revealed that the yield of neem seed oil by using soxhlet extraction is 28 %. E. coli was the predominant isolate isolated from the urine specimen followed by *Klebsiella sp., S. aureus, Pseudomonas Sp*. The antibacterial activity of neem oil was evaluated against the 4 urine clinical isolates by broth dilution method and agar well diffusion technique, and zone of inhibition was measured in mm. In our study, highest antibacterial activity was recorded in *S. aureus, Pseudomonas Sp*. Insecticidal activities of neem oil have been studied against gram pod borer and spider mites. Oil extracted from neem seed kernels showed greater lethal properties on the insects.

Keywords

Neem seed oil, antibacterial, insecticidal.

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INTRODUCTION:

Neem tree [*Azadirachta indica*] is a tree in the mahogany family Meliaceae, is evergreen tree found in most tropical countries. *Azadirachta indica* L. (neem) shows therapeutics role in health management due to rich source of various types of ingredients (15). The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, and quercetin. Seed kernel represents the most important source of active ingredient found in neem tree, such as azadirachtin and many others. Neem oil has the highest potential and production among the available wild oils. (13)

Antibacterial Activity against Urine clinical isolates:

The antibacterial activity of Neem oil is screened against the bacterial isolates using agar well diffusion and broth dilution method. The growth of microorganisms was inhibited by neem oil was observed to be significant with all the four isolates when compared with the control. Through this study it was found that neem oil is effective against the Urine clinical isolates. Many of the synthetic drugs present cause various side effects. Hence the drugs

which are developed through plant-based compounds have minimal side effects. The neem oil has got very good antibacterial activity.

Insecticidal Activity: The neem tree has over centuries been used as a herbal plant, including its effects against insect pests. Various parts of the neem tree: the bark, leaves, flowers, seeds and fruit pulp are used, mostly in the powdery or the extract form.

The aqueous extracts are sprinkled on field crops against various pests. The increasing demand for high quality food, free from chemical residues, makes it imperative that non-chemical means of protecting stored products or crops against insect damage be used. Pod borer is the most dangerous and major pest of gram. Insect pressure is intense during January – February as they tend to defoliate the whole plant, especially during the podding stage. The young pod borer bore inside the pod by making holes and then feeding on the developing grains. No gram field is free from its attack. It causes severe economic losses.

MATERIALS AND METHODS

I. EXTRACTION OF NEEM SEEED OIL:

Collection and processing of neem seed:

Neem seed were purchased from APMC shop, Paithan, Maharashtra. The dirt (soil and stone residues) is removed. Shells and hulls of fruits were removed by washing in clean water and then sundry in an open air for 2-3 days. The kernel was ground into powder using grinder. The powder was used for oil extraction (3).

Extraction of Neem Oil: The extraction of oil from neem seed was carried out by Two methods

1. **Soxhlet Extraction:** 50 g of neem seeds powder was mixed with 250 ml of Methanol in soxhlet extraction kit and heated at 60◦ C for six hours. The oil extracted was transferred to vaccum evaporator to remove solvent residue (13). The extracted oil was then centrifuged at 5000 rpm for 20 minutes to remove suspended particles. The process of extraction was repeated until sufficient quantity of the oil was obtained (3).
2. **Shake flask method:** The powder of neem seed kernel obtained (250 gm) was soked in one litre of petroleum ether and placed on a shaker for about 72 hours. Using a muslin cloth, the mixture was filtered, and the cake was kept. The filtrate obtained was made to undergo distillation to separate the oil obtained from the neem seed powder from the solvent (2).

Yield of obtained neem seed oil by above both the process was calculated by using following formula:

Yield of oil (%) =

Mass of oil ⁄ initial mass of sample× 100

II. DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF EXTRACTED OIL:

Following physicochemical properties of extracted neem oil was determined:

1. **pH:** The pH value of neem seed oil was analyzed using pH strip. The pH strip was dipped inside the neem oil and change in color of pH strip was observed carefully to determine the pH.
2. **Appearance:** The Appearance of neem seed oil is recorded by visual observation.
3. **Odor:** The odor of the neem seed oil is recorded manually by smelling it.
4. **Solubility in water:** 1 ml of neem seed oil and 1 ml of water was added in test tube and mixed well.

III. ANTIBACTERIAL ACTIVITY OF NEEM SEED OIL:

- **Isolation of urine clinical isolates:** Urine samples were cultured on CLED agar, medium and incubated over night at 37°C.
- **Culture characteristics:** Each of the color, size, elevation, margins and texture of colonies were screened. The morphological different colonies on CLED agar were sub-cultured on Nutrient agar, Mannitol, EMB and Mac Conkey agar medium selectively and further, in order to purify the isolated bacteria from urine sample. The isolates were subculture on nutrient agar medium.
- **Microscopic examination**: Pure isolates were examined microscopically, on the base of their cell wall composition (i.e. Gram staining).
- **Microbiological analysis:** According to gram staining technique, isolates were cultured on numerous selective and differential media to find out their color, colony morphology.
- **Biochemical test:** Selected colonies were identified and differentiate according to the culture characteristics, microscopic examination and microbiological analysis. Isolates were tested biochemically for their identification by following tests, such as; Catalase, Oxidase, Indole production test, MR test, VP test, Citrate utilization test, Urea hydrolysis, Sugar fermentation test (glucose, mannitol, maltose, lactose. Xylose, sucrose) (12).

The antibacterial effect of neem oil was checked by 2 methods as follows:

1. **Broth dilution method:** Nutrient broth tubes was prepared and inoculated with 100 µl test organisms (*Escherichia coli, Klebsiella Sp.*, *Staphylococcus aureus, pseudomonas Sp.*) broth culture (whose O.D. was 0.5 Mc Far land).A tube containing Muller Hinton broth but without an organism was used as control. The tubes were then examining for the presence or absence of growth using turbidity as a criterion. Colorimetric methods have the potential to generate clear cut endpoints based on visually detectable turbidity change. Other set of nutrient broth tubes were used as test, which contain both neem oil and test organism. The antibacterial activity of neem oil was measured by calculating the O.D. of 24 hr incubated culture.
2. **Agar well diffusion method:** A standardized inoculums was prepared in nutrient broth by growing Escherichia *coli, Klebsiella sp*, *Staphylococcus aureus, Pseudomonas sp.* to the turbidity of the 0.5 Mc Far land standard. Basal nutrient agar plates were prepared. Soft agar tubes were prepared and 100 µl test organisms' culture was added in it. Now this soft agar tube media is poured on basal agar plate. After solidification of media the wells of 6 mm was prepared by using sterile cork borer and 50 µl of neem seed oil was added in the wells while 50 µl of DMSO was added in another well as a control. Then the plates were incubated for 24 hours at 37° C and the result was observed by measuring the zone of inhibition.

IV. INSECTICIDAL ACTIVITY OF NEEM SEEDOIL:

The insecticidal activity of extracted neem oil was checked on cockroaches, Green gram pod borer (*Helicoverpa armigera*) and spider mite at various concentrations i.e. 10, 50, 80, 100 % resp. (various concentrations of neem oil was prepared by dissolving it in DMSO). DMSO & D/W was used as a control. The insecticidal activity was determined by recording the death time of the insects.

RESULT

I. EXTRACTION OF NEEM SEEED OIL: Neem seed kernel oil was extracted by using following two methods and yield of oil was calculated:

Oil extraction method	Shake flask Method	Soxhlet Method
Yield of oil (%)	14.06	28

Table 1

II. DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF EXTRACTED OIL: Physicochemical properties of extracted neem oil was determined as shown in table 2.

Property	Observation / result
pH	5
Appearance	Dark Brown
Odor	Strong Garlic
Solubility in water	Insoluble

Table 2

III. ANTIBACTERIAL ACTIVITY OF NEEM SEED OIL:

- Urine samples were cultured on CLED agar, medium and incubated over night at 37°C. From the observation of cultural characteristics of isolates on CLED agar medium, the obtained 4 isolates were suspected to be:
  1) isolate 1 (*Escherichia coli*)
  2) isolate 2 (*Klebsiella sp.*)
  3) isolate 3 (*Staphylococcus aureus*)
  4) isolate 4 (*Pseudomonas sp.*)
- For these 4 types of suspected urine isolates obtained on CLED agar medium, 4 types of respective selective media was used for isolation of their pure culture such as: EMB, Mac Conkeys, Mannitol salt agar, Nutrient agar etc. as shown in fig. A, B, and C respectively.

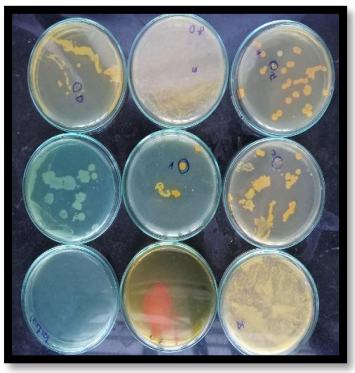
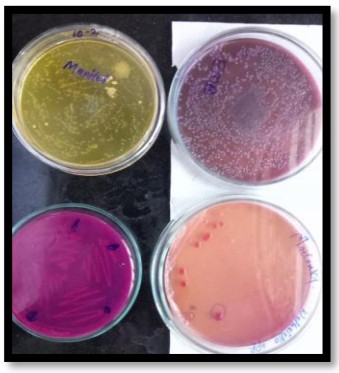
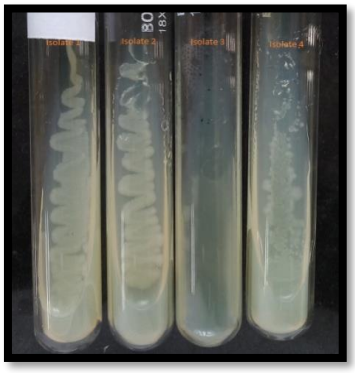

Fig.A **Fig.B** **Fig.C**

(Where, A = Urine clinical isolates on CLED agar medium, B = subculture of urine clinical isolates on selective medium, C = pure culture of urine clinical isolates on Nutrient agar slants).

- **Biochemical identification of urine clinical isolates:** Biochemical identification of 4 urine clinical isolates were performed by using different biochemical test as shown in table 3 and table 4. These isolates were then identified by ABIS online software.

Sr. No.	Gram Staining	Catalase	Oxidase	Indole	MR	VP	Citrate
Isolate 1	Negative	+	-	+	+	-	-
Isolate 2	Negative	+	-	+	-	+	+
Isolate 3	Positive	+	-	-	+	+	+
Isolate 4	Negative	+	+	-	-	-	+

Table 3

Sr. No.	Sugar Fermentation Test							Identified organism
	Sucrose	Mannitol	Glucose	Xylose	Lactose	Maltose	Sucrose	
Isolate 1	-	+	+	+	+	-	-	*E. coli*
Isolate 2	+	+	+	+	+	+	+	*Klebsiella sp.*
Isolate 3	+	+	+	-	+	+	+	*S. aureus*
Isolate 4	-	+	-	-	-	-	-	*Pseudomonas sp.*

Table 4

<u>The antibacterial effect of neem seed oil</u>: -

1. **Broth dilution method:** The antibacterial activity of neem oil against 4 types of urine clinical isolates (*E. Coli, Klebsiella, S. aureus, Pseudomonas*) was performed by broth dilution method as shown in fig. D, E, F, and G and absorbance was recorded as shown in table 5.

Sr. No.	Organism	Control	Test
1	*Klebsiella*	0.849	0.335
2	*S. aureus*	1.043	0.169
3	*Pseudomonas*	0.923	0.620
4	*E. Coli*	0.9	0.359

Table 5

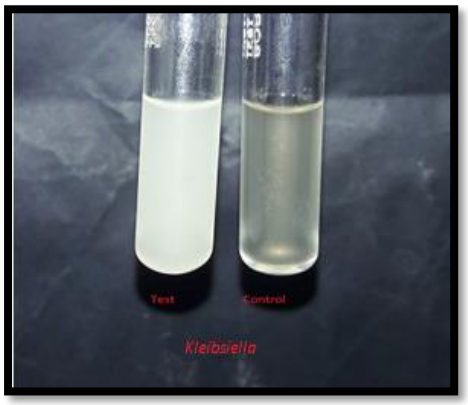

Fig. D

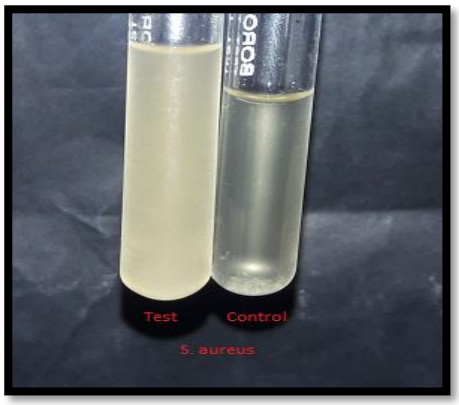

Fig. E

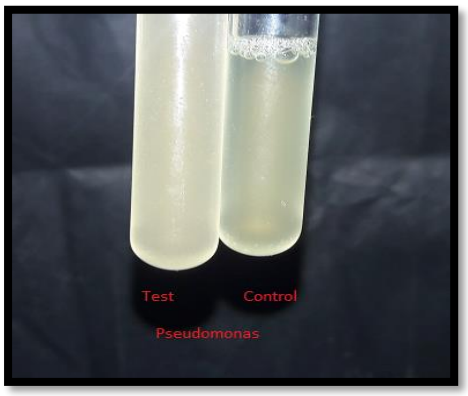

Fig. F

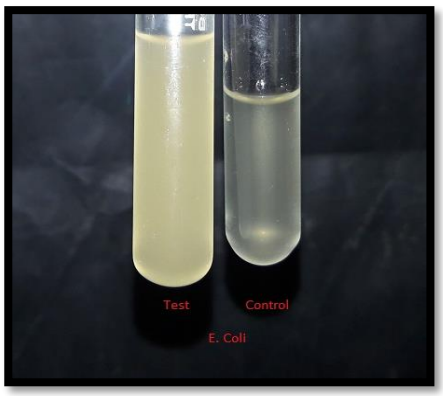

Fig. G

2. **Agar well diffusion method:** Isolated urine clinical isolates have shown effective zone of growth inhibition against neem oil. Isolated *S. aureus* and *Pseudomonas sp*. shown zone of inhibition against the neem seed oil. (Fig. H). The zone of inhibition was measured as shown in table 6:

Sr. No.	Organism	Zone of inhibition
1	*E. Coli*	-
2	*Klebsiella sp.*	-
3	*S. aureus*	13 mm
4	*Pseudomonas sp.*	13 mm

Table 6

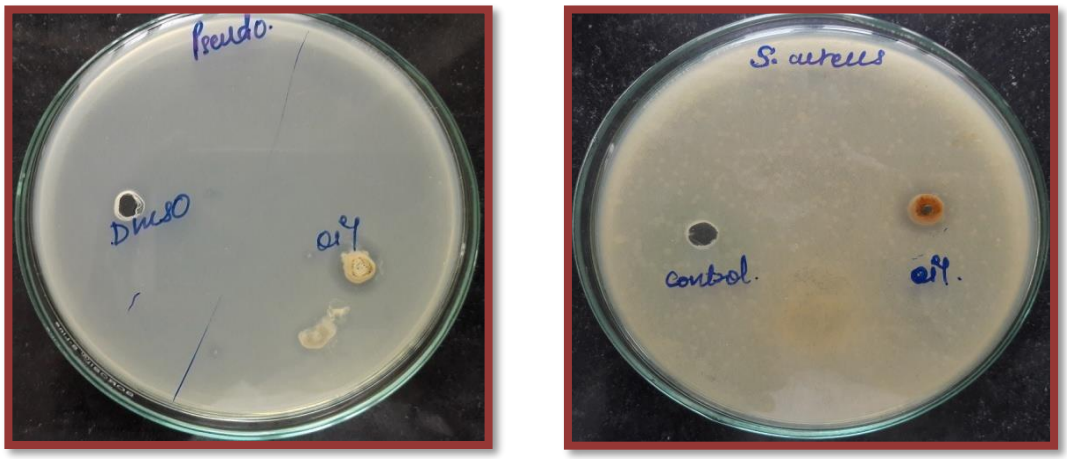

Fig H: Antibacterial activity of neem seed oil

IV. INSECTICIDAL ACTIVITY OF NEEM SEEDOIL:

The insecticidal activity was determined by recording the death time of the insects as shown in table 7 and 8 :

1) Gram pod Borer:

Sr. No.	Treatment	Death time (in sec) (Pod Borer)
1	Control 1 (DMSO)	-
2	Control 2 (D/W)	-
3	50 % Neem Oil	120
4	100 % Neem Oil	82
5	Neem based insecticide	60

Table 7

Fig. Insecticidal activity of neem seed oil against Gram Pod borer

2) Spider Mites:

Sr. No.	Treatment	Death Time (in min)
1	Blank (DMSO)	-
2	10 % Neem Oil	2:15
3	50 % Neem Oil	1:42
4	80 % Neem Oil	1:23
5	100 % Neem Oil	1:10

Table 8

Fig. Insecticidal activity of neem seed oil against mite

DISCUSSION

The use of soxhlet extraction process for the extraction of neem oil is generally preferred choice, due to very high oil yield. Result shown in table 4 also shows that yield of oil obtained by the soxhlet extraction technique is also high i.e. 28 % as shown in table 1. In our study significant antibacterial activity of neem oil is observed against *Klebsiella, Pseudomonas* and *S. aureus.* While the growth of *E. Coli* was not affected by the neem seed oil. Direct sprays of various concentrations of neem seed oil on insect like, gram pod borer, spider mites have shown that neem oil shows significant insecticidal activity (1).

CONCLUSION

In present study, oil is successfully extracted from neem seed kernel by using soxhlet extraction method and shake flask method. Physicochemical characteristics of extracted neem oil were studied. The antibacterial activity of this extracted neem oil was checked against urine clinical isolates which shows effective zone of growth inhibition against *Pseudomonas* and *S. aureus* Which indicates that Neem seed oil can be used for the treatment of several harmful infectious diseases caused by *Staphylococcus aureus , Pseudomonas sp.* Insecticidal activity is checked against insects (i.e. Gram pod borer & spider mite). It can be concluded that high concentration of neem seed oil can be used as good insecticide when sprays are given on infected green gram plant. However, field evaluation and identification is necessary to determine its efficacy under natural ecosystem.

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