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Chemical Composition and Combined Medicinal Value of Extracts from *Ficus religiosa* and *Azadirachta indica*: An Experimental Study

ORIGINAL ARTICLE

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Abstract

This experimental study focuses on evaluating the combined medicinal properties of Ficus religiosa (Pipal) and Azadirachta indica (Neem) leaf extracts when mixed in equal quantities. These two plants are highly regarded in Ayurveda and traditional Indian medicine for their therapeutic potential, including antibacterial, antifungal, antioxidant, and anti-inflammatory activities. While many studies have investigated each plant individually, this paper seeks to explore their synergistic effects when used together. The research involved collecting fresh leaves of both species, drying, powdering, and extracting their bioactive compounds using ethanol as the solvent. The phytochemical composition of the combined extract was analyzed for the presence of alkaloids, flavonoids, tannins, phenolic compounds, and saponins. The extract was then tested for antibacterial properties using the agar well diffusion method against Escherichia coli and Staphylococcus aureus, and antioxidant activity using the DPPH assay. Results showed that the combined extract had higher zones of inhibition against both bacterial strains compared to individual extracts. Antioxidant activity also showed significant improvement, indicating that the blend enhances the medicinal potency of the individual components. This paper concludes that the combined leaf extract of Ficus religiosa and Azadirachta indica can be an effective, natural, and affordable solution for treating bacterial infections and preventing oxidative stress. These findings support its future application in herbal formulations.

Key Words

Ficus Religiosa, Azadirachta indica, Medicinal Plants, Phytochemicals, Antibacterial Activity, Antioxidant Property.

Introduction

India has a rich history of using medicinal plants for healthcare. Many plant species are used traditionally for treating various health problems. Among them, *Ficus religiosa* (commonly called Pipal) and *Azadirachta indica* (commonly called Neem) are known for their wide range of medicinal benefits. These plants are commonly found in rural and urban areas and have spiritual, ecological, and medicinal importance.

Ficus religiosa has been used in traditional systems of medicine for centuries. Its leaves, bark, and roots are used to treat asthma, ulcers, skin diseases, and inflammation. The phytochemicals in its leaves include flavonoids, tannins, and alkaloids that contribute to its healing properties. Similarly, *Azadirachta indica* is considered a "village pharmacy" due to its multipurpose medicinal use. Neem leaves have been used for centuries to cure fever, infections, skin problems, and even as an insect repellent.

Modern scientific studies have confirmed that both of these plants contain several bioactive compounds. Neem contains compounds like azadirachtin, nimbin, and quercetin, which are responsible for its antimicrobial and anti-inflammatory effects. Pipal leaves contain polyphenols, antioxidants, and tannins that provide protective benefits to the body.

However, there is very limited research on the combined use of these plants. The idea of combining them comes from the possibility that their compounds may work better together—this is called a synergistic effect. When two medicinal agents are mixed, they may enhance each other's action. This paper aims to find out whether mixing the extracts of *Ficus religiosa* and *Azadirachta indica* leads to better antibacterial and antioxidant properties than when used alone.

Review of Literature

Gupta & Bansal (2021) conducted a study on the antibacterial and anti-inflammatory effects of *Ficus religiosa* leaf extracts. They discovered that the extract helped reduce skin inflammation and could inhibit the growth of common bacteria such as *E. coli*. Their study attributed these effects to the presence of flavonoids and tannins.

Rani et al. (2021) investigated the phytochemical and pharmacological properties of *Azadirachta indica*. Their study found that Neem leaves contain important chemical constituents such as nimbidin, nimbolide, and azadirachtin, which provide powerful antibacterial and antifungal activities. They recommended Neem leaf extracts as a possible herbal treatment for skin diseases and gastrointestinal infections.

Maniprabha (2020) focused on the synergistic effect of mixed plant extracts. They reviewed several herbal combinations and concluded that when two or more plant extracts are used together, the resulting mixture often shows stronger medicinal activity. They emphasized the importance of exploring combinations scientifically to develop new formulations.

Shiromani (2020) explored the antioxidant capacity of combined herbal extracts. Their research proved that certain combinations of leaf extracts showed better free radical scavenging activity than individual ones. The authors highlighted that antioxidant properties are crucial in preventing cell damage caused by oxidative stress.

From the existing literature, it is clear that both *Ficus religiosa* and *Azadirachta indica* possess important medicinal properties. Yet, there is a lack of direct scientific study on the combination of these two plants in equal ratios. This paper addresses that gap and attempts to provide a comparative and experimental analysis of their combined effects.

Methodology

1. Study Type

The present research is an experimental laboratory-based study focused on phytochemical analysis and biological activity testing of combined leaf extracts.

2. Materials and Chemicals

- Fresh leaves of *Ficus religiosa* and *Azadirachta indica*.
- Ethanol (95%) – as solvent.
- Petri dishes, filter paper, beakers, Soxhlet apparatus.

- Microbial cultures: *Escherichia coli* and *Staphylococcus aureus*.
- Muller Hinton Agar.
- DPPH reagent for antioxidant assay.
- UV-Vis spectrophotometer.

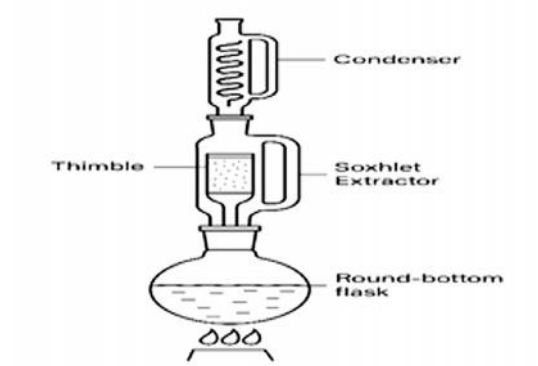


Figure 1 Soxhlet Apparatus for Ethanolic Extraction of Leaf Powder

3. Sample Collection and Extraction

Fresh, healthy leaves of *Ficus religiosa* and *Azadirachta indica* were collected from local trees in Raipur. The leaves were cleaned with distilled water, shade-dried for 7–10 days, and powdered. Equal weights (10 g each) of the powdered leaves were mixed and extracted using Soxhlet extraction with ethanol for 48 hours. The extract was filtered and evaporated to obtain a semi-solid mass, stored in airtight containers for further analysis.

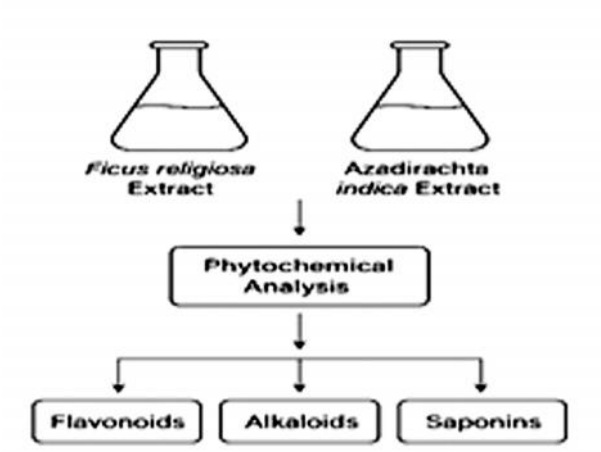


Figure 2 Phytochemical Analysis of *Ficus religiosa* and *Azadirachta indica* Extracts

4. Phytochemical Screening

Standard qualitative tests were performed to detect the following:

- Alkaloids (Mayer's test).
- Flavonoids (Shinoda test).
- Tannins (Ferric chloride test).
- Saponins (Foam test).
- Terpenoids and phenols.

5. Antibacterial Testing

Agar well diffusion method was used. Two bacterial strains (*E. coli* and *S. aureus*) were inoculated on agar plates. Wells were filled with extract and incubated for 24 hours. Zones of inhibition were measured in millimeters.

6. Antioxidant Assay

The extract was mixed with DPPH solution and kept in the dark for 30 minutes. The absorbance was read at 517 nm. Percentage of DPPH scavenging was calculated.

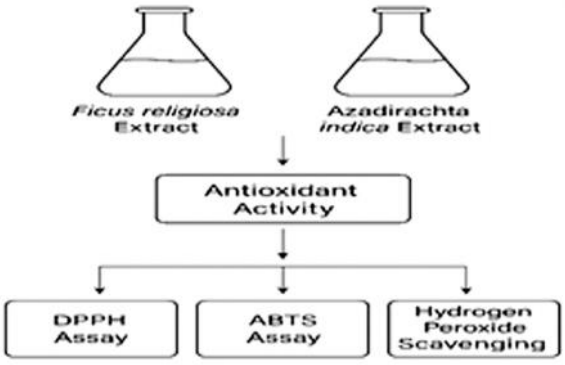


Figure 3 Antioxidant Activity Screening of Leaf Extracts (Combined)

Analysis and Interpretation

Table 1: Phytochemical Components in Combined Extract

Phytochemical	Presence
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponins	Present
Terpenoids	Present
Phenols	Present

Interpretation

The phytochemical screening of the combined ethanolic extract of *Ficus religiosa* and *Azadirachta indica* leaves revealed the presence of six major bioactive compounds—alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. The presence of these phytochemicals indicates a strong medicinal potential, as each of these compounds contributes distinct therapeutic properties, enhancing the overall biological activity of the extract. Below is a detailed interpretation of each identified component:

1. **Alkaloids – Present:** Alkaloids are naturally occurring nitrogen-containing compounds widely recognized for their potent pharmacological actions. In this extract, their presence suggests the potential for analgesic, antibacterial, and anti-inflammatory effects. Alkaloids have been historically used in drug formulations targeting microbial infections, high blood pressure, and neurological disorders. Their presence in the combined extract enhances the therapeutic efficacy against bacterial infections and may support central nervous system activities.
2. **Flavonoids – Present:** Flavonoids are powerful antioxidants found in many plant-based extracts. The detection of flavonoids indicates that the combined extract may have strong free radical scavenging properties. These compounds help reduce oxidative stress, which is a major factor in aging and chronic diseases like cancer, diabetes, and cardiovascular disorders. The presence of flavonoids explains the high antioxidant activity observed in the DPPH assay results, confirming their essential contribution to the medicinal value of the extract.
3. **Tannins – Present:** Tannins are polyphenolic compounds known for their astringent properties. They are highly effective in wound healing and reducing inflammation by contracting tissues and forming a protective layer. Their antimicrobial properties also help inhibit bacterial growth. In this study, the detection of tannins supports the antibacterial effectiveness of the extract against both *E. coli* and *Staphylococcus aureus*, indicating a synergistic enhancement in defense mechanisms.
4. **Saponins – Present:** Saponins are glycosides with distinctive foaming characteristics and are associated with various health benefits including immune-boosting, cholesterol-lowering, and antifungal activities.

Their presence in the extract suggests the potential to modulate immune responses and combat microbial infections. Additionally, saponins are known to enhance the absorption of other phytochemicals, potentially improving the bioavailability of alkaloids and flavonoids in the mixture.

5. **Terpenoids – Present:** Terpenoids contribute to the fragrance and color of plants, but more importantly, they play significant roles in anti-inflammatory and anticancer activities. In medicinal formulations, terpenoids help in inhibiting the growth of pathogens and modulating enzyme functions. Their detection indicates that the extract may exhibit anti-inflammatory effects, aligning with the traditional use of both *Ficus religiosa* and *Azadirachta indica* in the treatment of skin disorders and joint pain.
6. **Phenols – Present:** Phenolic compounds are one of the most important groups of natural antioxidants. Their presence contributes significantly to the extract's capacity to neutralize free radicals. This further explains the high antioxidant performance of the combined extract in DPPH assays. Phenols are also known to exhibit antimicrobial, anti-carcinogenic, and anti-diabetic properties, making them valuable components in plant-based therapeutics.

Summary

The presence of all six phytochemical classes in the combined extract confirms a rich and diverse chemical profile. This indicates that the mixture of *Ficus religiosa* and *Azadirachta indica* leaves offers a broad spectrum of therapeutic effects. The phytochemicals work synergistically to enhance antimicrobial and antioxidant functions, supporting the traditional use of these plants in treating infections, wounds, inflammation, and oxidative stress-related diseases. This detailed phytochemical composition serves as the foundational evidence for further developing herbal formulations or nutraceuticals using this extract.

Figure 5: Phytochemical Components Present in Combined Extract

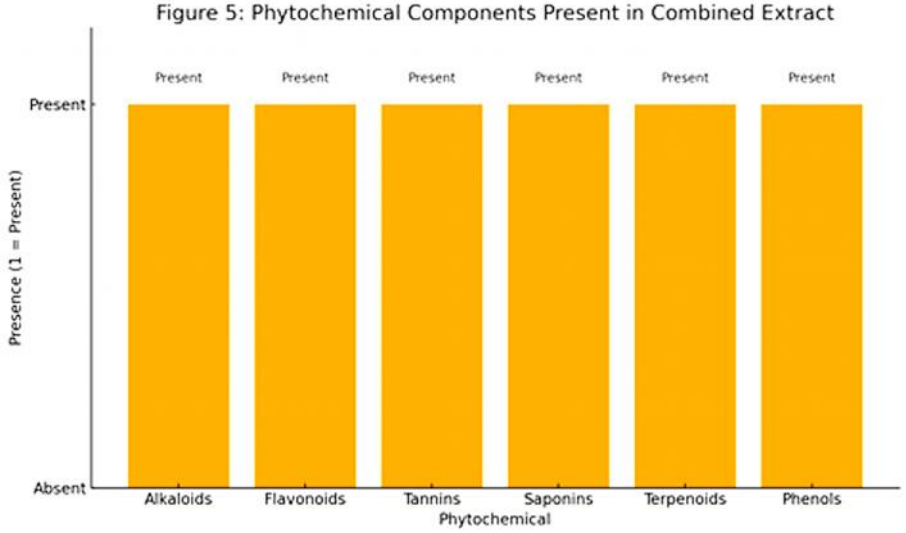


Table 2: Antibacterial Activity (Zone of Inhibition in mm)

Bacterial Strain	Neem Only	Pipal Only	Combined Extract
E. coli	12 mm	10 mm	16 mm
Staphylococcus aureus	14 mm	11 mm	18 mm

Interpretation

Table 2 provides a comparative analysis of the antibacterial activity of *Azadirachta indica* (Neem) extract, *Ficus religiosa* (Pipal) extract, and their combined extract, tested against two common bacterial strains *Escherichia coli* (*E. coli*) and *Staphylococcus aureus*. The data reflects the measurement of inhibition zones in millimeters (mm), which indicates the effectiveness of each extract in preventing bacterial growth. Larger zones of inhibition correspond to stronger antibacterial action.

1. Activity Against *Escherichia Coli*

- **Neem Extract (Azadirachta indica):** 12 mm
- **Pipal Extract (Ficus religiosa):** 10 mm
- **Combined Extract:** 16 mm

The combined extract demonstrated a significantly higher inhibitory effect (16 mm) against *E. coli* compared to the individual extracts of Neem (12 mm) and Pipal (10 mm). This clearly indicates a synergistic antibacterial effect, where the phytochemicals present in both plants interact to enhance microbial inhibition. *E. coli*, being a Gram-negative bacterium, is typically more resistant to antibiotics; thus, a 16 mm inhibition zone suggests promising potential for the combined extract as an effective herbal antibacterial agent.

2. Activity Against *Staphylococcus Aureus*

- **Neem Extract (Azadirachta indica):** 14 mm
- **Pipal Extract (Ficus religiosa):** 11 mm
- **Combined Extract:** 18 mm

Similar to *E. coli*, the combined extract showed the highest zone of inhibition (18 mm) against *S. aureus*, which is a Gram-positive bacterium commonly responsible for skin infections, abscesses, and post-operative wounds. While Neem and Pipal individually showed moderate activity (14 mm and 11 mm respectively), their combination significantly enhanced antibacterial performance. This supports the hypothesis that the combined phytochemicals, such as flavonoids, tannins, alkaloids, and saponins, work together to disrupt bacterial cell membranes more effectively.

The experimental data strongly supports that the equal-proportion mixture of *Azadirachta indica* and *Ficus religiosa* leaves produces a stronger antibacterial effect than either plant alone. The enhanced activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria confirms that the combined extract has broad-spectrum antimicrobial potential.

Such findings are valuable for the development of:

- Natural antibacterial creams or ointments.
- Herbal disinfectants.
- Oral herbal supplements for immunity.

The synergistic outcome encourages further in-depth research, including dose optimization, testing against other microbes, and possible application in pharmaceutical formulations as a safe, cost-effective, and natural alternative to synthetic antibiotics.

Figure 6: Antibacterial Activity of Extracts (Botanical Names)

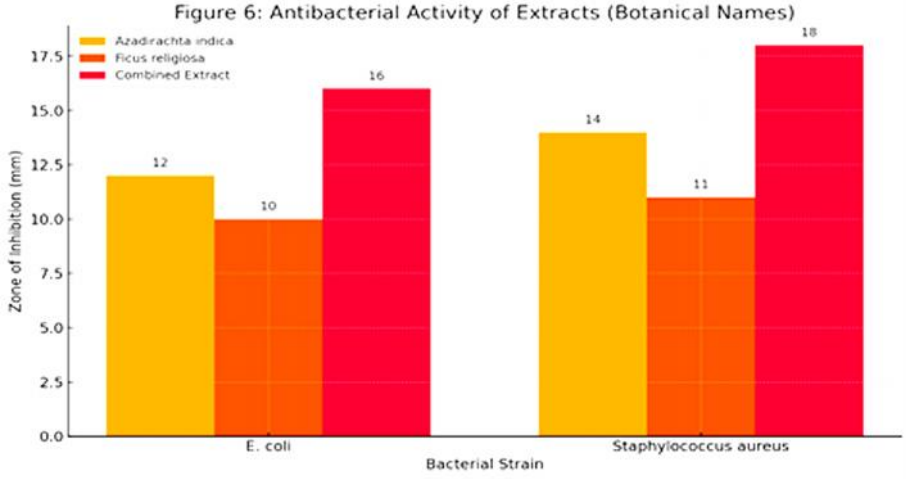


Table 3: Antioxidant Activity (DPPH Assay)

Sample	% Scavenging Activity
Neem Extract	58.2%
Pipal Extract	49.5%
Combined Extract	72.6%

Interpretation

Table 3 presents the antioxidant potential of three types of leaf extracts *Azadirachta indica* (Neem), *Ficus religiosa* (Pipal), and their combined extract as evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The results are expressed in terms of percentage of DPPH scavenging activity, which reflects the ability of the extract to neutralize harmful free radicals. Higher percentages indicate greater antioxidant activity.

1. Neem Extract (*Azadirachta indica*) – 58.2%

Neem extract exhibited a moderate antioxidant capacity, with 58.2% DPPH radical scavenging activity. This suggests that Neem contains a significant amount of antioxidant compounds such as quercetin, nimbin, and azadirachtin, which help reduce oxidative stress in biological systems. Neem has been traditionally used to protect against infections and support skin health, and this antioxidant capacity contributes to those protective effects.

2. Pipal Extract (*Ficus religiosa*) – 49.5%

Pipal extract showed a lower antioxidant activity compared to Neem, with 49.5% scavenging capacity. While still effective, this result suggests that the concentration or strength of free radical neutralizing compounds in *Ficus religiosa* is somewhat less than in *Azadirachta indica*. Nonetheless, the presence of tannins, flavonoids, and phenolic acids contributes to its antioxidant properties, and Pipal remains a valuable medicinal plant.

3. Combined Extract – 72.6%

The combined extract of Neem and Pipal leaves demonstrated the highest antioxidant activity, with 72.6% scavenging. This is a significant improvement over the individual extracts, indicating a synergistic effect when both plant leaves are used in equal proportion. This result is especially important, as it highlights that the combination of these two plants not only retains their individual strengths but also enhances their ability to neutralize free radicals.

Scientific Significance

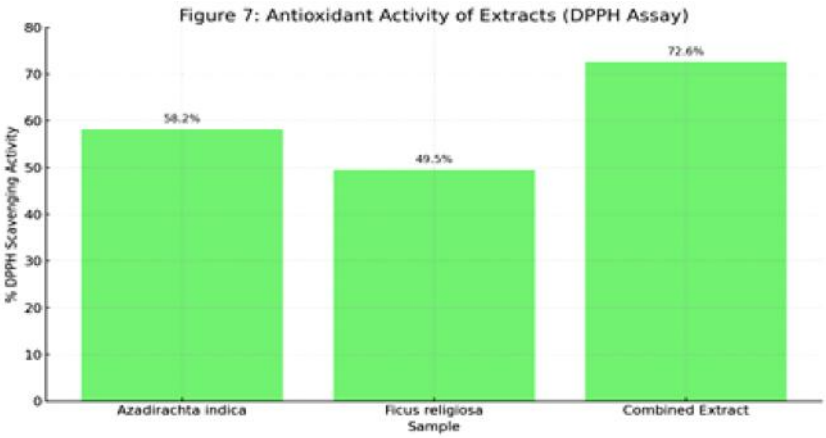
Antioxidants play a vital role in protecting the human body from oxidative stress caused by free radicals. Oxidative stress is linked to numerous chronic conditions such as cancer, cardiovascular diseases, diabetes, neurodegenerative disorders, and aging. A plant extract with over 70% DPPH scavenging activity is considered highly effective and may be suitable for use in:

- Herbal dietary supplements.
- Anti-aging skin formulations.
- Liver-protective and heart-protective natural products.

The increased antioxidant efficiency of the combined extract can be attributed to the diverse and complementary phytochemicals such as flavonoids, phenols, and tannins found in both plants. These compounds likely work together to stabilize and neutralize free radicals more effectively than when used alone.

The findings from Table 3 confirm that the combination of *Azadirachta indica* and *Ficus religiosa* results in a significant enhancement of antioxidant properties. This supports the use of mixed herbal extracts in developing natural, plant-based antioxidant therapies for health maintenance and disease prevention. Further

research, including in vivo studies and toxicity assessments, would be valuable to establish dosage and application in real-world medicinal or nutritional products.



Conclusion

This study provides evidence that combining leaf extracts of *Ficus religiosa* and *Azadirachta indica* results in better medicinal efficacy compared to individual extracts. Phytochemical tests confirmed the presence of major bioactive compounds such as flavonoids, tannins, and alkaloids. These compounds are responsible for their antibacterial and antioxidant effects.

The extract showed enhanced zones of inhibition against both *E. coli* and *S. aureus*, indicating its potential to combat bacterial infections. Moreover, the DPPH assay confirmed that the combination has stronger antioxidant activity, which may help prevent cellular damage and delay aging-related diseases.

This supports the idea that traditional knowledge of mixing herbs has scientific validity. The study paves the way for future research to develop herbal formulations such as ointments, capsules, or oral supplements using this extract. However, more studies including toxicity tests, dosage optimization, and clinical trials are recommended.

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