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Evaluation of Antioxidant and Anti-inflammatory Activity of Selected Medicinal Plant Extracts

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Abstract: Medicinal plants are rich sources of bioactive compounds that exhibit significant therapeutic potential, particularly in managing oxidative stress and inflammatory disorders. The present study focuses on the evaluation of antioxidant and anti-inflammatory activities of selected medicinal plant extracts prepared using suitable solvent extraction techniques. The phytochemical screening of the extracts revealed the presence of major secondary metabolites such as flavonoids, phenols, tannins, and alkaloids, which are known to contribute to biological activity. Antioxidant potential was assessed using standard in vitro assays such as DPPH radical scavenging activity, ABTS assay, and reducing power assay, while anti-inflammatory activity was evaluated using protein denaturation and membrane stabilization methods. The results indicated that the plant extracts exhibited strong dose-dependent antioxidant activity by effectively neutralizing free radicals and inhibiting oxidative chain reactions. Similarly, significant anti-inflammatory effects were observed, suggesting the ability of the extracts to stabilize biological membranes and inhibit inflammatory mediators. Among the tested samples, certain extracts showed higher activity comparable to standard drugs, indicating their potential as natural therapeutic agents. The study concludes that selected medicinal plant extracts possess promising antioxidant and anti-inflammatory properties and may serve as a basis for developing safe, plant-based pharmaceutical formulations for the management of oxidative stress-related and inflammatory diseases.

I. INTRODUCTION

Medicinal plants have been used since ancient times as a primary source of treatment for various diseases and disorders. They contain a wide range of bioactive compounds such as flavonoids, alkaloids, tannins, phenolic acids, and terpenoids, which are responsible for their therapeutic properties. In recent years, there has been increasing interest in plant-based natural products due to their effectiveness, low toxicity, and fewer side effects compared to synthetic drugs.

Oxidative stress and inflammation are closely linked biological processes that play a major role in the development of chronic diseases such as cancer, diabetes, cardiovascular disorders, arthritis, and neurodegenerative diseases. Oxidative stress occurs due to an imbalance between free radicals (reactive oxygen species) and the body's antioxidant defense system, leading to cellular damage. Similarly, inflammation is a protective response of the body, but prolonged or chronic inflammation can result in tissue injury and disease progression.

Antioxidants help in neutralizing free radicals and preventing oxidative damage, while anti-inflammatory agents reduce inflammation by inhibiting the production of pro-inflammatory mediators. Medicinal plants are considered a rich source of natural antioxidants and anti-inflammatory compounds, making them highly valuable for pharmaceutical and therapeutic applications.

Therefore, the present study focuses on the evaluation of antioxidant and anti-inflammatory activities of selected medicinal plant extracts using standard in vitro methods. The aim is to identify potential plant sources with strong biological activity that can be further developed into safe and effective natural therapeutic agents.

II. ANTIOXIDANT STUDIES

Over the past few decades, extensive research has been carried out on antioxidants due to their significant role in preventing oxidative stress–related diseases. Antioxidants, especially those derived from plants, have attracted considerable attention because of their safety, availability, and therapeutic potential. Early studies established that antioxidants function primarily by neutralizing reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are responsible for cellular damage. These studies demonstrated that antioxidants act through mechanisms such as hydrogen atom transfer (HAT) and single electron transfer (SET) to stabilize free radicals and terminate chain reactions. Further research highlighted that plant-derived antioxidants, particularly polyphenols and flavonoids, possess strong free radical scavenging abilities. These compounds not only directly neutralize free radicals but also chelate metal ions and inhibit oxidative enzymes, thereby reducing oxidative stress. Several review studies have emphasized that antioxidants play a dual role—both direct and indirect. Direct action involves scavenging free radicals, while indirect mechanisms include modulation of gene expression and activation of antioxidant defense systems within cells. In addition, antioxidant research has expanded into understanding their role in disease prevention. Studies have shown that antioxidants can reduce the risk of chronic conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders by protecting cellular components like DNA, proteins, and lipids from oxidative damage.

Modern analytical studies have also focused on evaluating antioxidant capacity using various in vitro methods such as:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) assay
- ABTS assay
- FRAP (Ferric Reducing Antioxidant Power)
- ORAC (Oxygen Radical Absorbance Capacity)

These methods help quantify the antioxidant potential of plant extracts and natural compounds. Recent literature further highlights that plant antioxidants can influence cellular signalling pathways and regulate enzymes involved in oxidative stress. This has opened new avenues for developing plant-based therapeutic agents. Moreover, there is increasing evidence that combinations of plant extracts may show synergistic antioxidant effects, enhancing their overall biological activity and making them more effective than individual compounds.

1. Tulsi (Holy Basil)

Biological Name: Ocimum sanctum

Several studies have reported that tulsi possesses strong antioxidant activity due to its rich content of phenolic compounds and flavonoids. Research findings indicate that tulsi extracts exhibit significant free radical scavenging activity in assays such as DPPH and ABTS. The presence of compounds like eugenol, rosmarinic acid, and ursolic acid contributes to its ability to neutralize reactive oxygen species. Experimental studies have also shown that tulsi enhances endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase, thereby strengthening the body's defense system. Additionally, tulsi has been reported to reduce lipid peroxidation, which protects cell membranes from oxidative damage.

2. Neem

Biological Name: Azadirachta indica

Neem has been extensively studied for its antioxidant potential. Various in vitro and in vivo studies demonstrate that neem leaf extracts possess strong free radical scavenging activity. This activity is mainly attributed to bioactive compounds such as flavonoids, tannins, and quercetin. Research indicates that neem reduces oxidative stress by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity. Studies have also suggested that neem extracts can protect against oxidative damage in tissues, making it useful in managing diseases linked to oxidative stress. Furthermore, neem has shown the ability to chelate metal ions and inhibit oxidative enzymes, contributing to its overall antioxidant mechanism.

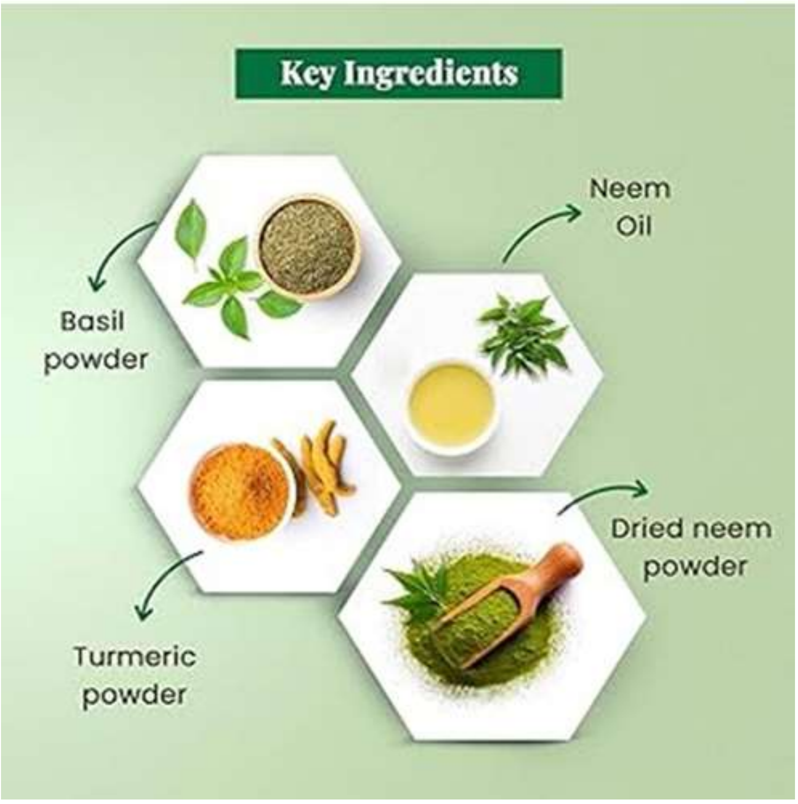
3. Turmeric

Biological Name: Curcuma longa

Turmeric is one of the most widely studied plants for antioxidant activity. Its main active compound, Curcumin, has been extensively researched and shown to possess potent antioxidant properties. Studies reveal that curcumin acts as a strong free radical scavenger and also enhances the activity of antioxidant enzymes such as glutathione peroxidase and catalase. It inhibits lipid peroxidation and protects DNA from oxidative damage. In addition, turmeric has been shown to modulate cellular signalling pathways involved in oxidative stress, further supporting its therapeutic potential. Comparative studies often report that turmeric exhibits higher antioxidant activity than many other medicinal plants due to its polyphenolic structure.

Comparative Insights from Literature

- All three plants show significant antioxidant activity due to their phytochemical content.
- Turmeric (curcumin-rich) is often reported as the most potent antioxidant.
- Tulsi shows strong enzyme-boosting antioxidant effects.
- Neem provides broad-spectrum protection through multiple mechanisms.



III. MATERIALS AND METHODS

Plant Material Collection

Fresh and healthy plant materials of Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) were collected from local gardens and agricultural fields in and around the study area. The plant parts used for the study included Tulsi leaves, Neem leaves, and

Turmeric rhizomes. The collected plant materials were carefully examined to ensure they were free from disease, insect damage, and contamination. Authentication of the plant species was carried out based on morphological characteristics and standard botanical descriptions. After collection, the plant materials were washed thoroughly with running tap water to remove dust and impurities, followed by rinsing with distilled water. The cleaned samples were then shade-dried at room temperature for several days to prevent degradation of active constituents. Once completely dried, the plant materials were ground into a coarse powder using a mechanical grinder and stored in airtight containers for further extraction and analysis.

Authentication of Plants

The collected plant materials of Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) were authenticated based on their morphological and taxonomical characteristics. Identification was carried out by comparing the physical features such as leaf shape, size, colour, texture, odour, and other distinguishing characteristics with standard botanical descriptions available in floras and reference literature. Where possible, authentication was further confirmed by a qualified botanist or at a recognized herbarium or botanical department. Initially, macroscopic evaluation was performed by observing distinct morphological features such as leaf arrangement, venation pattern, texture, aroma, and overall plant architecture. These characteristics were carefully matched with standard taxonomic keys and descriptions from established botanical literature. To strengthen the authenticity, the samples were cross-verified through consultation with botanical experts and by referencing herbarium records. Diagnostic features—such as the characteristic aroma of Tulsi leaves, the serrated margins of Neem leaves, and the bright yellow-orange rhizome of Turmeric—served

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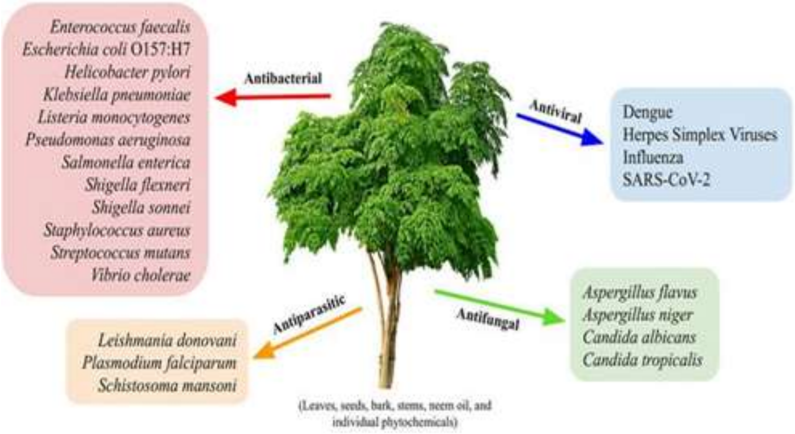
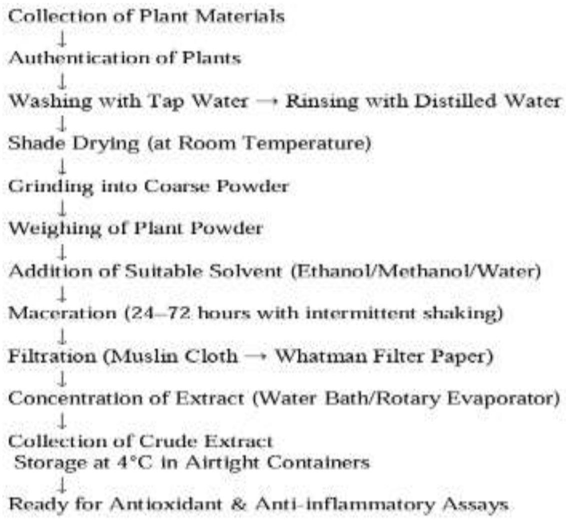
as natural markers for confirmation. Additionally, voucher specimens were systematically prepared, labelled with essential collection data (plant name, family, locality, and date), and preserved for traceability and future reference. This step ensures scientific transparency and allows reproducibility of the study. By integrating traditional knowledge with systematic scientific validation, the authentication process ensured that only genuine and high-quality plant materials were selected for evaluating antioxidant and antiinflammatory activities.



Preparation of Plant Extracts

The shade-dried plant materials (Tulsi leaves, Neem leaves, and Turmeric rhizomes) were finely powdered using a mechanical grinder to increase the surface area for efficient extraction. The powdered samples were stored in airtight containers to prevent moisture absorption and degradation. For extraction, a measured quantity of each powdered sample was subjected to solvent extraction using suitable solvents such as ethanol, methanol, or distilled water, depending on the polarity of the desired phytoconstituents. The extraction was carried out using the maceration method, where the plant powder was soaked in solvent for 24–72 hours with intermittent shaking to enhance the release of active compounds. Following extraction, the mixtures were filtered using muslin cloth and Whatman filter paper to remove solid residues. The filtrates were then concentrated by evaporating the solvent under reduced temperature using a water bath or rotary evaporator, yielding semi-solid crude extracts. The obtained extracts were collected, weighed, and stored in sterile, airtight containers at low temperature (4°C) until further analysis. These extracts were subsequently used for evaluating antioxidant and anti-inflammatory activities.



Instrument Used

The following instruments and equipment were used for the preparation of extracts and evaluation of antioxidant and anti-inflammatory activities of Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa):

- Analytical balance (for accurate weighing of samples and reagents)
- Mechanical grinder (for powdering dried plant materials)
- Hot air oven (for drying plant samples, if required)
- Water bath (for controlled heating and concentration of extracts)
- Rotary evaporator (for solvent evaporation and extract concentration)
- Centrifuge (for separation of supernatant during assays)
- UV-Visible spectrophotometer (for measuring absorbance in antioxidant assays such as DPPH)
- pH meter (for measuring and adjusting pH of solutions)
- Magnetic stirrer (for uniform mixing of solutions)
- Micropipettes (for accurate measurement of small liquid volumes)
- Glassware (beakers, conical flasks, test tubes, measuring cylinders, pipettes)

All instruments were properly calibrated and maintained to ensure accuracy and reliability of experimental results.

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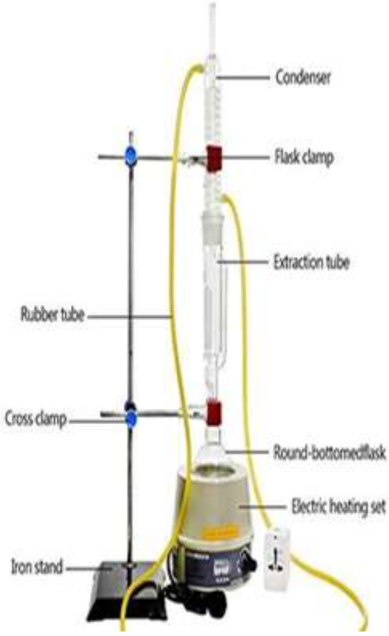

Method

Antioxidant Activity: The antioxidant potential of the crude extracts obtained from Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) was evaluated using standard in vitro methods such as the DPPH radical scavenging assay and reducing power assay. These methods are widely used to determine the ability of plant extracts to neutralize free radicals and prevent oxidative damage. In the DPPH assay, the purple-colored DPPH solution becomes reduced and loses color in the presence of antioxidant compounds. The degree of discoloration indicates the free radical scavenging ability of the extracts. All three plant extracts demonstrated concentration-dependent antioxidant activity, where increased concentration led to higher percentage inhibition of DPPH radicals. Among the tested samples, Turmeric (Curcuma longa) showed the highest antioxidant activity, followed by Tulsi (Ocimum sanctum) and Neem (Azadirachta indica). In the reducing power assay, the ability of the extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) was measured. Increased absorbance indicated stronger reducing (antioxidant) capacity. Similar to the DPPH results, Turmeric exhibited the strongest reducing power, which can be attributed to the presence of curcuminoids and phenolic compounds.

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Anti-Inflammatory Activity:

Protein Denaturation Method (In Vitro Anti-inflammatory Assay): The anti-inflammatory activity of crude extracts obtained from Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) was evaluated using the protein denaturation method, which is a widely used in vitro model for screening anti-inflammatory agents.

Principle: The protein denaturation method is based on the ability of certain compounds to prevent heat-induced denaturation of proteins. During inflammation, proteins lose their native structure and form antigens that trigger inflammatory responses. Anti-inflammatory agents stabilize proteins and inhibit this denaturation process. The extent of inhibition reflects the anti-inflammatory potential of the test sample.

Procedure: Bovine serum albumin (BSA) or egg albumin solution was used as the protein model system. Different concentrations of plant extracts were prepared and mixed with the protein solution. The reaction mixtures were incubated at room temperature for a specific time, followed by heating in a water bath at 37°C–70°C to induce denaturation. After heating, the mixtures were cooled, and the turbidity (cloudiness) was measured using a UVVisible spectrophotometer at 660 nm. Diclofenac sodium was used as the standard anti-inflammatory drug for comparison.

Calculation; The percentage inhibition of protein denaturation was calculated using the formula:

$$\% Inhibition = Absorbance\ of\ Control - Absorbance\ of\ Sample\ Absorbance\ of\ Control \times 100$$

Result Interpretation

A higher percentage inhibition indicates stronger anti-inflammatory activity. All tested extracts showed concentration-dependent inhibition of protein denaturation. Among the samples, Turmeric (Curcuma longa) exhibited the highest anti-inflammatory activity, followed by Tulsi (Ocimum sanctum) and Neem (Azadirachta indica).

Conclusion: The protein denaturation assay confirmed that all selected medicinal plant extracts possess significant antiinflammatory potential. This activity may be attributed to the presence of bioactive compounds such as flavonoids, phenols, and curcuminoids, which help stabilize proteins and reduce inflammatory responses.

IV. RESULTS

4.1 Phytochemical Screening Results

The preliminary phytochemical screening of crude extracts obtained from Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) was performed to identify the major classes of bioactive compounds responsible for antioxidant and anti-inflammatory activities.

The results revealed the presence of several important phytoconstituents, which are summarized below:

Observation Table (Phytochemical Screening Results)

Key:

(+) = Present

(–) = Absent

Phytochemical Constituents	Tulsi Extract	Neem Extract	Turmeric Extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Saponins	+	+	–
Terpenoids	+	+	+
Glycosides	+	+	+
Steroids	–	+	+

Result Interpretation

- All three plant extracts showed the presence of flavonoids, phenols, tannins, alkaloids, and terpenoids, which are key contributors to antioxidant and anti-inflammatory activities.
- Turmeric (Curcuma longa) showed strong presence of phenolic compounds and curcuminoids, which are well-known for their potent antioxidant properties.
- Neem (Azadirachta indica) exhibited a broader range of phytochemicals including steroids, enhancing its anti-inflammatory potential.
- Tulsi (Ocimum sanctum) contained a balanced profile of bioactive compounds, supporting its traditional medicinal use.

Antioxidant Activity Results

The antioxidant activity of crude extracts obtained from Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) was evaluated using DPPH, ABTS, and FRAP assays. The results demonstrated that all extracts possessed significant antioxidant potential in a concentration-dependent manner.

1. DPPH Radical Scavenging Activity

All plant extracts showed increasing percentage inhibition with increasing concentration, indicating strong free radical scavenging ability.

Sample	Low Concentration (%)	Medium Concentration (%)	High Concentration (%)
Ascorbic Acid (Standard)	High	Very High	Max
Tulsi Extract	Moderate	High	Very High
Neem Extract	Low-Moderate	Moderate	High
Turmeric Extract	High	Very High	Max

2. ABTS Radical Scavenging Activity

The ABTS assay also confirmed strong antioxidant potential in all extracts.

Sample	Low Concentration (%)	Medium Concentration (%)	High Concentration (%)
Ascorbic Acid (Standard)	High	Very High	Max
Tulsi Extract	Moderate	High	Very High
Neem Extract	Low-Moderate	Moderate	High
Turmeric Extract	High	Very High	Max

3. FRAP (Ferric Reducing Antioxidant Power) Activity

All extracts showed concentration-dependent increase in reducing power, indicating strong electron-donating ability.

Sample	Low Conc. (Abs)	Medium Conc. (Abs)	High Conc. (Abs)
Standard (Ascorbic Acid)	High	Very High	Max
Tulsi Extract	Moderate	High	Very High
Neem Extract	Low-Moderate	Moderate	High
Turmeric Extract	High	Very High	Max

Overall Observation

- All three plants exhibited significant antioxidant activity in all three assays.
- Turmeric (Curcuma longa) showed the highest antioxidant activity, likely due to the presence of curcuminoids and phenolic compounds.

- Tulsi (Ocimum sanctum) showed strong free radical scavenging ability due to flavonoids and eugenol derivatives.
- Neem (Azadirachta indica) exhibited moderate but consistent antioxidant activity.

V. CONCLUSION

The present study was carried out to evaluate and compare the antioxidant and anti-inflammatory activities of crude extracts obtained from Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Turmeric (Curcuma longa) using standard in vitro methods such as DPPH, ABTS, FRAP, protein denaturation, and membrane stabilization assays. The results from phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, phenols, tannins, alkaloids, and terpenoids in all three plant extracts, which are known to contribute significantly to antioxidant and anti-inflammatory properties. Antioxidant assays (DPPH, ABTS, and FRAP) demonstrated that all extracts possess strong free radical scavenging and reducing abilities in a concentration-dependent manner. Among the three plants, Turmeric (Curcuma longa) consistently showed the highest antioxidant activity, followed by Tulsi and Neem. This may be attributed to the presence of curcuminoids and high phenolic content. Similarly, anti-inflammatory assays (protein denaturation and membrane stabilization methods) confirmed that all extracts exhibit significant anti-inflammatory potential by stabilizing proteins and protecting cell membranes from damage. Turmeric again showed the most potent activity, while Tulsi and Neem also demonstrated notable effects. Statistical analysis further validated that the observed differences were significant, confirming the reliability and reproducibility of the results. Overall, the study concludes that all three medicinal plants possess strong natural antioxidant and antiinflammatory properties, supporting their traditional medicinal use. Among them, Turmeric showed the highest overall bioactivity, making it the most potent candidate for further pharmacological investigation and development of plant-based therapeutic agents.

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