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Research Paper

A Review: Effect of Neem Extracts (Azadirachta indica) On Cavity Causing Microorganism

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ABSTRACT: *Herbal medicines are quickly becoming popular because they provide patients with a safer, more cost-effective, and more efficient form of treatment. Neem (Azadirachta indica) has long played a significant role in dental health and hygiene. It has demonstrated efficacy as a cancer-preventive, anti-inflammatory, and antibacterial agent.It is now of vital relevance to examine the biological activities of some of the isolated neem compounds, the pharmacological effects of neem extracts, clinical trials, and potential neem therapeutic applications, as well as their safety. It has long been a crucial component of oral hygiene in India and is still a well-liked dental care product there. Neem extract is included in dental care products as a natural remedy for oral-dental issues and for maintaining oral health. Dental health affects one's whole quality of life, and chronic diseases and systemic problems are associated with poor oral health. To stop the proliferation of dental pathogenic organisms, this study examined the antibacterial properties of neem leaf extract. Neem leaves were used in the research to generate an extract by maceration and the soxhlet method, which was then used to investigate the extract's antibacterial activity and assess the inhibitory zones using the agar well diffusion method.*

KEYWORDS*: Neem(Azadirachta indica), Aqueous extract, Phytochemicals**,** oral hygiene, antibacterial activity.*

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I. INTRODUCTION

The evergreen neem tree is grown throughout the subcontinent. Since ancient times, every part of the tree has been employed as a traditional medicine for domestic remedies against a variety of human illnesses. In Ayurveda, Unani, and homeopathic medicine, neem has been utilized extensively. The neem tree is known as " Arishtha" in Sanskrit, which means healer of illness. That is why it is known as "Sarbaroganibarini". In Pakistan, the tree is still regarded as the local medical facility. [1]. De Jussieu first identified the neem tree as A. indica in [2], and its taxonomic status is as follows:

- Order - Rutales
- Suborder - Rutinae
- Family - Meliaceae (mahogany family
- Subfamily - Melioideae
- Tribe –Melieae
- Genus - Azadirachta
- Species – indica

Figure 1: Neem-leaves [3]

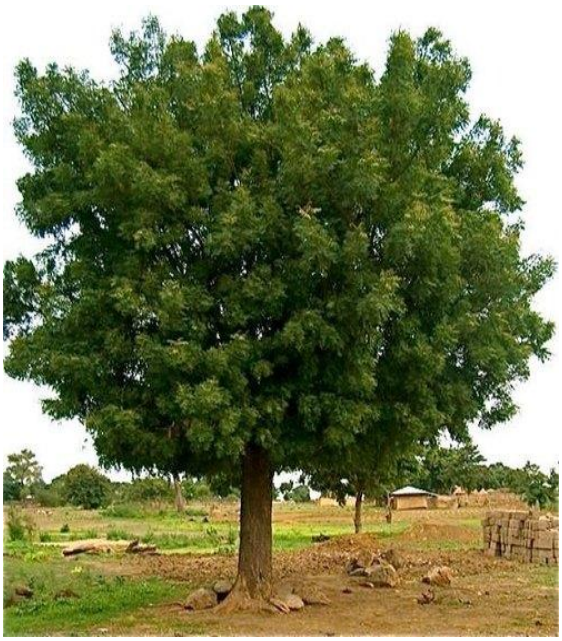

Figure 2: Neem tree [4]

Neem was used for the first time by the Harappa culture, which predates us by 4500 years, in ancient India.[5]Azadirachta indica (Neem) is a species of the genus Azadirachta, which belongs to the mahogany family Meliaceae. [6] Neem is a perennial plant that grows in India, Pakistan, and Bangladesh's tropical and semi-tropical climates. [7] Azadirachta indica is a plant that comes from the Azadirachta genus. Azadirachta indica is derived from the Persian words "Azad" which means "free" and "dirakat" which means "tree". Indica means "Indian origin, therefore it means "free tree of India". Since prehistoric times, neem has been an indispensable herb in Ayurveda, Unani, and other traditional medical therapies. Neem is still a widely used, affordable remedy in Indian traditional medicine for a variety of illnesses, earning it the nickname "village dispensary"[8]. In 1942, Siddiqui was the first to recognize the therapeutic qualities of neem. He extracted Nimbin, Nimbinin, and Nimbidin, a bitter substance from neem. He claimed that whereas the first two have antipyretic and anti-inflammatory characteristics, nimbidin has antiarthritic, antiulcer, and anti-inflammatory properties[9]. Neem plant components with anti-inflammatory, antipyretic, analgesic, antibacterial, anti-tumorogenic, antioxidant, antiulcer, and immunostimulant activities have been identified. Neem extract's main ingredients, azadirachtin, and nimbinin are responsible for their antibacterial properties[10]. Particularly in S. mutans and lactobacilli species, neem leaf extract significantly reduced plaque index and bacterial count[11]. Neem oil has anti-plaque activity, as Elavarasu et al. showed in their study[12]. Neem, propolis, turmeric, licorice, and sodium hypochlorite were tested as root canal irrigants for their antibacterial activity against E. faecalis and C. albicans by Hedge & Kesaria. Neem extracts showed exceptional effectiveness in inhibiting the most resistant species of E. fecalis and candida during root canal disinfection[13]. Adyanthaya et al investigated the methanol neem twig extract's antimicrobial effects. They discovered that neem extract is effective at reducing bacteria that cause periodontal disease and cariogenic infections, and they recommended adding neem twig methanol extract to oral care products.[14] Neem oil and leaves have been shown to have antifungal effects

and have been successful in reducing denture-related stomatitis [15]. Since ancient times, neem twigs and bark have been used as natural toothbrushes. Significant anti-cariogenic activities have been observed in neem bark extract on a variety of cariogenic bacteria inhabiting oral flora. Neem extract in acetone had an anti-carcinogenic action in S. sorbinus, according to Bhuiyan et al. [16]. S. mutans and S. faecalis were inhibited by neem bark extract at a 50% concentration, according to Almas Khalid's study [17]. Neem extract has demonstrated effectiveness in lowering both acute and chronic inflammation [18]. In their investigation, Bothell et al. suggested that neem-based mouthwashes could be utilized to treat gingival and periodontal illnesses since neem extract significantly reduced plaque and gingival inflammation [19]. Neem extract-based mouthwashes are effective in preventing plaque-induced gingivitis and have fewer long-term negative effects than mouthwashes containing chlorhexidine, according to research by Chatterjee et al. [20]. Neem has an anticancer effect on cancerous cells by preventing them from proliferating, inducing cell death, preventing cancer angiogenesis, restoring the balance of cellular reduction/oxidation (redox), and boosting the host immune system's defenses against cancerous cells [21]. The Azadirachta indica leaf has anti-inflammatory and antihyperglycemic characteristics, making it useful for treating a variety of skin ailments like eczema, ringworm, acne, as well as conditions that can lead to the development of gangrene and diabetic foot. Azadirachta indica is thought to have been used to cleanse the blood, fight free radicals, and remove toxins from the body[22]. Smallpox and chicken pox are traditionally treated with the leaves' paste, which also functions as an antiviral treatment [23]. The leaf juice is used as a tonic to boost appetite and get rid of intestinal worms. [24] It is also used to treat fever and has hypoglycaemic, hypolipidemic, hepatoprotective, and hypotensive properties [25] The antibacterial activity of the leaf extract against dental infections is employed therapeutically [26].

Additionally, the chosen plant is employed in the Ayurvedic medical system to cure malarial fever [27]. The manufacture of tablets that repel mosquitoes using neem oil is now possible in northeast India. has a variety of therapeutic applications as well, such as AIDS, cancer, skin illnesses, and digestive disorders therapies [28]. As an antibacterial agent, the leaf extract is frequently utilized. Neem is also used as an anthelmintic, antibacterial, and in toothpaste, lotions, and soaps for therapeutic purposes [29, 30].Several researchers have examined the plant Azadirachta indica's medicinal qualities. They included antipyretic properties [31], anti-malarial and anti-tumor effects [32].anti-ulcer properties, anti-diabetic properties [33]. anti-fertility properties[34]

The term "microflora" describes all of the bacteria and other microorganisms that are present in a specific ecosystem.A single organism, a human host, or a piece of an animal's body can serve as the ecosystem. Billion different types of bacteria reside in our bodies. The mouth is a complex ecosystem with a large number of microorganisms that have not yet been fully researched. Its mechanism and chemistry are also not entirely known. Bacteria from over 30 genera have been found in human mouths. The oral microbiota may be advantageous or harmful. They support the host's defenses by defending against exogenous bacteria and supplying certain nutrients and digestive enzymes including amylase, lipase, and proteases [35]. The entire quality of life is influenced by dental health, and systemic disorders and chronic conditions are linked to poor oral health. There is a strong correlation between oral disease and the more than 750 species of bacteria that live in the oral cavity [36].The mouth cavity has a wider diversity of bacteria than any other part of the body. [37] The most common bacteria detected in the oral cavity are *Streptococcus mitis, Streptococcus oral, Streptococcus sanguis, Streptococcus mutans, Streptococcus Gordonii, Staphylococcus aureus, Staphylococcus epidermidis, Veillonella sp., Neisseria sicca, Fusobacter. S. mutans* should be the primary target of any preventive strategy, according to all current evidence. [38]Dental caries (tooth decay), [39] periodontitis (gum disease),[40] and the creation of dental plaques are all caused by microorganisms from the oral cavity. The microbial flora found in tooth canals following failed endodontic therapy appears to be a fairly small subset of the bacteria found in untreated root canals. [41] Dental caries affect 60-65 percent of the population in India [42]. while periodontal disease affects 50-90 percent of the population, depending on age. [43]

Dental caries is a localized, transmissible infectious condition that ends up in the destruction of hard dental tissue. It is caused by plaque build-up on the teeth's surface and biochemical activities of complex micro-communities. *Streptococcus apps. and Staphylococcus aureus* one of the most common opportunistic organisms in dental caries, and it plays a key role in digesting carbohydrates, producing acid, and demineralizing tooth enamel.[44]Numerous biologically active substances can be isolated from neem's chemical components, which include alkaloids,glycosides, flavonoids, saponins, anthraquinone, tannic acid, phenolic compounds,and carotenoids, steroids, and ketones. [45] Salannin, volatile oils, meliantriol, and Nimbin are other substances found in Azadirachta indica that have biological effects [46]. Organic nitrogenous compounds are known as alkaloids. These have an extraordinary range of pharmacological effects and are naturally alkaline.Carbon, hydrogen, and oxygen make up the complex and active compounds known as glycosides.[47]. The Latin word "Sapo," which means soap, is where the word "sapoonins" originates. Similar to other cholesterol-lowering medications, saponins combine with bodily cholesterol to produce potent insoluble complexes that block reabsorption while increasing excretion, leading to the depletion of the substance. Additionally, saponins exhibit anti-bacterial, anti-fungal, and anti-protozoal properties [48]. Chemically speaking, anthraquinones are 9, 10

dione Madder, a climbing plant that contains anthraquinone and glycosides and is used as a dyeing agent [49,50,51]Higher plants have a variety of flavonoids. The flavonoids function as antioxidants to defend against free radicals, which can harm tissues and cells. [52,53]. Astringent, fragrant, acidic glycosides are tannins.These can be found in the roots, stems, bark, leaves, fruits, and even the hairs of plants. Tannins aid in wound healing. Alkaloids, glycosides, flavonoids, and saponins are examples of phytoconstituents, which are also antimicrobial components found in plants. Plants use these antibiotic principles as a defense mechanism against many diseases [54].

- **Collection of clinical samples:**

Seven dental plaque samples were collected from the adult patients from various dental clinics.

- **Isolation and identification of dental pathogens:**

The sample of the dental plaque was spread on blood agar plates and left to grow for 18 to 24 hours at 37oC using the streak plate technique. The pathogens were then found using Bergey's manual [55].

- **Processing of neem:**

The neem leaf was picked, cleaned, dried in the shade, and then put in a blender to make a powder. The powder that was made was then put in a clean container that kept air out and kept at room temperature.

- **Preparation of neem extract:**

To make the extracts, five solvents such as chloroform, ethanol, methanol, toluene, and distilled water were used.

- Two methods were used for the preparation of neem extract:
  1. Soxhlet method
  2. Macerationmethod

1. The sample was finely powdered and put into a Soxhlet neem extraction (SNE) using methanol, chloroform ethanol, petroleum ether, and isoamyl alcohol in succession. The resultant liquid extracts were concentrated under reduced pressure (in a vacuum at 40°C) after being put through a Rotary evaporator. The recovered residues were labeled and kept in the refrigerator as crude extracts for additional research [56]. The resulting dried plant extract residues were redissolved in 0.1 percent dimethyl sulfoxide (DMSO) to obtain a concentration of 100 mg/ml, filtered through a 0.45 m membrane filter, and then stored in sterile brown bottles at 20°C in a freezer until the bioassay.

2. The samplewas finely powdered and it was then allowed to undergo a maceration neem extraction (MNE) using methanol, ethanol, chloroform, distilled water, and toluene. 20gm of neem leaf powder was put in a separate sterile conical flask with 200ml of each solvent. The contents were mixed

3. well and kept in a shaker for 24 hours. After 24 hours, the contents of each flask were filtered through Whatman no. 1 filter paper and then concentrated by evaporation**.**

- **Analysis of the phytochemistry of Azadirachta indica extract:**

Alkaloids, saponins, tannins, steroids, flavonoids,Phenols, Carbohydrates, and glycosides are just a few of the phytoconstituents that have been measured both qualitatively and quantitatively [57].

i. **Alkaloids**: Combine 1 ml of each solvent's extract with 1 ml of the marquis reagent, then thoroughly mix the mixture. If the mixture becomes a dark orange or purple color, alkaloids are present in the sample.

ii. **Saponins** - In this procedure, 2 ml of each solvent's extract and 6 ml of distilled water were added to a test tube and thoroughly mixed. The development of bubbles or persistent foam is a sign that saponins are present.

iii. **Tannins**: To test for tannins, we added 10% alcoholic ferric chloride to a 2 ml extract of each solvent. If a brownish blue or black hue forms, tannins are present.

iv. **Terpenoids:** Add 0.5 ml of chloroform and a few drops of strong sulfuric acid to 1 ml of each solvent's extract, followed by terpenoids. Terpenoids are present in the sample as evidenced by the formation of a reddish-brown precipitate.

v. **Flavonoids**: Add a few drops of 20 percent sodium hydroxide to 2 ml of each extract. It is seen that bright yellow color is developing. A few drops of hydrochloric acid that has been diluted by 70% were added to this, and the yellow tint vanished. The appearance and disappearance of yellow color in the sample suggests the presence of flavonoids.

vi. **Cardiac glycosides**: Add 3 drops of 1% aqueous ferric chloride solution to 1 ml of each solvent's extract, along with 0.5 ml of glacial acetic acid. The emergence of a brown ring at the interface is a sign that the sample contains cardiac glycosides.

vii. **Carbohydrates**: Take 1 ml of the extract from each solvent at the side of the tubes, followed by a few drops of Molish's reagent. After that, the mixture was left to stand for two to three minutes.The presence of carbohydrates in the sample is indicated by the formation of a red or dark violet color.

viii. **Phenols**: Add 2 ml of 5 % aqueous ferric chloride to 2 ml of each solvent's extract. The development of blue color in the sample denotes the presence of phenols. [58].

II. ANTIBACTERIAL ACTIVITY:

The cup diffusion method was used on Muller Hinton agar medium to assess the antibacterial activity of the various extracts. [59] Cork borer (5 mm in diameter) is used to create wells in Muller Hinton agar plates, and sterile cotton swabs are dampened with the bacterial suspension to disperse inoculums of bacteria on the solid plates. Using a micropipette, twenty microliters of the working suspension or solution of plant extracts and the equivalent volume of extraction solvent for the control were added to the wells. Plates were incubated at 37 C for 24 hours after being left to allow the extract to diffuse into the medium for a while. After incubation, the plates were examined for the presence of a zone of inhibition (ZOI), and the diameter of the inhibition zone was measured and noted.

- **Formulation of herbal toothpaste:**

The herbal toothpaste was made using the instructions given by [60]. The ingredients and amounts were mixed for 100gm paste. (Table1).

- **Evaluation of toothpaste:**

A total of 10 formulations with different Soxhlet neem extraction (SNE) and maceration neem extraction (MNE) concentrations were created and tested for antibacterial activity alongside commercial neem paste, the same as was done for the evaluation of neem extract and neem powder.

Ingredient	Quantity in %	Property
Calcium Carbonate	56.0 gm	Abrasive
Sodium lauryl sulfate	1.0 gm	Surfactants
Glycerine	22.0 gm	Anticrusting agent
Gum tragacanth	1.5 gm	Gelling agent
Purified water	19.4 gm	Vehicle
saccharine	0.1 gm	Sweetener
flavor	q.s.	Flavoring agent
preservative	q.s.	Preservative
Plant Extract	Various conc.	Active Ingredient

Table 1: Formulation of Toothpaste

- **Evaluation of toothpaste's activity against the isolates:**

The antimicrobial activity of toothpaste made with the essential extract was tested using the Agar cup Method. [61,62] On Mueller-Hinton agar plates, swabs were taken from the broth cultures of the isolates. A sterile borer was used to dig holes for wells. Toothpaste formulas were put in the wells, and the plates were kept at 37oC for 24 hours. Control was kept for each strain using a formulation that didn't have the active ingredient. After incubation, the plates were examined for the presence of a zone of inhibition (ZOI), and the diameter of the inhibition zone was measured and noted.

III. APPLICATIONS

Dental applications of Neem

I. Antibacterial activity:

A natural antibacterial agent is neem. numerous academic research has shown that it has antimicrobial properties. [63] The antibacterial results against *S. mutans* and *S. faecalis* have been documented. [64] Significant antibacterial activity was reported in neem leaves, sticks, and bark ethanol extract. [65,66] In comparison to *S. salivarius, S. mitis, and S. sanguis*, dried Neem chewing sticks had the strongest antibacterial effects against *S. ssmutans*. [67]

II. Anti-candida activity:

Neem leaf extract in both ethanol and water has shown potent anti-candidal activity against Candida albicans. [68] A clinical trial showed how Neem's leaf aqueous extract affected adhesion, cell surface hydrophobicity, and biofilm formation, which may have an impact on Candida albicans colonization.[69]

III. Anti-plaque activity:

The gallotannin-enriched extract from Melaphis Chinensis and aqueous extract of neem stick suppress the formation of insoluble glucan, which causes bacterial aggregation. It lessens streptococci's capacity to colonize tooth surfaces. [70] Neem oil shows significant
antibacterial activity and has been suggested for use in treatingdental plaque.[71]

VI. Anti-cariogenic activity:

Neem extract showed antibacterial properties against *S. Mutans S. salivarius S. sanguis andS.mitis*. Combining chewing sticks has been discovered to be effective in getting rid of the bacteria that causes tooth caries. [72] Dental caries can be treated with the help of a chloroform extract of neem leaf, which inhibited *Streptococcus mutans* and *Streptococcus salivarius. [*73] Acetone extract from neem bark is bactericidal to S. sobrinus, indicating that it has anti-cariogenic properties. [74]

IV. CONCLUSION

The omnipotent neem tree is nature's gift to mankind for the prevention and cure of many different illnesses. Alkaloids, saponins, tannins, steroids, glycosides, essential oils, and flavonoids are a few phytochemicals found in plants that have strong antibacterial properties. Neem's effectiveness as a good and affordable antibacterial, anti-inflammatory, and anticancer agent has been demonstrated by years of comprehensive research on its medicinal effects in treatingoral and dental issues. Since the plant is widely available and the extract is easily prepared using a straightforward maceration and soxh let procedure, it may be a genuine and more affordable alternative totraditional medications. Neem has already been recommended as a source for the creation of contemporary non-toxic medications. These encouraging outcomes motivate us to carry out the current research project. It's time for modern oral and dental care products to contain neem extracts.

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