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FORMULATION AND EVALUATION OF NEEM ANTIMICROBIAL LINCTUS

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ABSTRACT

Neem (Azadirachta indica), a medicinal plant with extensive antimicrobial properties, has been widely studied for its therapeutic potential against various human pathogens. This comprehensive review explores the formulation and evaluation of neem-based antimicrobial linctuses, focusing on extracts derived from different parts of the neem tree, including leaves, bark, seeds, and oil. Neem's bioactive compounds, such as azadirachtin, nimbolide, and catechin, exhibit significant antibacterial, antifungal, and antiviral activities. The formulation process involves optimizing excipients for stability, palatability, and bioavailability while maintaining the efficacy of neem extracts. Evaluation studies demonstrate neem's effectiveness against pathogens like Staphylococcus aureus, Escherichia coli, Candida albicans, and Salmonella typhi. In silico studies reveal strong ligand-receptor interactions between neem phytochemicals and inflammatory markers, such as cyclooxygenase-2 (COX-2) and C-reactive protein (CRP), supporting its anti-inflammatory activity. In vitro tests confirm neem's ability to inhibit microbial growth and biofilm formation, with minimum inhibitory concentrations comparable to conventional antibiotics. Stability tests indicate that neem-based linctuses retain their antimicrobial efficacy over time. Additionally, toxicity assessments highlight their safety for oral administration. This review underscores the potential of neem-derived linctuses as a natural alternative to synthetic antimicrobials, particularly in combating drug-resistant pathogens. Future research should focus on standardizing formulations and conducting clinical trials to establish neem's therapeutic significance in infectious disease management.

KEYWORDS

1. Neem Extracts
2. Antimicrobial Linctus
3. Bioactive Compounds
4. Drug Resistance
5. Phytotherapy

INTRODUCTION

This study investigates the formulation and evaluation of a novel neem (Azadirachta indica)-based linctus for the treatment of oral infections and the promotion of oral hygiene. Neem's established antimicrobial properties make it a promising candidate for pharmaceutical applications. This research focuses on developing a therapeutic formulation and rigorously evaluating its efficacy and safety profile, aiming to contribute to the advancement of natural product-based oral healthcare solutions.

NEEM: A PROMISING SOURCE FOR ANTIMICROBIAL THERAPEUTICS

Azadirachta indica, commonly known as neem, is a traditional medicinal plant with a rich history of use in various cultures. Its widespread recognition stems from its potent antimicrobial properties, encompassing antibacterial, antiviral, antifungal, anti-inflammatory, and anti-biofilm activities.[1] Numerous studies have investigated the therapeutic potential of different neem components, including leaves, seeds, and bark, for the treatment of infections and diseases. The antimicrobial efficacy of neem extracts makes them a promising source for developing novel antimicrobial formulations, such as linctus.[1]

PHARMACOLOGICAL PROPERTIES AND APPLICATIONS OF NEEM

Neem is a versatile tree native to the Indian subcontinent, valued for its medicinal, cosmetic, and agricultural uses. Its various parts, including leaves, seeds, and bark, are rich in antioxidants and possess anti-inflammatory, antibacterial, antifungal, and antiparasitic properties.[5] Traditional medicine has long utilized neem for treating a wide range of conditions, including ulcers, fever, and infections.[5] Neem extracts have shown therapeutic potential for various health conditions, including skin disorders, dental issues, and infections.[11] Neem oil, extracted from seeds, is a natural pesticide and insect repellent.[11] However, it is important to note that ingestion of neem by pregnant women can be harmful.[6]

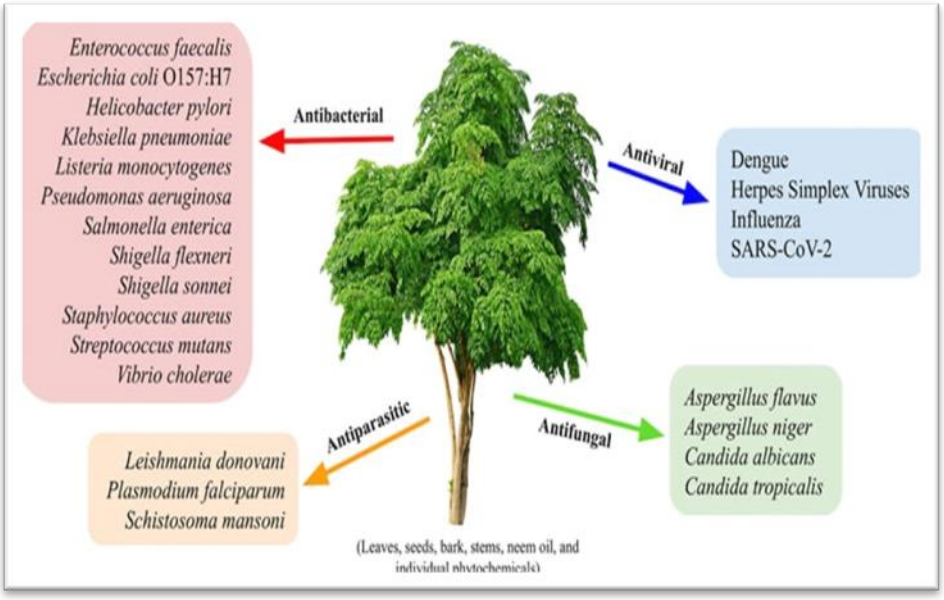

Fig. no. 1: Neem.

PLANT PROFILE OF NEEM (Azadirachta indica)

TAXONOMY

- **Scientific Name:** Azadirachta indica
- **Family:** Meliaceae
- **Genus:** Azadirachta
- **Species:** indica.

DESCRIPTION

Neem is a fast-growing, evergreen tree native to the Indian subcontinent and dry regions of South Asia. It can grow up to 30 meters in height with a rounded crown and thick furrowed bark. The leaves are compound with toothed leaflets, and the small white flowers are fragrant and bisexual. The fruit is a smooth yellow-green drupe with sweet-flavored pulp. Neem thrives in poor, rocky soils and tolerates a wide range of environmental conditions except freezing temperatures.

PHYTOCHEMISTRY

Neem contains over 300 bioactive compounds, including:

- **Limonoids**: Azadirachtin, nimbolide, gedunin
- **Triterpenoids**: Nimbin, nimbidin
- **Non-isoprenoids**: Tannins.

These compounds exhibit antimicrobial, antioxidant, antifungal, antiviral, and anti-inflammatory properties. Limonoids are particularly effective against biofilm-forming pathogens such as Staphylococcus aureus and methicillin-resistant S. aureus (MRSA).

ANTIMICROBIAL POTENTIAL

Neem extracts (e.g., ethanol, methanol) are widely studied for their antimicrobial activities. Specific findings include:

- Inhibition of MRSA biofilm formation at concentrations of 62.5–125 μg/ml.
- Reduction of MRSA biofilm adherence by up to 83.8% using petroleum ether extract.
- Neem has demonstrated efficacy against bacteria (E. faecalis, Pseudomonas aeruginosa), fungi (Candida albicans), viruses, and parasites.

APPLICATIONS IN ANTIMICROBIAL LINCTUS

Neem's antimicrobial properties make it suitable for linctus formulations targeting respiratory infections or oral pathogens. Active compounds like azadirachtin and nimbolide can be incorporated into ethanol or methanol-based linctus preparations to enhance therapeutic efficacy against drug-resistant microbes.

PLANT PROFILE OF NEEM (AZADIRACHTA INDICA) FOR THE FOMULATION AND DEVELOPMENT OF ANTI-INFLAMMATORY AND ANTIOXIDANT LINCTUS

PHYTOCHEMICAL COMPOSITION

- **Contains over 140 active compounds, including:**
- **Flavonoids:** Quercetin and β-sitosterol
- **Triterpenoids:** Nimbin, nimbolide, nimbidin
- **Limonoids:** Azadirachtin, gedunin
- **Antioxidants:** Ascorbic acid and polyphenols**.**

PHARMACOLOGICAL PROPERTIES

1. ANTI-INFLAMMATORY ACTIVITY

- Compounds like limonoids inhibit inflammatory mediators (e.g., prostaglandins and cytokines), reducing inflammation and pain.
- Nimbolide and nimbidin have been shown to suppress inflammation by modulating pathways such as NF-κB and COX enzymes**.**

2. ANTIOXIDANT ACTIVITY

- Neem extracts scavenge free radicals and enhance the body's natural antioxidant defenses.
- Active compounds like nimbolide, azadirachtin, and quercetin show potent free radical scavenging activity**.**
- Protects cells from oxidative damage, which is crucial in preventing chronic inflammation and related diseases.

APPLICATIONS IN LINCTUS FORMULATION

- Neem-based linctus can be developed to target inflammatory conditions (e.g., sore throat) and oxidative stress-related ailments.
- The inclusion of ethanol or aqueous neem leaf extracts ensures effective delivery of bioactive compounds with anti-inflammatory and antioxidant properties.
- Synergistic effects of multiple phytochemicals enhance therapeutic efficacy.

TRADITIONAL USES

- Treats inflammation-related conditions such as arthritis, fever, and respiratory infections.
- Used for its antioxidant benefits in detoxifying the body and promoting overall health.

COMPOSITION FOR NEEM-BASED ANTIMICROBIAL LINCTUS

The composition for the formulation and evaluation of an antimicrobial linctus using neem extract derived from various parts of the neem tree involves incorporating bioactive compounds from leaves, bark, seeds, flowers, and fruit. Below is a suggested composition based on the medicinal properties and antimicrobial activities of neem.

ACTIVE INGREDIENTS

1. NEEM LEAF EXTRACT (ETHANOLIC OR METHANOLIC)

- Contains azadirachtin, nimbin, and cyclic trisulphides with antibacterial and antifungal properties.
- Effective against E. coli, Staphylococcus aureus, and fungal pathogens like Candida albicans.
- Concentration**:** 5–10% w/v.[1][2]

2. NEEM BARK EXTRACT (METHANOLIC)

- Rich in margolone, margolonone, and polysaccharides with antibacterial and anti-inflammatory activities.

- Concentration: 3–7% w/v.[1]

3. NEEM SEED OIL

- Contains nimbidin, azadirachtin, and gedunin with antifungal and antibacterial effects.
- Concentration: 2–5% v/v.[1]

4. NEEM FLOWER EXTRACT (AQUEOUS)

- Known for its cough-relieving properties and mild antimicrobial activity.
- Concentration: 1–3% w/v.[1]

5. NEEM FRUIT EXTRACT

- Contains compounds beneficial for insecticidal and antibacterial purposes at higher concentrations.
- Concentration: 2–4% w/v.[1]

EXCIPIENTS AND ITS LIMITATIONS

1. **Solvent:** Ethanol or Methanol (for extraction) in combination with purified water.

2. **Viscosity Enhancer:** Xanthan gum or hydroxypropyl methylcellulose (0.5–2%) to achieve syrup-like consistency.

3. Preservative: Sodium benzoate or parabens (0.1–0.2%) to prevent microbial contamination.

4. **Sweetener:** Sucrose or artificial sweeteners (5–10%) to mask neem's bitterness.

5. **Flavoring Agent:** Mint or lemon flavor (0.2–0.5%) for better palatability.

6. **Stabilizer:** Ascorbic acid (0.1–0.5%) to prevent oxidation of active compounds.

MEDICINAL USES

1. SKIN CARE

Neem is known for its ability to purify the blood and detoxify the body, which helps in managing skin issues. It acts as an exfoliant, removing dead skin cells and preventing blemishes.[27] Neem oil is often applied to wounds and infections due to its antiseptic properties.[29] Treats acne, psoriasis, eczema, and fungal infections due to its antimicrobial and anti-inflammatory properties.[25]

2. WOUND HEALING

The application of Neem leaves and extracts are applied to wounds and ulcers for their antiseptic and healing effects, due to its tissue regeneration properties.[26]

3. DIGESTIVE HEALTH

The anti-inflammatory properties of neem aid in reducing gastrointestinal issues such as ulcers, constipation, and flatulence. It helps balance digestive functions and promotes overall gut health.[28]

4. BLOOD SUGAR REGULATION

Neem has been shown to lower blood sugar levels, making it beneficial for diabetic patients. It can be taken in various forms to help manage diabetes effectively.[27],[29]

5. IMMUNE SYSTEM SUPPORT

Neem boosts immunity through its antimicrobial effects, helping the body fight off infections and diseases. Regular consumption can enhance overall health by eliminating free radicals and regulating hormones.[25]

6. PAIN RELIEF

Application of neem oil or extracts can alleviate pain associated with conditions like arthritis due to its anti-inflammatory effects.[25][29]

7. DETOXIFICATION

Neem aids in detoxifying the body both internally (through consumption) and externally (through topical application), stimulating liver and kidney functions to eliminate toxins.[25]

8. ANTI – INFLAMMATORY EFFECTS

Neem contains compounds that exhibit anti-inflammatory properties, which can help reduce inflammation in the body. This makes it useful for managing conditions such as arthritis and other inflammatory disorders.[6][32]

9. ANTIMICROBIAL ACTIVITY

Neem is effective against a variety of pathogens due to its strong antimicrobial properties. It can help prevent infections by inhibiting the growth of harmful bacteria and fungi.[31]

10. POTENTIAL ANTI – CANCER PROPERTIES

Preliminary studies suggest that neem may have anti-cancer effects by inhibiting the growth of cancer cells and preventing tumor formation.[30][32]

COSMETIC USES

11. HAIR CARE

Neem's antifungal properties make it useful in treating dandruff and promoting healthy hair growth. It is commonly found in shampoos and conditioners.[25][27]

12. DENTAL HYGIENE

Neem is used in oral hygiene products due to its effectiveness against dental pathogens like Streptococcus mutans and Enterococcus faecalis.[6] Used in toothpaste and mouthwashes to prevent plaque formation and gum diseases; neem twigs are also used as toothbrushes in rural areas.[6][28]

TRADITIONAL APPLICATIONS

- Neem tea is consumed to lower fever and relieve headaches.
- Poultices made from neem leaves treat septic sores, burns, and skin ulcers.[26]

LINCTUS

Linctus is a type of thick, sweet medicinal liquid syrup that is taken orally and used primarily to relieve coughs and soothe sore throats. It is often prescribed to treat dry coughs by suppressing the cough reflex in the brain. Linctuses can also provide relief from minor throat irritation and allergy symptoms like runny nose and watery eyes.[12] Linctuses can contain various active ingredients depending on their formulation, such as antitussives (cough suppressants), expectorants (to help loosen mucus), or demulcents (to soothe the throat).[11][13] Linctuses are typically syrup-based, which not only enhances palatability but also acts as a preservative due to high sugar content. However, formulations containing water may require additional preservatives to prevent microbial growth.

PHYSICOCHEMICAL PROPERTIES

1. pH

- Range: Typically between 4.2 and 5.3 for cough linctuses, which is slightly acidic to neutral.[27] This range supports the stability of many herbal extracts and antimicrobial agents.
- Importance: pH affects drug solubility, stability, and absorption. A slightly acidic pH can enhance the antimicrobial activity of certain compounds.

2. VISCOSITY

- Range: Varies based on excipients; glycerin-based formulations tend to have higher viscosity (around 87.42 mPa·s), while simple syrup formulations have lower viscosity (around 48.70 mPa·s).[27]
- Importance: Viscosity influences the flowability and contact time of the linctus with mucous membranes, potentially affecting drug delivery and bioavailability.

3. SPECIFIC GRAVITY

- Range: Typically around 1.195 to 1.254 for cough linctuses, depending on the formulation.[27]
- Importance: Specific gravity is a measure of density relative to water, affecting the formulation's flowability and stability.

4. ANTIMICROBIAL ACTIVITY

- Factors: The presence of compounds like neem extracts or honey can provide antimicrobial effects. Honey's acidity (pH 3.2-4.5) and low water activity contribute to its antibacterial properties.

5. STABILITY AND SHELF LIFE

- Importance: Ensuring the stability of the formulation over time is crucial for maintaining its efficacy and safety. Factors like pH, viscosity, and storage conditions influence shelf life.

ADVANTAGES OF LINCTUS

- **Relief from Cough Symptoms**

Linctus is effective in relieving symptoms of dry cough and sore throat by exerting a soothing action on the mucous membranes of the throat.[14]

- **Soothing Properties**

Ingredients like glycerol in linctus provide demulcent properties, which help in soothing the throat and reducing irritation, making it a safe option for both children and adults.[15]

- **Easy to Use**

Linctus is typically available in a syrup form, making it easy to administer, especially for those who have difficulty swallowing tablets.[14]

- **Versatility in Formulations**

Linctus can be formulated with various flavors and ingredients, such as lemon or honey, which can make it more palatable and appealing to patients.[15]

- **Rapid Action**

Some linctuses, like those containing Levocloperastine Fendizoate, act quickly to suppress cough reflexes, providing fast relief.[17]

- **Combination Therapy**

Certain linctuses, such as Sinarest, combine multiple active ingredients to address not just cough but also other symptoms like sneezing and runny nose associated with allergies.

- **Accessibility**

Many types of linctus are available over-the-counter or by prescription, making them accessible for a wide range of patients.

DISADVANTAGES OF LINCTUS

- **Side Effects**

Common side effects include dizziness, drowsiness, nausea, vomiting, constipation, and lightheadedness. These can be temporary but may require medical attention if severe.[18]

- **Dependence and Addiction**

Codeine-based linctuses can lead to dependence and addiction, especially if used improperly or in higher doses than prescribed.[19]

- **Withdrawal Symptoms**

Stopping codeine-based linctus abruptly can cause withdrawal symptoms such as anxiety, sweating, and tremors.

- **Interactions with Other Medications**

Linctus can interact with other medications, potentially worsening side effects or reducing efficacy. It's crucial to inform your doctor about all medications you're taking.[18]

- **Contraindications**

Certain linctuses, especially those containing codeine, are not suitable for everyone, such as individuals with chronic respiratory conditions or those prone to addiction.[16][20]

- **Impact on Daily Activities**

The sedative effects of some linctuses can impair driving ability and mental focus, requiring caution when performing tasks that require alertness.[12][18]

- **Risk of Overdose**

Misuse of codeine-based linctus can lead to serious health issues, including respiratory depression and even death.[21]

- **Potential for Allergic Reactions**

Some individuals may experience allergic reactions to ingredients in linctus, which can range from mild to severe.

ANTIMICROBIAL LINCTUS

An antimicrobial linctus would be a cough syrup formulation that includes ingredients with antimicrobial properties. While traditional linctuses primarily contain demulcents, expectorants, and antitussives, incorporating antimicrobial agents could enhance their effectiveness against infections.[24] Antimicrobial linctus refers to a type of cough syrup that contains ingredients with antimicrobial properties, aimed at alleviating cough symptoms while potentially combating infections. These formulations typically include a mixture of active ingredients that can soothe the throat, reduce irritation, and provide relief from coughs.

ROLE OF EXCIPIENTS IN FORMULATION AND DEVELOPMENT

- Excipients play a critical role in the formulation development of neem-based antimicrobial linctus by ensuring stability, efficacy, and patient acceptability.
- Excipients are non-active components included in formulations to enhance the manufacturing process, stability, and functionality of the active pharmaceutical ingredient (API). In the case of neem-based linctus, excipients serve the following purposes:

1. Stabilizers: Neem extracts, which contain bioactive compounds like azadirachtin, nimbolide, and limonoids, are prone to degradation. Stabilizing excipients such as antioxidants (e.g., ascorbic acid) can protect these compounds from oxidation.[1],[3]

2. Solubilizers: Neem's active compounds are often extracted using solvents like ethanol or methanol. Solubilizers (e.g., glycerin or propylene glycol) help dissolve these extracts uniformly in the linctus base.[2],[3]

3. Viscosity Agents: To achieve a syrup-like consistency for linctus, viscosity enhancers such as cellulose derivatives (e.g., hydroxypropyl methylcellulose) or xanthan gum are used.[1],[3]

4. Sweeteners and Flavoring Agents: Neem has a bitter taste; sweeteners (e.g., sucrose or artificial sweeteners) and flavoring agents improve palatability for patient compliance.[1],[3]

5. Preservatives: Antimicrobial preservatives like parabens or sodium benzoate prevent microbial growth in the formulation, ensuring shelf-life stability.[1][3]

6. Emulsifiers: For neem oil-based formulations, emulsifiers like lecithin or polysorbates ensure uniform dispersion of oil in the aqueous medium.[3]

ANTIMICROBIAL PROPERTIES OF NEEM

Neem (Azadirachta indica) is widely recognized for its antimicrobial activity against bacteria, fungi, and viruses. Its bioactive compounds include:

- **Limonoids:** Effective against drug-resistant pathogens and biofilm-forming organisms.
- **Tannins:** Contribute to antimicrobial activity by disrupting microbial membranes.
- **Azadirachtin and Gedunin:** Known for their antibacterial and antifungal properties.[2]

Neem extracts have been shown to outperform some traditional medicinal plants in antimicrobial efficacy against pathogens like E. coli, Salmonella, S. aureus, and Vibrio cholerae. These properties make neem a promising candidate for therapeutic applications in infectious diseases.

EVALUATION AND QUALITY CONTROL TESTS

Evaluating and ensuring the quality of antimicrobial linctus formulations using neem extract involves several critical steps. These include physical, chemical, and microbiological tests to guarantee the product's efficacy, stability, and safety.

1. Physical Tests

- Appearance and Color: Ensure the linctus has a uniform appearance and color.
- Viscosity: Measure using a viscometer to ensure it meets the desired consistency.
- pH: Check to ensure compatibility with oral mucosa.

2. Chemical Tests

- Assay of Active Ingredients: Use techniques like HPLC or TLC to quantify neem extract components.
- Solvent Residue Test: Ensure that solvents used in extraction are within acceptable limits.
- Stability Studies: Conduct accelerated stability tests to assess degradation over time.

3. Microbiological Tests

- Antimicrobial Activity: Use agar well diffusion or broth dilution methods to assess efficacy against target pathogens.
- Sterility Test: Ensure the formulation is free from microbial contamination.
- Preservative Efficacy Test: Verify that preservatives prevent microbial growth.

4. Toxicity and Safety Tests

- Acute Toxicity Studies: Conduct in vivo studies to assess safety in animal models.
- Cytotoxicity Tests: Use cell cultures to evaluate potential cytotoxic effects.

5. Formulation Evaluation

- In Vitro Dissolution Test: Evaluate the release of neem extract from the linctus.
- Bioavailability Studies: Assess how effectively the neem extract is absorbed in the body.

EXAMPLE OF QUALITY CONTROL PROTOCOL

Protocol for Antimicrobial Activity Evaluation

1. Preparation of Neem Extract

Extract neem using a suitable solvent (e.g., ethanol) and dry to obtain a solid mass.

2. Preparation of Linctus Formulation

Mix the neem extract with excipients (e.g., glycerin, sweeteners) to achieve the desired consistency.

3. Antimicrobial Assay

- Use the agar well diffusion method to assess activity against bacteria like E. coli and S. aureus.
- Prepare Mueller-Hinton agar plates and inoculate with test organisms.
- Create wells and fill with different concentrations of neem linctus.
- Incubate at 37°C for 24 hours and measure inhibition zones.

Example of Stability Testing

- Accelerated Stability Test: Store the linctus at elevated temperatures (e.g., 40°C) and humidity (e.g., 75%) for a specified period (e.g., 3 months).
- Physical and Chemical Analysis: Regularly assess appearance, pH, viscosity, and active ingredient content.

LITERATURE REVIEW

1. *Ivan K. Simpson et al., (2022)* This study investigated the use of glucose syrup from high-quality cassava flour (HQCF) as a vehicle or sweetener in the preparation of paracetamol syrup and simple linctus. Four formulations were prepared using glucose syrup, while two controls used sucrose syrup. All formulations passed microbial load and drug content tests. The study found that glucose syrup from HQCF could be a suitable alternative to sucrose syrup in oral liquid formulations, potentially reducing costs.

2. *Marina R. Wylie et al.*, (2022) Azadirachta indica, a traditional medicine plant, has antimicrobial properties in dentistry, food safety, bacteriology, and parasitology. Its extracts show promise against drug-resistant pathogens. Further research is needed to understand its mechanisms, clinical efficacy, and safety.

3. *Jose Francisco Islas et al.*, (2020) Global health practices are integrating alternative medicine with evidence-based approaches to understand metabolic processes. Azadirachta indica (Neem) offers health benefits like insect repellent, anti-inflammatory effects, diabetes management, and cancer prevention. However, improper extraction methods can pose health risks.

4. *Adamu Muhammad et al.*, (2019) The study at Federal University Dutse examined the antimicrobial activity of neem leaf extract against Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus. The extract showed greater effectiveness against Gram-negative bacteria, with inhibition zones of 21 mm and 14 mm respectively.

5. *Arshad Husain Rahmani et al.*, (2018) Neem, a Meliaceae tree, contains bioactive compounds like azadirachtin, quercetin, and limonoids, which have antioxidant, hepatoprotective, antimicrobial, and anticancer effects. These compounds modulate cell signaling pathways, suggesting potential therapeutic applications in cancer management.

6. *Harmest Kaur et al.,* (2017) This study compared the pharmacognostical and physico-chemical profiles of Ashtanga Maleha powder (AP) and linctus (AL) for asthma treatment, revealing significant differences in herbal ingredients and the impact of preparation methods on the final product's properties.

7. *Shradha M. Patel et al.,* (2016) Neem, also known as "the wonder tree" or "nature's drug store," is known for its medicinal properties, including cancer prevention. Its phytochemicals, found in leaves, bark, and seeds, exhibit biological and pharmacological activities through free radical scavenging, DNA repair, apoptosis, and immune modulation.

8. *YUVANESWARAN KRISHNAN et al.,* (2015) The study by Yuvaneswaran Krishnan et al. (2015) assessed the cytotoxic and antimicrobial properties of Azadirachta indica leaf extracts. The acetone extract showed stronger inhibition against Gram-negative bacteria, while chloroform extract showed stronger inhibition against Gram-positive bacteria. Cytotoxicity was assessed using the Artemia salina lethality assay, with acetone extract showing higher toxicity than chloroform. These findings suggest A. indica leaves have potential as antimicrobial and cytotoxic agents.

9. *Ranjit R. Raut et al*., (2014) The study evaluated the antimicrobial properties of neem leaves and bark against gram-negative and gram-positive bacteria using an agar well diffusion method. Results showed significant inhibition zones for V. cholerae and B. subtilis, while E. coli and S. typhi showed lower susceptibility.

10. *Sherein et al.,* (2014) The study evaluated neem extract's antimicrobial activity against 13 animal strains using agar-well diffusion. Results showed significant bactericidal effects at lower concentrations, particularly against Gram-negative bacteria. However, it did not show activity against Gram-positive bacteria. The extract also showed fungicidal properties against Candida albicans and Aspergillus flavus.

11. *Ashish A. Gawai et al.,* (2014) The study aimed to validate the cleaning process in a facility producing Paracetamol Suspension and Simple Linctus BP, preventing cross-contamination. Results showed no visible residues, chemical residues below acceptable limits, and microbial counts within acceptable thresholds. Proper cleaning prevented

cross-contamination between the two products, highlighting the importance of strict cleaning protocols and validation processes.

12. *Venugopalan Santhosh Kumar et al.,* (2013) Neem, also known as the "divine tree," is a plant native to the Indian subcontinent with a long history in traditional medicine dating back to prehistoric times. The World Health Organization estimates that 50% of people in developing countries rely on traditional medicine for primary healthcare. Over half of the global population still relies on plants for medicinal purposes. This review highlights the extensive use of neem for treating various ailments throughout history.

13. *WasudeoNamdeoGhonmode et al.,* (2013) Enterococcus faecalis is a common microorganism in root canal treatments, requiring thorough debridement and elimination. Traditional irrigants have antimicrobial properties, but the rise of antibiotic-resistant strains and synthetic drug side effects has led to interest in herbal alternatives. This study aims to evaluate their antimicrobial efficacy compared to sodium hypochlorite.

14. *S.Susmitha et al.,* (2013) The study tested aqueous extracts of Azadirachta indica (Neem) against Escherichia coli and Salmonella sp. using the cup diffusion method. The leaves showed effectiveness against both pathogens, with E. coli showing a Minimum Bactericidal Concentration of 5 mg/l. The study highlights neem's potential as an antimicrobial agent for combating infectious diseases.

15. *Imam Hashmat et al.,* (2012) This review explores the biological activities, isolated compounds, pharmacological actions, clinical studies, potential medicinal uses, and safety evaluations of neem, a key plant in traditional medicine in India. It highlights the progress made in understanding its chemistry, biological activity, and applications, making it a valuable natural product source.

16. *I.P. Ogbuewu et al.,* (2011) The rising costs of chemical fertilizers and agrochemicals in developing countries have led to increased poverty and debt. Neem trees, rich in bioactive compounds, offer non-wood products with diverse applications. This review compiles research on neem's potential in agriculture, industry, medicine, and environmental sustainability, highlighting its potential in addressing these challenges.

17. *Maurice Iwu et al.,* (2009) Traditional cough treatments often use linctuses with sedative, expectorant, and antimicrobial ingredients, with syrup for soothing the throat. Medicinal

herbs like Garcinia kola, Zingiber officinale, Aframomummelegueta, and Ocimum viride are used for cough formulations due to their antimicrobial, anti-inflammatory, and expectorant properties. This study evaluates the physical properties of a cough linctus using herbal extracts.

18. *Girish K. et al.,* (2008) Neem, also known as Indian Lilac or Margosa, is a versatile tree in the tropics with numerous non-wood products like leaves, bark, flowers, seeds, oil, and neem cake. These products have numerous biological activities, making them useful in medicine, agriculture, and industry, earning it the title of a "green treasure."

19. *R. Suhapriya et al.,* (2005) Neem, also known as Azadirachta indica, is a medicinal plant with over 140 biologically active compounds found in its leaves, flowers, seeds, fruits, roots, and bark. Its therapeutic effects include anti-inflammatory, antimicrobial, antiviral, and antioxidant properties, and have potential for treating conditions like diabetes, malaria, and skin diseases.

20. *Sara J. Boeke et al.,* (2004) Neem, also known as Azadirachta indica, is a plant with pesticidal and health benefits like blood sugar regulation and anti-inflammatory properties. However, it also has toxic effects, with non-aqueous extracts being the most toxic. Despite these, neem-based insecticides can be safely used with proper care, especially for long-term exposure.

21. *Goutam Brahmachari et al.,* (2004) Neem, a "wonder tree" in India, has diverse uses in disease treatment, agriculture, and pest control. Its active chemical constituents and commercial products are discussed, along with safety evaluations of various parts and formulations. The extensive scientific knowledge on neem contributes significantly to global interest in its applications.

22. *V Natarajan et al.,* (2003) The study investigated the effectiveness of Azadirachta indica leaf and seed extracts against dermatophytes. Clinical isolates of Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporumcanis were treated with neem extracts. The MIC was 31 µg/ml, and at a concentration below the MIC, neem seed extract significantly altered fungi growth patterns.

23. *Kausik Biswas et al.,* (2002) Neem, a valuable traditional medicinal plant in India, has shown significant progress in understanding its chemistry, biological activity, and

applications over the past five decades. It is now recognized as a valuable source of natural products for developing medicines and industrial products, with a focus on safety evaluations.

EXTRACTION AND STANDARDIZATION

2.1 EXTRACTION OF NEEM

- **AQUEOUS EXTRACTION**

This method is often used for preparing neem leaf extract.

Procedure

➔ Collect fresh neem leaves and wash them thoroughly.

➔ Dry the leaves in the shade.

➔ Mix 50 grams of dried neem leaf powder with 500 ml of distilled water.

➔ Boil the mixture for about 30 minutes.

➔ Filter the solution using Whatman No. 1 filter paper to obtain a clear aqueous extract.

➔ Store the extract at 4°C until use.[40]

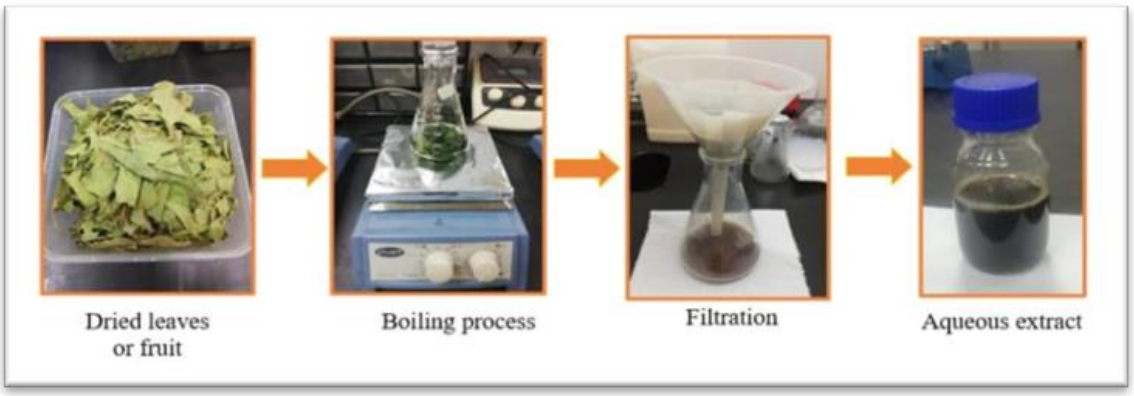

Fig. no. 2: Aqueous Extraction.

- **MECHANICAL EXTRACTION**

This method is commonly used for extracting neem oil from seeds.

Procedure

➔ Collect neem seeds and clean them.

➔ Use hydraulic pressing equipment to apply pressure on the seeds to extract oil. The process typically starts at a pressure of 138 bars and can go up to 412 bars until oil flow ceases.

➔ The extracted oil is then purified to remove impurities.[41]

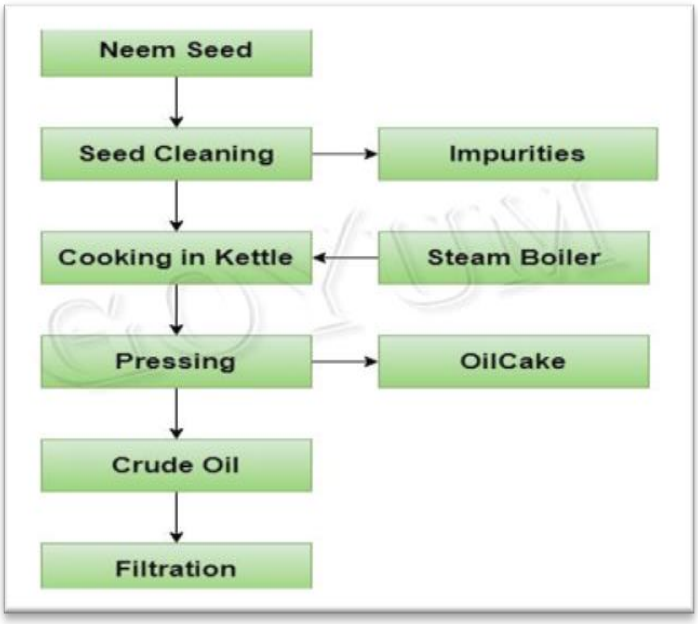

Fig. no. 3: Mechanical Extraction.

- **STEAM PRESSURE EXTRACTION**

This technique involves using steam to facilitate oil extraction from seeds.

Procedure

➔ Neem seeds are subjected to steam heating, which swells them and makes it easier to extract oil under high pressure.

➔ This method enhances the efficiency of oil extraction by reducing the mechanical pressure required.[41]

- **ETHANOLIC EXTRACTION**

Ethanol is widely used to extract bioactive compounds from neem leaves due to its efficiency in isolating antimicrobial agents.

Procedure

➔ Fresh neem leaves are collected, washed, and dried at 35–40°C.

➔ The dried leaves are pulverized into powder using an electric grinder.

➔ The powder is placed in a Soxhlet apparatus with 96% ethanol and heated at 78°C for 18 hours.

➔ The ethanolic extract is concentrated using a rotary evaporator to obtain a solid mass, which is stored at 4°C.

- **ABSOLUTE ETHANOL EXTRACTION**

This method involves maceration and filtration for neem leaf extracts.

Procedure

➔ Fresh neem leaves (25 g) are mixed with 50 mL of absolute ethanol and macerated for 1–2 minutes.

➔ The mixture is filtered through muslin cloth and filter paper to remove coarse residues.

➔ The residue is re-extracted with additional ethanol, and both extracts are pooled.

➔ Alcohol is evaporated using a water bath until the final volume reaches approximately 25 mL. The extract is stored in airtight amber containers.

- **PETROLEUM ETHER AND ETHYL ACETATE EXTRACTION**

Neem leaves can also be extracted using petroleum ether or ethyl acetate for specific bioactive compounds.

Procedure

➔ Neem powder is mixed with the solvent (petroleum ether or ethyl acetate) and rotated for one day.

➔ The mixture is filtered using Whatman No. 1 filter paper, and the residue undergoes repeated extraction cycles.

➔ The filtrate is dried to evaporate the solvent, leaving behind fine extracts.

2.2 YIELD CALCULATION

➔ The extraction yield percentage was calculated using the formula

➔ The ethanolic extract typically showed higher yields compared to aqueous extracts.[38][39]

3. FORMULATION OF NEEM ANTIMICROBIAL LINCTUS:

Ingredients

➔ Neem extract (aqueous or ethanolic)

➔ Sweeteners (e.g., sucrose or honey)

➔ Thickeners (e.g., glycerin or xanthan gum)

➔ Preservatives (e.g., sodium benzoate)

➔ Flavoring agents (e.g., menthol or lemon oil)

Procedure

➔ Neem extract was mixed with other ingredients in specified proportions.

➔ The mixture was homogenized to ensure uniform distribution of active compounds.

➔ The linctus was stored in amber-colored bottles to prevent light degradation.

4. ANTIMICROBIAL ACTIVITY TESTING

4.1 DISK DIFFUSION METHOD

➔ Sterile paper disks were impregnated with neem extract at varying concentrations (e.g., 100, 50, 25 mg/mL).

➔ Disks were placed on Mueller-Hinton agar plates inoculated with bacterial cultures such as Staphylococcus aureus, Escherichia coli. Pseudomonas aeruginosa, and Candida albicans.

➔ Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured.[39]

4.2 MINIMUM INHIBITORY CONCENTRATION (MIC)

➔ The broth microdilution method was used to determine MIC values.

➔ Serial dilutions of neem extract were prepared in Mueller-Hinton broth, and bacterial suspensions were added.

➔ MIC was defined as the lowest concentration at which no visible growth occurred after incubation.[39]

4.3 MINIMUM BACTERICIDAL CONCENTRATION (MBC)

MBC was determined by subculturing samples from MIC wells onto fresh agar plates to identify the lowest concentration that killed the bacteria completely.[39]

5. STABILITY STUDIES

➔ Stability testing was conducted under various conditions:

➔ Temperature: Room temperature (25°C), refrigerated conditions (4°C), and accelerated conditions (40°C).

➔ Humidity: Controlled humidity chambers were used.

➔ Physical appearance, viscosity, pH, and antimicrobial activity were monitored over time.[39]

6. TOXICITY ASSESSMENT

➔ Perform acute toxicity studies in animal models to ensure Safety.

➔ Hemolytic assays were conducted to evaluate cytotoxicity against red blood cells.

➔ Neem extracts showed no significant toxicity in tested models.[38][39]

7. STATISTICAL ANALYSIS

Data from antimicrobial activity tests and stability studies were analyzed using statistical tools like ANOVA to determine significance levels.

CONCLUSION

The study on the formulation and evaluation of the neem antimicrobial linctus shows significant promise as a natural alternative for managing oral infections. Neem's broad-spectrum antimicrobial properties, including antibacterial, antiviral and antifungal effects, support its potential as a viable treatment. Further research is needed to standardize preparations, assess toxicity, and conduct clinical trails. Neem-based formulations could offer a safer alternative to synthetic antimicrobials, reducing resistance and side effects. Overall, neem holds great potential in the development of effective oral health products.

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