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TO STUDY THE ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACT OF AZADIRACHTA INDICA (NEEM) LEAF

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ABSTRACT

Antimicrobial activity of different extract of neem (*Azadirachta indica*) is the objective of current invstigation. Azadirachta Indica currently known as Neem leaf. *Azadirachta indica* has several medicinal uses, especially its leaves. This study examined the antimicrobial activity of Neem leaf extracts against human pathogenic bacteria. To evaluate the antimicrobial impacts of neem leaf extracts., various bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Bacillus* species, were treated with alcoholic, diethyl ether, water, and fresh juice extracts. The antimicrobial assays were performed using the spread plate technique. After 24 hours of incubation at 37°C, clear zones of inhibition were observed, indicating suppression of bacterial growth. Among the extracts, the ethanol extract of neem leaves demonstrated the strongest antibacterial effect on *Bacillus* compared to its effects on *S. aureus*, *E. coli*, & *Streptococcus pneumoniae*.

KEY WORDS: *Azadirachta indica*, *E. coli*, *Staphylococcus aureus*, *Pneumonia*, *Bacillus.*

INTRODUCTION

Plants contain numerous biologically active compounds with substantial potential for development into medicinal agents. While herbal medicines have long been the cornerstone of health care in developing countries, there has been a significant recent increase in their use in developed nations as well [1]. Plants offer an alternative approach in the quest for new treatments. Many plants, renowned in traditional healing practices, are believed to possess protective and therapeutic properties [2]. It is probable that plants will remain a valuable source of new molecules, which, with potential chemical modification, could lead towards the creation of new and improved drugs [3]. Bacterial resistance to antibiotics poses a significant

challenge for clinicians and the therapeutic industry. Considerable efforts are being made to counteract this issue, including the extensive screening of medicinal plants from traditional medicine systems. This approach aims to discover new, safer, and more effective agents to combat infectious diseases [4]. Each part of the neem tree holds a range of medicinal properties. Non-woody parts, such as the leaves, bark, oil, flowers, fruits & seeds, exhibit remarkable properties including antiallergic, antifungal, antibiotic, antidemartic, antibacterial, anti-inflammatory, insecticidal, larvicidal, antimalarial, antiulcer, and other biological activities [5]. Certain water-soluble Azadirachta indica extracts Azadirachta indica extracts are highly significant for their hyperglycemic, hypolipidemic, and hypotensive activities [6].

Neem, commonly utilized in traditional Indian healing practices, is a rich source of various therapeutic compounds and flourishes in tropical regions. In the Indian subcontinent, its twigs are often used as natural chewing sticks. Research on Neem has shown that it contains active substances with various medicinal properties. Azadirachta indica is utilized in folklore medicine for treating diabetes and has demonstrated potential antidiabetic activity. Neem leaf extracts have shown promise as agents for lowering blood sugar levels in both type 1 and type 2 diabetes. Beyond their medicinal uses, neem leaves are also valued for their capacity to maintain cleanliness in residential areas. Traditionally, neem seeds have been employed to cure infections, particularly those affecting the eyes and ears. Alcoholic extracts from neem flowers have been observed to affect the estrous cycle and partially prevent ovulation in rats, suggesting their potential as contraceptives. Moreover, Neem aqueous extract has demonstrated significant potential as an effective chemotherapeutic and antiviral agent. The research sought to evaluate the antibacterial effectiveness of neem leaves against various human pathogens, including Escherichia coli, Staphylococcus aureus, Bacillus species, and Streptococcus pneumoniae.

MATERIALS AND METHODS

1 Selection of plant

The Neem plant (Azadirachta indica) was selected for this study. Leaves were gathered from the garden at Sonekar College in Koradi, Nagpur, and were subsequently identified and authenticated by the Botany Department of Rashtrasant Tukadoji Maharaj University, Nagpur. The herbarium sheet authentication number is 013.

2 Extraction.

The completely shade-dried material was coarsely powdered and subjected to Soxhlet extraction using ethanol and diethyl ether successively. Additionally, a water extract was prepared through maceration, and fresh juice was obtained from fresh Neem leaves.

3 PHYTOCHEMICAL ANALYSIS

The following tests were conducted:

3.1 **Alkaloids Test:** Five grams of the evaporated extract were boiled with 5 ml of dilute HCl in a water bath for 5 minutes. After cooling and filtering, Dragendoff's reagent was added to the filtrate. The presence of alkaloids was indicated by the formation of a red precipitate.

3.2 **Flavonoids Test:** Three grams of the extract were subjected to ethyl acetate treatment, followed by heating in a water bath. After cooling and filtration, the filtrate was mixed with a 1% aluminum chloride solution. The development of a yellow color indicated the presence of flavonoid compounds.

3.3 **Saponins Test:** One gram of the extract was boiled with 5 ml of distilled water, filtered while hot, and the filtrate was shaken vigorously with a few drops of olive oil. The formation of an emulsion indicated the presence of saponins.

3.4 **Tannins Test:** Two grams of the evaporated extract were boiled with 45% ethanol, cooled, and filtered. Lead acetate solution was added to the filtrate, and the formation of a gelatinous precipitate indicated the presence of tannins.

3.5 **Glycosides Test:** One gram of the evaporated extract was boiled with 15 ml of distilled water, cooled, and filtered. Fehling's solutions A and B were added to the filtrate and boiled. A brick-red color indicated the presence of glycosides.

3.6 **Reducing Sugars Test:** The plant extract was treated with Fehling's solution, and a color change from deep blue to brick red indicated the presence of reducing sugars.

3.7 **Steroids Test:** The aqueous extract was mixed with concentrated sulfuric acid and acetic anhydride. A color change to violet indicated the presence of steroids.

3.8 **Oils Test:** About 0.2 g of the plant extract was pressed between filter papers and examined for transparency. Transparency indicated the presence of oils.

3.9 **Terpenoids Test:** Four milligrams of the extract were treated with acetic anhydride and chloroform, followed by the gradual addition of concentrated sulfuric acid. A red-violet color indicated the presence of terpenoids[11-12].

4. MICROORGANISM

Pathogenic strains of Escherichia coli, Staphylococcus aureus, Bacillus, and Streptococcus pneumoniae were utilized. These strains were sourced from the laboratory at Sonekar

College of Pharmacy in Koradi, Nagpur.

4.1 *Staphylococcus aureus* is a Gram-positive bacterium linked to a variety of clinical conditions. It is prevalent in both community and healthcare settings and can lead to several types of infections, such as skin abscesses, boils, and cellulitis. In more serious cases, it can result in bloodstream infections, pneumonia, and infections of the bones and joints [13].

4.2 *Escherichia coli* (*E. coli*) is a Gram-negative, facultative anaerobic bacterium commonly found in the intestines of warm-blooded animals. Although most strains are harmless, some can cause severe foodborne illnesses and contribute to food contamination outbreaks, potentially leading to product recalls. *E. coli* is typically present in fecal matter and tends to grow rapidly under aerobic conditions initially, but its numbers decrease over time [14].

4.3 *Bacillus* is a genus of Gram-positive, rod-shaped bacteria that encompasses both obligate aerobes, which need oxygen to survive, and facultative anaerobes, which can live in the absence of oxygen. *Bacillus* species are positive for the catalase enzyme when exposed to or using oxygen. [15].

4.4 *Streptococcus pneumoniae*, commonly referred to as pneumococcus, is a Gram-positive, spherical bacterium that frequently appears in pairs (diplococci). It is non-motile and does not produce spores. Recognized in the late 19th century as a significant cause of pneumonia, *S. pneumoniae* can be present without symptoms in healthy carriers and usually inhabits the respiratory tract, sinuses, and nasal cavity. [16].

5. ANTIMICROBIAL ASSAY

The antimicrobial assay involved using water, ethanol, diethyl ether, and fresh juice extracts from neem leaves, applying the agar well diffusion method. Nutrient agar plates were inoculated with the target microorganisms using the spread plate technique. Each extract ethanolic, diethyl ether, fresh juice, and water was placed in distinct wells on the agar plates to enable diffusion into the medium. After incubation at 37°C for 24 hours, clear, circular zones of inhibition were noted, signifying the suppression of bacterial growth. The diameters of these inhibition zones were measured to determine the antimicrobial activity. [17-18].

RESULT AND DISCUSSION

Table.1 Phytochemical result of Neem leaf extracts

Phytonutrients	Water	Ethanol Extract
Flavonoids	++	++
Alkaloids	++	++

Oils	++	++
Saponins	++	++
Tannins	++	++
Glycosides	++	++
Steroids	++	++
Terprenoids	++	++
Reducing sugars	++	++

The qualitative assessment for different extract was performed by measuringdiameter of ZOI on four different kind of bacteria.

Table No. 2- Various Bacteria Measuring Zone of Inhibition

Different extract	S.aureus ZOI(mm)	E. coli ZOI (mm)	*Bacillus ZOI (mm)*	S. pneumonia ZOI (mm)
Water extract	20mm	0	0	0
Ethanolic extract	16mm	15mm	20mm	13mm
Diethyl ether extract	14mm	31 mm	0	12mm
Fresh juice of Neem	17mm	25mm	11mm	0

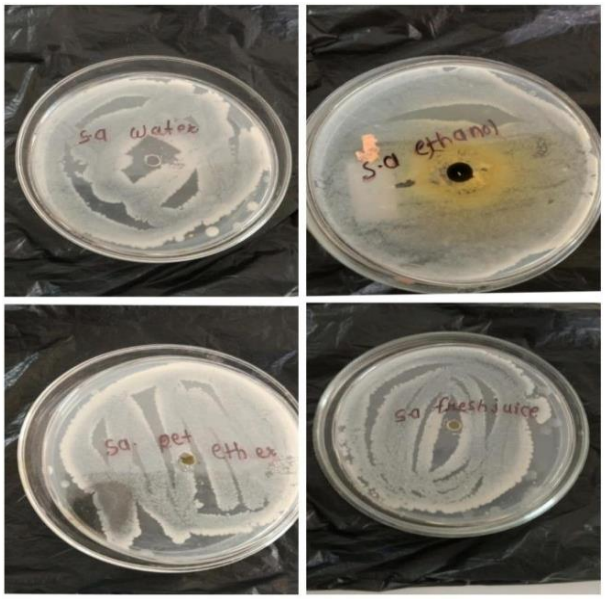

Figure 1 - Zone of inhibition in s. aureus

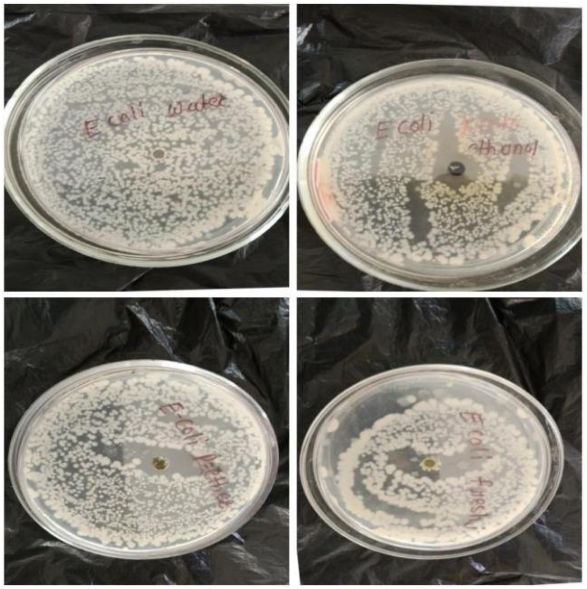

Figure 2 - Zone of inhibition in E. coli

Figure 3 - Zone **of inhibition in S. Pneumonia.**

Figure 4 - Zone **of inhibition in bacillus**

CONCLUSION

Neem extracts, sourced from different parts of the *Azadirachta indica* plant, displayed significant antimicrobial activity against a variety of bacterial strains. Among the extracts, the ethanolic extract from neem leaves proved to be the most effective, showing the strongest antibacterial activity, especially against *Bacillus*. In contrast, the water extract was only effective against *Staphylococcus aureus*, demonstrating limited antimicrobial potential. The diethyl ether extract exhibited notable antimicrobial efficacy, particularly against *Escherichia coli*, outperforming the other extracts in this regard. Additionally, the fresh juice of neem leaves was highly effective against *E. coli*, showing superior activity compared to its effects on *S. aureus* and *Bacillus*.

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NO CONFLICTS OF INTERESTS

"We declare no conflicts of interest."

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