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## **Therapeutic and Pharmacological Potential of Azadirachta indica**

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### **ABSTRACT**

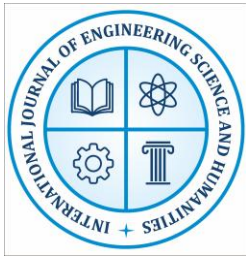
Azadirachta indica (neem) is a widely recognized medicinal plant with extensive use in traditional systems such as Ayurveda. The present study highlights the therapeutic and pharmacological potential of neem based on its rich composition of bioactive compounds, including azadirachtin, nimbin, nimbidin, and quercetin. Various parts of the plant—leaves, bark, seeds, and oil—exhibit significant biological activities such as antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anticancer, and immunomodulatory effects. Neem has demonstrated effectiveness against a broad spectrum of pathogens, supports blood glucose regulation, and protects against oxidative stress-induced cellular damage. Additionally, its role in promoting skin health, wound healing, and oral hygiene has been well documented. Despite its promising pharmacological profile, concerns regarding toxicity at higher doses necessitate controlled usage and further clinical validation. Overall, Azadirachta indica represents a valuable natural resource with significant potential for the development of novel therapeutic agents in modern medicine.

**Keywords:** Azadirachta indica, Antimicrobial activity, Anti-inflammatory properties, Phytochemicals, Pharmacological applications

### **I. INTRODUCTION**

Azadirachta indica, commonly known as neem, is an evergreen tree belonging to the Meliaceae family and is widely distributed across tropical and subtropical regions, particularly in the Indian subcontinent. It has been an integral part of traditional medicinal systems such as Ayurveda, where it is often referred to as the “village pharmacy” due to its extensive therapeutic applications. For centuries, neem has been utilized in the treatment of various ailments ranging from skin disorders and infections to metabolic and inflammatory conditions. Its long-standing use in folk medicine has attracted significant scientific interest, leading to extensive research on its pharmacological properties and bioactive constituents.

The therapeutic potential of Azadirachta indica is attributed to its rich and diverse phytochemical profile. Different parts of the plant, including leaves, bark, seeds, flowers, and roots, contain a variety of biologically active compounds such as azadirachtin, nimbin, nimbidin, nimbolide, and



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flavonoids like quercetin. These compounds are known to exhibit multiple pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anticancer, and immunomodulatory effects. The presence of such a wide range of phytochemicals makes neem a promising candidate for the development of plant-based therapeutic agents.

In recent years, there has been a growing global interest in natural products and herbal medicines as alternatives to synthetic drugs. This shift is largely driven by concerns over the side effects, high costs, and increasing resistance associated with conventional pharmaceuticals. In this context, *Azadirachta indica* has gained considerable attention due to its safety profile, affordability, and wide availability. Scientific studies have validated many of its traditional uses, demonstrating its effectiveness against a variety of bacterial, fungal, and viral pathogens. Additionally, neem extracts have shown potential in managing chronic conditions such as diabetes mellitus by regulating blood glucose levels and improving insulin sensitivity.

Another significant aspect of neem's pharmacological importance is its role as an antioxidant. Oxidative stress is a major contributing factor in the development of numerous chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. The antioxidant properties of neem help in neutralizing free radicals, thereby protecting cells from oxidative damage. Furthermore, neem has demonstrated promising anticancer activity in various experimental studies, where it has been shown to inhibit tumor growth and induce apoptosis in cancer cells. These findings suggest that neem may play a crucial role in future cancer prevention and therapy.

Apart from its internal therapeutic uses, neem also holds great importance in dermatology and oral healthcare. Neem-based formulations are widely used for treating skin conditions such as acne, eczema, and psoriasis due to their antimicrobial and anti-inflammatory properties. Similarly, neem twigs and extracts have been traditionally used for maintaining oral hygiene, as they help reduce plaque formation and prevent gum diseases.

Despite its numerous health benefits, it is important to consider the safety and dosage of neem-based products. While neem is generally regarded as safe when used appropriately, excessive consumption, particularly of neem oil, may lead to adverse effects. Therefore, further clinical studies are necessary to establish standardized dosages and ensure its safe application in modern medicine.

## II. REVIEW OF LITERATURE REVIEW

A total of 82 Indian medicinal plants traditionally used in medicines were subjected to preliminary antibacterial screening against several pathogenic and opportunistic microorganisms. Aqueous, hexane and alcoholic extracts of each plant were tested for their antibacterial activity using agar well diffusion method at sample concentration of 200 mg/ml. The results indicated

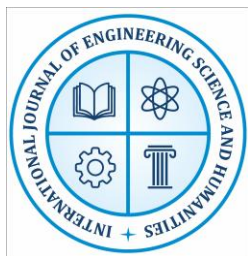


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that out of 82 plants, 56 exhibited antibacterial activity against one or more test pathogens. Interestingly, extracts of five plants showed strong and broad spectrum activity as compared to rest of 51 plant extracts which demonstrated moderate activity. On the whole the alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts. Among various extracts, only alcoholic extracts of *Emblica officinalis*, *Terminalia chebula*, *Terminalia bellerica*, *Plumbago zeylanica* and *Holarrhena antidysenterica* were found to show potentially interesting activity against test bacteria. These active crude alcoholic extracts were also assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity. Nimbin is one of the many substances found in neem seeds and is reported to have several medicinal properties and uses. For example, it is an anti-pyretic, can be used to treat arthritis, hypoglycaemia, peptic ulcers, anti-secretory activity, and it can also be used as an antibiotic. In this paper, we present the results of a preliminary experimental study to extract nimbin from neem seeds using CO<sub>2</sub> supercritical fluid extraction (SFE). The operating pressure in the extraction was varied from 10 to 26 MPa, the temperature was varied from 308 to 333 K and the flow rate was varied from 0.24 to 1.24 ml/min. An optimum extraction rate was observed at a pressure of 23 MPa when operating at 308 K. Best extraction conditions occurred at 23 MPa, 308 K and a flow rate of 1.24 ml/min for a 2 g sample of neem. The measured extraction rate was found to be about 0.18 mg of nimbin/g neem seed per hour of operation which is equivalent to about 0.35 kg nimbin extracted per kg nimbin present in neem seeds. The future work needs to focus on the interaction between the various operating parameters such as temperature, pressure and flow rate of supercritical carbon dioxide. In addition physical properties i.e., particle size, porosity need to be determined in order that a model can be developed and tested.

Chewing sticks (Miswak) is most commonly used in the Middle East and Indian Subcontinent. *Salvadora persica* (Arak) and *Azadirachta indica* (Neem) are commonly used as oral hygiene tools in different parts of the world. Several studies have demonstrated the anti-plaque, anticariogenic and antibacterial effect of these sticks. The aim of this study was to compare the effectiveness of antimicrobial activity of Neem and Arak chewing stick's aqueous extracts at various concentrations. The microbial inhibition was measured using blood agar and ditch plate method up to 48 hours. The pH of Neem extract was 6.1 and of Arak was 4.9. Data suggested that both chewing stick extracts are effective at 50% concentration on streptococci and *Streptococcus faecalis*. Arak extract was more effective at lower concentrations for *Streptococcus faecalis*. The effect may be due to the difference of their chemical composition and variability in their pH. Further research is needed to extrapolate other plants used for oral hygiene. Chewing sticks are recommended as oral hygiene tools for health promotion in developing countries.



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## III. MATERIALS AND METHODS

**Method:** - Samples of the most commonly used Neem (*Azadirachta Indica*), Zaitoon (*Olea europaea*) Kikar (*Accacia arabica*), Peelu (*Salvadora persica*), Ban (*Glycosmic pentaphylla*), Khiran (*Capparis aphylla*) and Arak (*Salvadora persica*) from Saudi Arabia were bought from the open market. The leaves were identified by its colour and scent and recognized by agriculturist and vendor. The leaves were air shipped to Riyadh, Saudi Arabia. The experiments were carried out at the College of Dentistry Research Center, King Saud University, Riyadh.

**Preparation of extracts:** - 100 gm of each of the chewing sticks were used in the experiment. The chewing sticks were kept sun dried for 2 weeks at 30°C before extract preparation. The sticks were cut into small pieces and ground to powder in a ball mill. The powder was kept separately in sterile, dry screw-capped bottles, which were stored in a dry cool place for one week before aqueous extraction. Each successive 10 gm quantity was put into a sterile screw-capped bottle to which 100 ml of sterile deionized distilled water was added. The extracts were allowed to soak for 48 hours at 40°C before the mixtures were centrifuged at 2,000 rpm for 10 minutes. The supernatants were passed through a 0.45 mm membrane filter, the extracts were prepared at 5, 10 and 50 % concentrations (v/v) and stored in 5 ml portions at 20°C. The pH of the chewing sticks extracts was determined. Normal saline solution was used as control for antimicrobial activity.

### Collection and Preparation of Neem Leaf Extract

The fresh leaves of *Azadirachta indica* were collected, dried and finely chopped, then dissolved in tap water, at a concentration of 50 g of dried leaves per liter of water, for 24 hours at room temperature based on the methods of Cruz et al. (2004). The mixture was filtered and the extract (50 g/l) was used immediately in the experiments, in different dilutions.

### Experimental Fish

Apparently healthy African cat fish; *Clarias gariepinus* weighing  $70.2 \pm 4.5$ g, were collected from the Fish Hatchery of the University of Agriculture Makurdi, Laboratory and acclimated in indoor tanks supplied with de-chlorinated tap-water and continuous aeration for 2 weeks. Total volumes of the composite samples were approximately 20L. Water was filtered through a 40µm mesh collection net, sub samples were removed from this volume during mixing.

## IV. EVALUATION OF NEEM

### Physical evaluation –

**Total ash content:** Approximately 2 g of air dried drug powder was weighed accurately and placed in previously ignited and tared silica crucible. It was then ignited by gradually increasing the temperature to 500-6000 C until the material was white, indicating the absence of carbon. It



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was cooled in a desiccator and weighed. Total ash content was calculated in mg/gm of air dried material.

## **Acid insoluble ash**

Approximately 2 g of air dried drug powder was weighed accurately and burned in a crucible at 500- 6000 C. The ash so obtained was boiled with 25 ml of 2 M Hcl for 5 minutes. It was then filtered through ashless filter paper and the residue was washed with hot water until the water become neutral. The filtered ash and filter paper were placed in a crucible, dried and then burnt at 5000 C. until constant weight. The loss in weight of the powder as percentage of the initial weight was calculated as acidinsoluble ash in the sample.

## **Petroleum ether soluble extractives**

5 g of drug powder with 100 ml of pet.ether (60-80o C.) was put in a glass bottle, sealed and kept for 24 hrs at room temperature with intermittent stirring. The solution was filtered and 25 ml of the filtered solution was filled in a wide mouth glass bottle and solvent evaporated at room temperature. It was then dried in the oven at 1050C for 1 hr and the percentage yield of pet.ether extractive was calculated.

## **Ethanol soluble extractives**

5 g of drug powder with 100 ml of 95 % ethanol was put in a glass bottle, sealed and kept for 24 hrs at room temperature with intermittent stirring. The solution was filtered and 25 ml of the filtered solution was filled in a wide mouth glass bottle and solvent evaporated at room temperature. It was then dried in the oven at 1050C for 1 hr and the percentage yield of 95% ethanol extractive was calculated.

## **Water soluble extractives**

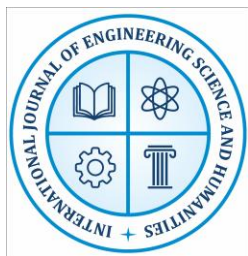
5 g of drug powder with 100 ml of water was put in a glass bottle, sealed and kept for 24 hrs at room temperature with intermittent stirring. The solution was filtered and 25 ml of the filtered solution was filled in a wide mouth glass bottle. The solvent was then evaporated in the oven at 1050C and the percentage of water soluble extractive was calculated.

## **V. RESULT AND DISCUSSION**

### **Phytochemicals of Azadirachta indica: -**

The phytochemicals of A. indica is presented in table1. Flavonoid has the highest concentration (5.79±0.01mg/L) followed by alkaloid (3.12±0.00 mg/L) while the least is hydrogen-cyanides (0.44±0.01 mg/L).

TABLE 1: Phytochemicals of Neem Leaf Extract Azadirachta indica



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Phytochemicals	Concentrations (Mean±SEM)
Alkaloids	3.12±0.00
Flavonoids	5.79±0.01
Saponins	0.86±0.01
Hydro-cyanides	0.44±0.01

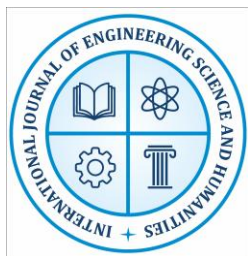
The phytochemical screening of *A. indica* extract indicated the presence of saponin, flavonoid, alkaloid and hydrogen cyanides (Table 1), the presence of these compounds may be responsible for the antibacterial activities of the extracts of *A. indica* on the test organisms. The phytochemical components of the *A. indica* have been established in previous studies and these include tannins, saponins, alkaloid, phenols, flavonoids, (Biswas et al., 2002, El-Mahmood, et al., 2010). Several studies have linked presence of these bioactive compounds in plant materials to antimicrobial activity.

### Variations in Physicochemical Properties of Water: -

The variations in the water quality in the various concentrations of Neem leaf extract is presented in table 2. The mean temperature ( $29.10 \pm 0.00$  oC) was uniform for all the treatment set-up from control (0.00 mg/L) to 150 mg/L. The pH decreased as the concentration of neem leaf extract increased. The least pH was recorded in the 350 mg/L-concentration,  $6.87 \pm 0.01$  while the highest was observed in the 50 mg/L- concentration  $8.32 \pm 0.00$ .

The variation were statistically significant ( $p < 0.05$ ). The electrical conductivity increased with an increase in concentration of the neem leaf extract. The least value ( $822.00 \pm 1.00$   $\mu$ S/cm) was from the control while the highest value of EC ( $951.50 \pm 1.50$  mg/L) was recorded in the 350 mg/L-concentration of neem leaf extract. The variation was statistically significant ( $p < 0.05$ ). The total dissolved solids equally increased with an increase in the concentration of the neem leaf extract. The least value was from the control ( $408.00 \pm 1.00$  mg/L) while the highest was recorded in the 350 mg/L-concentration,  $473.50 \pm 1.50$  mg/L. The dissolved oxygen decreased as the concentration of the extract increased. The control ( $5.75 \pm 0.05$  mg/L) had the highest concentration while the highest concentration was in the 350 mg/L-concentration,  $4.05 \pm 0.05$  mg/L.

TABLE 2: Water Quality Parameters of the Concentrations Media of Neem Leaf Extract



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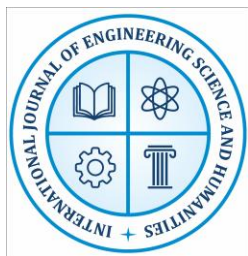
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Concentrations of Neem Leaf Extract	Water Quality Variables				
	T (°C)	pH	EC (µS/cm)	TDS (mg/L)	DO (mg/L)
Control(0.00M g/l)	29.10±0.00	8.29±0.02 <sup>a</sup>	822.00±1.00 <sup>g</sup>	408.00±1.00 <sup>d</sup>	5.75±0.05 <sup>a</sup>
50 Mg/L	29.10±0.00	8.32±0.02 <sup>a</sup>	903.00±1.00 <sup>f</sup>	412.50±6.50 <sup>d</sup>	5.55±0.05 <sup>b</sup>
100 Mg/L	29.10±0.00	8.12±0.01 <sup>b</sup>	906.00±1.00 <sup>f</sup>	439.00±1.00 <sup>c</sup>	5.00±0.00 <sup>c</sup>
150 Mg/L	29.10±0.00	8.02±0.01 <sup>c</sup>	916.50±1.50 <sup>e</sup>	440.00±2.00 <sup>c</sup>	4.25±0.05 <sup>d</sup>
200 Mg/L	29.10±0.00	7.26±0.02 <sup>d</sup>	927.00±1.00 <sup>d</sup>	454.50±1.50 <sup>b</sup>	4.25±0.05 <sup>d</sup>
250 Mg/L	29.10±0.00	7.22±0.02 <sup>d</sup>	931.00±1.00 <sup>c</sup>	459.00±1.00 <sup>b</sup>	4.15±0.05 <sup>dc</sup>
300 Mg/L	29.10±0.00	6.93±0.01 <sup>e</sup>	935.00±1.00 <sup>b</sup>	467.50±0.50 <sup>a</sup>	4.05±0.05 <sup>e</sup>
350 Mg/L	29.10±0.00	6.87±0.01 <sup>f</sup>	951.50±1.50 <sup>a</sup>	473.50±1.50 <sup>a</sup>	4.05±0.05 <sup>e</sup>
<i>P-value</i>	-	0.00	0.00	0.00	0.00

During the study, it was observed that total dissolve solid increases with increase in concentrations. This may be due to the amount of suspended matter in the water as concentrations increases. Dissolve Oxygen is very important in limnological studies indicating levels of pollution in water bodies (Wicken, 2008). In the present study dissolve oxygen ranged between (4.05±0.05- 5.75±0.05mg/L) indicating a decreases with increase in concentration of the neem leaf extract. As the concentration increased, there may have been increased organic load of the extract which required larger amounts of dissolved oxygen for breakdown.

## VI. CONCLUSION

From the study, it was concluded that neem leaf extract can be used to control external microbial load on *Clarias gariepinus*. The practice is environment friendly and has no bio-accumulative effect in the tissues of consumers. The phytochemical screening of neem leaf extract was conducted to assess the nature and concentrations of phytochemicals useful in treating the microbial load on the body of the juveniles of *Clarias gariepinus*. Flavanoid (5.79±0.01 mg/L), saponins (0.86±0.01 mg/L), alkaloids (3.12±0.00) and hydro-cyanides (0.44±0.01) were analysed from the extracts. A static toxicity tests were run to determine lethal concentrations (96-hrs LCs) of neem leaf extract to *Clarias gariepinus*. The LC50 was 239.86mg/L for TVC and 226.38 mg/L for TCC. The LC99 was 690.95 mg/L for TVC and 595.19 mg/L for TCC. The LC99 concentration was considered as the effective treatment concentrations of the external microbial load on *Clarias gariepinus* using neem leaf extract. The Total Viable Counts (TVC, 5.1 X 10<sup>6</sup> cfu/g) and Total Coliform Counts (TCC, 3.7 X 10<sup>6</sup> cfu/g) were highest in 50ml/L and least



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(TVC,  $1.7 \times 10^6$  cfu/g, TCC,  $1.1 \times 10^6$  cfu/g) in 350ml/L. This showed that the TVC and TCC decreases with increase in concentration of aqueous extract of *A. indica*.

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