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Evaluation of Antibacterial Properties of Neem Seed Oil

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ABSTRACT

Neem is a potent blood purifier, detoxifier, and fever reducer according to Ayurvedic scriptures. Neem's astringent properties aid in the promotion of healing even further. Medicated neem oil is considered one of the best for treating skin conditions. Neem works well as an anti-inflammatory for discomfort in the muscles and joints. Diterpenesuginol, nimbiol (bark), triterpenes, beta-sitosterol, stigmastrol (leaf), Limonoids, Malliantriol (seed oil), Nimbendiol (seed oil), and azardiractin (seed) are among the groups of substances that have been isolated from the plant in good amounts. These compounds are sulphurous. In summary, this investigation unequivocally demonstrated the antibacterial efficacy of neem oil against bacterial strains isolated from various environments. Today, more research on antimicrobials originating from plants is required. To identify the antibacterial chemicals found in these plants and to ascertain the full range of their activity, more investigation is required. Nonetheless, the current investigation into the in vitro antibacterial assessment of specific plants serves as a foundation for future phytochemical and pharmacological research aimed at finding novel antibacterial medications.

Keywords- Neem seed oil, Antimicrobial properties, Herbal medicine, Zone of inhibition.

I. INTRODUCTION

Within the Meliaceae family, neem (*Azadirachta indica*) is one of the most useful and appropriate tree species found in India. Being able to thrive in a broad variety of soil conditions, including pH 10, this tree is very adaptable and significant on the Indian subcontinent. Owing to its numerous use, Indian farmers have been growing it since the Vedic era, and it is now ingrained in Indian culture. Neem trees are frequently seen growing sporadically in farmer's fields and along field boundaries without having an impact on the crops. Neem seed oil posses great antibacterial activity which was well studied by various researcher like Kannusamy and Rengaswamy (2016), Ojha (2016), Sewani and Qureshi (2016), Tanwar et al. (2016) [1-4]. Anticancer activity of neem seed oil was study by Singh et al. (2014) [5]. Anti diabetic activity of neem seed oil was study by Dholi et al., (2011) [6]. These studies proves that neem seed oil have multidimensional application. In this present work there was an attempt had been made to demonstrate antimicrobial activity of neem seed oil.

II. MATERIAL AND METHOD

Collection of plant method:

Fresh seeds of plant were collected from rural area of Nuapada district, western Odisha, India in the month of June at morning time when the dew was evaporated.

Depulping of Neem fruits:

Depulping is a process to remove seed coat and pulp from the Neem seed. It is done by hand and using mechanical depulper. The ripe Neem fruits were rubbed between palms in the bucket of water and wash the seed by use clean water for depulping [7].

Separation of the kernels[33]:

The dried Neemseeds are grand slightly by the hand and the outer shell of the seed is removed, kernels are present inside the shell which are separated and then made into powder using a grinder. It should be powdered

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such this no oil comes out of it. This coarse powdered is used for further studies for the extraction of oil[7]."

Extraction of oil:

Neem seeds need grinding according to the amount of oil content in the seed and size as well, after which they are sun dried and feed into the neem oil pressing machine. As the screw moves forward, neem oil is pressed out under high pressure and strong force. Then it is collected in a drum. [8]

Purification of oil:

After extraction of oil filtration is done to remove the various unwanted particles left in the extracted oil in order to obtain pure neem oil. The oil is then heated continuously to remove volatile material from it. [8]

Percentage yield:

1kg of seed was taken and the oil was extracted by compression method.

Evaluation of oil:

Physical test: - The oil is taken in a small transparent bottle and checks its colour, odour and tests.

Solubility: - The oil is put for solubility test on different solvent like water, alcohol, chloroform, acetone, petroleum ether take in a test tube. To find the solubility solvent is taken and 1 drop of oil is put on it.

Viscosity:- The viscosity was observed on Brookfield viscometer No: 8557400.

Refractive index: - Refractive index of neem oil was observed on Abbes Refractometer. [9]

Preparation of ointment

Formulation

White soft paraffin-50%
Liquid paraffin-20%
Polyethyleneglycol-30%
Neem oil-5ml

White soft paraffin, Liquid paraffin and Polyethyleneglycol was mixed at 80˚C temperature. Then it was cooled and at 40˚C Neem oil was mixed with continuous stirring. [10]

Evaluation of Ointment:

Colour and Odour- Physical parameters like colour and odour were examined by visual examination.

Consistency- Smooth and no greediness are observed by rubbing to skin.

P^H - P^H of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated.

LOD- LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105˚C.

Solubility- The oil is put for solubility test on different solvent like water, alcohol, chloroform, acetone, petroleum ether take in a test tube.

Washability -Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test- Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability study- Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2oC, 25oC and 37oC. The herbal ointment was found to be physically stable at different temperature i.e. 2oC, 25oC, 37oC within four weeks. [11]

III. ANTIBACTERIAL ACTIVITY OF OIL AND FORMULATION

Bacteria used for the study:

The bacterial strains of *Escherichia coli* (BKCP- 1), *Pseudomonas Sp.* (BKCP- 2), *Staphylococcus Sp.* (BKCP- 3) were isolated from different environments of the college campus, samples diagnosed and identified with help of cultural characteristic, gram staining and biochemical test.

Media used for the culture of bacteria:

Media used for this study were procured from HI-MEDIA Company, Mumbai. Nutrient agar and broth, Mac Conkey agar and broth, Mannitol agar and broth were used as General media as well as selective media for respective bacterial strains.

Qualitative test:

The disc diffusion method was followed. For this purpose, the bacterial lawn was made on Nutrient agar plates from 10^3 CFU/ml of respective bacterial cultures. The neem oil and herbal ointment impregnated paper discs (Whatman filter paper No 1) were put in triplicate to get concordant readings. The zones of inhibition depicted (if any) by the activity of drug were measured in mm. For standard each paper discs was impregnated with cefixime 10µg. [11]

Quantitative Assay:

The tube dilution method was followed to determine the minimum inhibitory concentration of neem oil and its ointment. The lowest concentration of drug inhibiting the bacterial growth was treated as MIC of the drug. For this purpose, a range of concentrations (25/ml, 50µl/ml, 75µl/ml, 100µl/ml, and 125µl/ml) of neem oil and herbal ointment containing neem oil was taken with the diluents Sodium taurocholate salt (0.4%).

Bactericidal / Bacteriostatic effect of the Neem oil:

The cidal / static effect of the oil was determined by making sub-cultures on Nutrient agar plates by streaking (Reference). The plates were then incubated for 24 hours at 37^0 C and growth or no growth was observed. [11]

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IV. RESULTS AND DISCUSSIONS

Result:

Extraction of oil: Percentage of yield was found is 170ml.

Evaluation of oil:

Physical Test:

Colour-yellow

Odour-characteristics

Taste-bitter

Table 1: Solubility test of oil

Sl no	Name of solvent	Soluble or insoluble
1	Water	Insoluble
2	Chloroform	Soluble
3	Ether	Soluble
4	Aceton	Soluble
5	Alcohol	Soluble
6	Petrolium ether	Soluble

Table 2: Viscosity test of oil

Sl no	No of rotation	Viscosity
1	100rpm	53.7
2	60rpm	47.0
3	50rpm	46.8 & 47
4	30rpm	40
5	20rpm	31

Refractive index –The refractive index was found to be1.401

Evaluation ointment

Table 3: Physical Evaluation

Sl No	Test	Observation
1	Colour	Pale yellow
2	Odour	Characteristic
3	Consistency	Smooth
4	P^H	5.6
5	LOD	5%
6	Washability	Washable
7	Irritability	Non Irritant
8	Stability	Stable

Solubility: Insoluble in water, soluble with alcohol, ether, chloroform.

Table 4: Antibacterial Activity of Oil and Formulation

Bacterial strain	MIC of oil	MIC of Formulation	Zone of inhibition of oil in mm	Zone of inhibition of Formulation in mm	Zone of inhibition of Standard drug in mm
AIR BACTERIAS	75µl	40µg	15	14	20
HAND BACTERIAS	50µl	30µg	21	23	24
WATER BACTERIAS	25µl	40µg	24	20	25

Bactericidal / Bacteriostatic effect:

From the sub culture there was no development of bacterial colony found revealed that both formulation and oil had bactericidal effect on bacterial strain.

V. DISCUSSION

Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects[40]. The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens[41]. Neem Oil from the seeds possesses a wide spectrum of antibacterial activity action against gram-negative and gram-positive microorganisms.

Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The seed oil was subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria *E. coli* and *Staphylococcus Sp.* and *Pseudomonas Sp.*. It was clear from the present results, that both seed oil and formulation of Neem exhibited pronounced activity against all the tested bacteria. The results show that the seed oil of *A indica* showed more inhibitory effect against *E. coli* whereas ointment of Neem oil showed best activity against *Pseudomonas Sp.*. Nimbidin, Nimbendiol and azadirachitin showed antibacterial activity[42]. The minimum inhibitory concentration (MIC) for bacteria was ranged between 30-40 mg/ml for neem oil was 25-75µl/ml when tested with seed oil of Neem.

VI. CONCLUSION

In summary, this investigation unequivocally demonstrated the antibacterial efficacy of neem oil against bacterial strains isolated from various environments. Today, more research on antimicrobials

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originating from plants is required. To identify the antibacterial chemicals found in these plants and to ascertain the full range of their activity, more investigation is required. Nonetheless, the current investigation into the in vitro antibacterial assessment of specific plants serves as a foundation for future phytochemical and pharmacological research aimed at finding novel antibacterial medications.

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