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EXTRACTION OF NEEM OIL FROM SEEDS: ANTIMICROBIAL ACTIVITY

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ABSTRACT

The extraction of neem oil from neem seeds (Azadirachta indica) has garnered significant interest due to its wide range of applications in medicine, agriculture, and cosmetics. This study aims to explore the extraction methods of neem oil and evaluate its antimicrobial properties. Neem seeds were collected, dried, and subjected to solvent extraction using n-hexane to obtain the neem oil. The extracted oil was then analyzed for its antimicrobial activity against common pathogenic microorganisms, including Escherichia coli, Staphylococcus aureus, and Candida albicans, using the disc diffusion method.

The results indicated that neem oil exhibited significant antimicrobial activity against all tested microorganisms, with varying levels of inhibition. The oil showed the strongest antimicrobial effect against Staphylococcus aureus, suggesting its potential as a natural antibacterial agent. The antimicrobial efficacy was dose-dependent, with higher concentrations of neem oil leading to stronger inhibition zones. This study underscores the potential of neem oil as an effective, eco-friendly alternative to synthetic antimicrobial agents. Further research into its active components and applications in pharmaceutical and agricultural industries is recommended.

KEYWORDS: *Neem oil, Azadirachta indica, extraction, antimicrobial activity, Staphylococcus aureus, Escherichia coli, Candida albicans.*

INTRODUCTION

The people of India have long revered the neem tree; for centuries, millions have cleaned their teeth with neem twigs, smeared skin disorders with neem-leaf juice, taken neem tea as a tonic, and placed neem leaves in their beds, books, grain bins, cupboards, and closets to keep away troublesome bugs. The neem oil is used for hair for growth of hair Given the use of various neem-derived products as pesticides and the realistic chances of residues derived from the treatments still being present at the time of consumption, there may be a risk for consumers. Therefore, in this study we present a review of the toxicological data from human and animal studies with oral administration of different neem- based preparations. These preparations can consist of crude plant parts, the seed oil, aqueous extracts of parts of the tree, extracts obtained with non-aqueous solvents, the pure bioactive insecticide ingredients and commercially available neem-based pesticides.

The word NEEM is derived from Sanskrit Nimba which means "bestower of good health. It has also been known as Ravisambha sun ray like effects in providing health. The Neem tree has been venerated through the ages in the Indian countryside as it provided hope in any situation and the faith in the miraculous healing powers of this amazing tree led patients with incurable diseases to adopt neem as way life. They lived in the shade of the tree, drank infusion of various part (Leaf, bark, etc) as advised by Ayurvedic tradition. They used young twigs for oral hygiene first thing in the morning, ate tender leaves as salad or cooked leaves with vegetable as food. Neem gums was used as lozenges for dryness of throat and allay thirst. In summer, sweet, ripe fruit were sucked for their sweetish pulp. All this together, probably strengthened their immune system to meet any challenge!!!

Neem oil is generally recommended for skin diseases while neem leaves are used for beauty purposes. Neem oil is a vegetable oil obtained from the fruits and seeds of the Neem tree *(Azadirachta indica*). It has been used for centuries in traditional Indian medicine and is now used in a wide range of applications due to its many beneficial properties. In recent years, extraction of Neem oil has become increasingly common, and the methods used to extract the oil vary. 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 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. This research paper aims to investigate the extraction of neem oil with different methods, as well as the potential applications of the extracted oil in different fields. Neem oil is a vegetable oil obtained from the leaves, bark and seed kernels of the neem tree. The review will evaluate the different extraction methods and discuss their efficacy, cost effectiveness, and safety. Furthermore, the paper will assess the potential uses of neem oil in various fields and discuss the benefits and risks associated with them. The extraction of Neem oil is a complex process and there are

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several methods used to extract the oil from the seeds. These include mechanical pressing, which is the most common method, solvent extraction, and steam pressure extraction. Each method has its own advantages and disadvantages, and it is important to choose the right. Neem leaf extracts have a powerful antiseptic, anti-fungal, antiviral and anti-bacterial effect. Unlike synthetic chemicals that often produce side effects such as allergic reactions, rashes etc. Neem is gentle and does not create any the complications. Unlike Neem seed oil, Neem leaves have a pleasant odor. An extract from neem leaves can be prepared as an alcoholic tincture or as tea. The alcohol extract has a dark green colour and is effective for several weeks. It can be used in anti-ageing nourishing formulas, mouthwashes, face washes, shower gels, soothing gels, face masks, skin toners, etc.

PLANT PROFILE



Fig. No. 01 Neem Plant Profile.

BIOLOGICAL IMPORTANCES OF NEEM

More than 150 compounds have been isolated from different parts of neem. The compounds have been divided into two major classes: isoprenoid (***Chatterjee and Pakrashi, 1991***) like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins, carbohydrates, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. Nimbidin, is responsible for crude bitter principle extracted Azadirachata, chemistry, medicinal properties, neem, A. indica which possess several biological activities, from this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated (***Siddiqui, 1942; Schumacher et al., 2011; Naik et al., 2014***). Further, Biswas et al. (2002) have reviewed the biological activities some of the neem compounds, pharmacological actions of the neem extracts, clinical study and plausible medicinal applications of neem along with their safety evaluation. Further, neem also possess compounds acts as Anti-inflammatory, Antiarthritic, Antipyretic, Hypoglycaemic, Antigastric ulcer. Spermicidal, Antifungal, Antibacterial, Diuretic, Antimalarial, Antitumour, Immunomodulatory
Medicinal Uses:

They are said to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. Neem products are also used in selectively controlling pests in plants. Neem is considered a part of Ayurvedic medicine. Neem is also known as the village pharmacy'. All parts of neem are used for preparing many different medicines, especially for skin disease. A compound from the Neem tree can be used as aspermicide Neem oil is used for preparing cosmetics (soap and shampoo, ozone as well as lotions and others), and is useful for skin care such as acne treatment. Neem oil has been used effectively as a mosquito repellent.

1.Leaves

Neem preparations are reportedly efficacious against a variety of skin diseases, septic sores, and infected burns. The leaves, applied in the form of poultices or decoctions, are also recommended for boils, ulcers, and eczema. The oil is used for skin diseases such as scrofula, indolent ulcers, and ringworm.

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2.Neem Flowers



Dried neem flowers are powdered and mixed with the leaves to prepare beauty packs for treating excess oil secretion, reducing acne and pimple and also to cure itching problems. Dried neem flowers help in treating blackheads. You need to mix neem oil with dried neem leaves and apply these directly on the blackheads.

3.Neam Bark



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Powder promotes skin health by encouraging natural skin rejuvenation, healthy turnover, and maintaining optimal moisture levels. It supports digestive health and healthy metabolism with its naturally soothing properties that rekindle the digestive fire while maintaining Pitta levels.

4.Gum



- Moisturizes the skin.
- Soothes inflamed and irritated skin.
- Fight multiple signs of premature aging.....
- Tackles blackheads and whiteheads.
- Treats uneven skin tone.
- Fights acne and pimples...
- Protects against environmental damage.

5.Seed

.



Treats acne. The antibacterial properties of neem fight acne-causing bacteria, which helps in the treatment and prevention of acne...
Pacify irritated skin.
Fight signs of ageing.....
Benefit of neem
High in fat content, neem oil improves the quality of your hair. Use neem oil if your scalp is dry because it nourishes the scalp, and its antifungal agents help treat dandruff.
Adding a few drops of neem oil to your hair can help with an itchy scalp.
Using neem oil can help you have a healthier scalp and in turn more robust locks.

MEDICINAL USE OF NEEM

Antimicrobial Activity

An antimicrobial kills or inhibits the growth of microbes, They used for cures of microbial infections. Various types of

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antimicrobials are used such as antibiotics, synthetic and natural compounds. However, prolonged use of mostly antibiotics can decrease the number of gut flora and causes. resistance, which can have a negative impact on health. Prolonged courses of antibiotic also cause serious side effects. Antifungal agents work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes cells. These cells are similar at the molecular level. Consequently, there are often side-effects to some of these drugs
Antibacterial and Anti-Fungal Activities:

The antibacterial activity of Neem leaves oilagainst various bacterial and fungal strains. The Neem oil showed considerably activity against bacterial [Gram-positive bacteria: example. Staphylococcus species and the Gram-negative bacteria; example Escherichia colil and fungal strains. The antibacterial activity against microbial cultures namely: Bacterial Strain; Escherichia coli, Bacillus cerus, Proteus vulgaris, Salmonella typhi, Klebsiella pneumoniae. Shigella dysenterae and Fungal strain; Fusarium oxysporum, Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Candida albicans, Cladosporium sp., Microsporum canis, Microsporum gypseum. Trichophyton rubrum, Trichophyton mentagrophytes, Penicillum notatum etc. The oil was not able to inhibit Proteus vulgaris. It was observed that the oil exhibited inhibitor effects against most of the microorganisms tested. The antifungal activity of neem oil against above fungal strains showed considerably activity. Moreover, the aqueous extract of plant has been previously reported to show antifungal activity, In this study the antibacterial and antifungal activities of the extracts from leaves oil. The crude oil is generally active against bacteria and fungi. In the light of these results we can conclude that level of antimicrobial activities of the Neem oil was compared with the chemical composition: activity relationship of extract.

Anticancer Activity
Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz[a] anthracene (DMBA), as revealed by reduced incidence of neoplasm (Gogati and Marathe, 1989). Pramanik et al. (2016) has conducted a study in chemoprotective neem compounds viz., azadirachtin, nimbolide and limonoid enrich extractson models of buccal carcinogenesis in hamsters. Overall studies were tested positive to reduce the expression and cell proliferation antigens. Further, researchers have shownprominent anti-cancerous activities from limonoid-derived compounds from neem. Amongst these, both 1-0-deacetylohchinolide B and 15-0-deacetylnimbolindin-B are proved to be beneficial to hinder cell growth in human cervical adenocarcinoma (Zhu et al., 2017; Chen et al., 2018). A very recent study discovered that alkaloid-derived limonoid, azadiramide-A, is primarily found in Neem leaf ethanolic extracts, showed to stop cell growth and induce apoptosis in both the estrogen independent MDAMB-231 and estrogen dependent MCF-7 cell lines of breast cancer in human beings (Elumalai et al., 2012; Zhu et al.,2017).

Antioxidant Activity
The antioxidant activity of neem seed extract has been demonstrated in vivo during horse- grain germination which is associated with low levels of lipooxygenase activity and lipid peroxides (Balasenthil et al., 1999). An antioxidant principle has also been isolated, which is a potent inhibitor of plant lipooxygenases. Anti-oxidants derived from neem is simple and cost effective way to supplement with natural extracts like those derived from Neem, in forms such as teas and oils, seem to be a simple and cost-effective way to introduce antioxidants (Alzohairy, 2016). 061 International Journal of Economic Plants2022

MATERIAL AND METHOD

MATERIAL

1. Neem Seeds: Fresh or dried seeds of Azadirachta indica.
2. Solvents: Methanol, ethanol, acetone, or water for extracting bioactive compounds.
3. Microorganisms: Bacteria (*E. coli, S. aureus, P. aeruginosa*), fungi (C. albicans, A. niger), and viruses (HIV, HSV).
4. Culture Media: Nutrient agar, Mueller-Hinton agar, or Sabouraud dextrose agar.
5. Tulsi leaf and coconut oil

- **Extraction of neem oil**

  Material
  1. Neem seeds
  2. Solvent (methanol, ethanol, or water)
  3. Grinder or mortar and pestle
  4. Filter paper
  5. Rotary evaporator or lyophilizer (optional)

  Protocol:
  1. Dry Neem seeds at 50°C for 2 hours
  2. Grind seeds into a fine powder.
  3. Weigh 10g of powder and transfer to a flask.
  4. Add 100mL solvent and stir for 2 hours.
  5. Filter mixture using filter paper.
  6. Concentrate extract using rotary evaporator or lyophilizer (if desired).

METHOD

- **Agar dilution method**

1. Prepare agar medium according to manufacturer's instructions.
2. Autoclave the agar medium at 121°C for 15-20 minutes.
3. Cool the agar medium to 45-50°C.
4. Add Neem seed extract to the agar medium at various concentrations (e.g., 100, 500, 1000 µg/mL).
5. Mix well and pour the agar medium into sterile petri dishes.
6. Allow the agar to solidify.
7. Inoculate microorganisms onto the agar surface using a sterile inoculum.
8. Incubate the plates at 37°C (bacteria) or 25°C (fungi) for 24-48 hours.

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CONCLUSION

Neem oil extracted from Azadirachta indica seeds exhibits potent antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and viruses. The oil's bioactive compounds, particularly azadirachtin, nimbin, and nimbidin, contribute to its antimicrobial properties.

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