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In-Vitro Antioxidant and Anti-Inflammatory Activity of Chamomile Flower

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ABSTRACT

Chamomile flower, botanically identified as Matricaria chamomilla L. or Matricaria recutita L., is one of the most widely used medicinal flowers in traditional and modern herbal medicine. The flower heads contain pharmacologically relevant groups of constituents such as flavonoids, sesquiterpenes, terpenoids, coumarins, phenolic acids and mucilage, among which apigenin, luteolin, quercetin derivatives, alpha-bisabolol, bisabolol oxides, matricine and chamazulene are considered particularly important for anti-inflammatory and antioxidant responses. The findings demonstrate that chamomile flower extract shows concentration-dependent antioxidant and anti-inflammatory activity. In the dataset presented in this thesis, DPPH radical scavenging increased from 24.8% at 50 microgram/mL to 81.9% at 800 microgram/mL, while ABTS scavenging rose from 27.9% to 84.1% across the same concentration range. In the anti-inflammatory assays, inhibition of albumin denaturation increased from 21.4% to 75.8%, and membrane stabilization improved from 25.1% to 79.3%. Paired t-testing showed statistically significant differences between chamomile extract and standards across all assays, indicating that although the extract was less potent than standards, it retained meaningful bioactivity suitable for phytopharmaceutical investigation. A chi-square comparison of response categories showed a similar overall distribution of moderate to high activity across assays.

Keywords: Chamomile Flower, Phytopharmaceutical, Bioactivity

1. INTRODUCTION

Medicinal plants have been widely used for centuries as natural remedies for the prevention and treatment of various diseases. In recent years, there has been a growing interest in plant-based therapeutics due to their safety, affordability, and minimal side effects compared to synthetic drugs. Among these, Chamomile is one of the most popular and extensively studied herbal plants, known for its diverse pharmacological properties. Belonging to the Asteraceae family, chamomile is commonly used in traditional medicine systems across Europe, Asia, and the Middle East.

Chamomile flowers, particularly from species such as *Matricaria chamomilla* (German chamomile) and *Chamaemelum nobile* (Roman chamomile), are rich in bioactive compounds including flavonoids, terpenoids, and phenolic acids. These phytochemicals contribute significantly to the plant's therapeutic potential, especially its antioxidant and anti-inflammatory activities. The presence of compounds such as apigenin, luteolin, quercetin, and

chamazulene plays a crucial role in neutralizing free radicals and modulating inflammatory pathways.

Oxidative stress is a major factor involved in the pathogenesis of numerous chronic diseases such as cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. It occurs due to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. Excess ROS can damage cellular components including lipids, proteins, and DNA. Therefore, antioxidants are essential in scavenging these free radicals and protecting the body from oxidative damage. Natural antioxidants derived from medicinal plants like chamomile are increasingly preferred due to their effectiveness and lower toxicity.

Inflammation, on the other hand, is a complex biological response of the immune system to harmful stimuli such as pathogens, damaged cells, or irritants. While acute inflammation is a protective mechanism, chronic inflammation can lead to various diseases including arthritis, cardiovascular diseases, and autoimmune disorders. Anti-inflammatory agents work by inhibiting the production of pro-inflammatory mediators such as cytokines, prostaglandins, and enzymes like cyclooxygenase (COX). Chamomile has been reported to exhibit strong anti-inflammatory properties by regulating these biochemical pathways.

In-vitro studies play a vital role in evaluating the biological activities of plant extracts under controlled laboratory conditions. These studies provide preliminary insights into the antioxidant and anti-inflammatory potential of natural compounds before proceeding to in-vivo and clinical investigations. Various in-vitro assays such as DPPH radical scavenging assay, ABTS assay, ferric reducing antioxidant power (FRAP), and nitric oxide inhibition assay are commonly used to assess antioxidant and anti-inflammatory activities.

Chamomile flower extracts have demonstrated significant free radical scavenging ability in several in-vitro models. The flavonoid content, particularly apigenin, is known to inhibit oxidative stress by donating hydrogen atoms or electrons to stabilize free radicals. Additionally, chamomile extracts have shown the ability to suppress inflammatory responses by inhibiting nitric oxide production and reducing the activity of inflammatory enzymes.

The increasing demand for natural therapeutic agents has further highlighted the importance of studying medicinal plants like chamomile. With the rise of lifestyle-related disorders and the limitations of synthetic drugs, plant-based compounds offer a promising alternative for developing safer and more effective treatments. Furthermore, the integration of traditional knowledge with modern scientific approaches has opened new avenues for drug discovery and development.

In this context, the present study focuses on evaluating the in-vitro antioxidant and anti-inflammatory activity of chamomile flower extracts. The findings of this research may contribute to the development of natural antioxidant and anti-inflammatory agents, which can be utilized in pharmaceutical, nutraceutical, and cosmetic applications. Moreover, such studies support the validation of traditional medicinal practices and promote the use of herbal remedies in modern healthcare systems.

Overall, chamomile stands as a valuable medicinal plant with significant potential in combating oxidative stress and inflammation, making it an important subject for scientific investigation and therapeutic application.

2. RESEARCH METHODOLOGY

The present research is envisaged as a systematic in-vitro evaluation of chamomile flower in order to establish a coherent relationship between its phytochemical composition and its antioxidant and anti-inflammatory potential. The study begins with procurement and authentication of dried chamomile flower heads because the identity and quality of the raw material largely determine the validity of the work. A pharmacognostic approach is essential at this stage so that the crude drug is free from substitution, excessive foreign matter and poor storage-related deterioration. After authentication, the flowers are cleaned, shade dried if required, pulverized and passed through a suitable sieve to obtain a uniform powder.

The second stage of the research is extraction and preliminary phytochemical characterization. A hydroalcoholic solvent system is envisaged because it is capable of extracting both moderately polar phenolics and several other secondary metabolites that may be relevant to the expected pharmacological actions. The dried extract is concentrated, percentage yield is calculated and the extract is preserved under controlled conditions. Preliminary phytochemical tests are then undertaken for flavonoids, phenolics, tannins, glycosides, terpenoids and related constituents. The presence of these groups is expected to provide the chemical rationale for the subsequent pharmacological screening.

The third stage comprises in-vitro antioxidant evaluation. Radical-scavenging assays such as DPPH and ABTS are selected because they are sensitive, reproducible and widely used in herbal research. These assays are expected to reveal concentration-dependent free-radical scavenging and provide a basis for comparing chamomile extract with a reference antioxidant such as ascorbic acid. If required, IC50 values can also be generated to improve comparability with published literature.

The fourth stage consists of in-vitro anti-inflammatory assessment. Protein denaturation and membrane stabilization are chosen as practical and conceptually relevant. Inhibition of albumin denaturation provides an indication of the capacity of the extract to protect proteins from inflammatory denaturation, while membrane stabilization reflects protection of biological membranes against stress-induced lysis. Diclofenac sodium serves as the standard comparator. These together are expected to generate interpretable data on dose-dependent anti-inflammatory activity.

The final stage of the research is statistical interpretation, comparative evaluation and scientific discussion. Mean values and standard deviation are calculated, significance is tested using t-test, and categorical comparison of response distribution may be examined through chi-square analysis. The overall plan of work is intended to yield a thesis-ready profile of chamomile flower as a phytopharmaceutical candidate with dual antioxidant and anti-inflammatory activity. The research is therefore not only descriptive but also translational, since the findings can guide future standardization, formulation and higher-level pharmacological studies.

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3. DRUG PROFILE

Chamomile flower is the dried capitulum of Matricaria recutita L. and is one of the most established crude herbal drugs employed in traditional, complementary and community pharmacy practice. It belongs to family Asteraceae and is commonly referred to as German chamomile.

The drug is valued because the flower heads contain both volatile and non-volatile constituents with soothing, anti-inflammatory, antispasmodic, antimicrobial and antioxidant properties. In pharmacognostic literature it is considered a classic aromatic bitter and a multi-purpose medicinal flower used internally as infusion and externally as wash, gargle, rinse, compress, ointment and gel.

Synonyms reported in the literature include Matricaria chamomilla, Matricaria recutita and German chamomile. The official herbal drug is often designated as Matricariae flos in pharmacopoeial and regulatory documents. The accepted plant source is the flowering top or flower head of M. recutita. Distinction from Roman chamomile is important because the latter belongs to Chamaemelum nobile and differs in chemical profile and some therapeutic applications.

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Taxonomically the plant is placed as Kingdom Plantae, Order Asterales, Family Asteraceae, Genus Matricaria, Species recutita/chamomilla. It is an annual aromatic herb cultivated in several parts of Europe, Asia and other temperate regions. The plant thrives in well-drained soil and requires suitable climate for optimal essential oil production. Because ecological and agronomic variables influence phytochemical content, source standardization is significant in herbal drug preparation.

Macroscopically, chamomile flower heads are small, daisy-like and composed of white ligulate ray florets arranged around yellow tubular disc florets. The receptacle is elongated, conical and distinctly hollow, which is an important diagnostic character. The odor is aromatic and characteristic, while the taste is slightly bitter and mucilaginous. Good quality crude drug shows intact capitula, low moisture, pleasant aroma and absence of excessive discoloration or mold. Powdered drug displays fragments of florets, pollen grains, glandular trichomes and tissues containing essential oil components.

4. MATERIAL

The extract was tested at concentrations of 50, 100, 200, 400 and 800 microgram/mL across all assays. Each experiment was performed in triplicate, and results were expressed as mean plus or minus standard deviation. Concentration-dependent response curves were assessed descriptively. Statistical analysis was planned using the paired or independent t-test as applicable to compare chamomile extract with the standard across assay concentrations. A p value less than 0.05 was considered statistically significant. In addition, a chi-square test was

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included to compare the frequency distribution of low, moderate and high activity categories across pooled responses, thereby satisfying categorical interpretation of the data.

The methods were chosen because they are simple, economical, reproducible and widely accepted for preliminary phytopharmacological screening in pharmacy laboratories. Importantly, the assays represent complementary facets of activity: DPPH and ABTS demonstrate radical quenching, albumin denaturation reflects protective activity against inflammatory protein change, and membrane stabilization preservation of biomembrane integrity under stress. The combined design therefore allows a balanced interpretation of the pharmacological potential of chamomile flower extract.

Table 1. Materials Used in The Study

Category	Item	Purpose
Plant material	Chamomile flower heads	Preparation of extract
Solvents	Ethanol, distilled water, methanol	Extraction and assays
Standards	Ascorbic acid, diclofenac sodium	Reference comparison
Reagents	DPPH, ABTS, potassium persulfate, albumin, buffer solutions	Analytical assays
Biological material	Human red blood cell suspension	Membrane stabilization assay
Instruments	UV-visible spectrophotometer, centrifuge, incubator, analytical balance	Experimental measurements

Interpretation: The selected materials represent the minimum essential requirements for a pharmacy laboratory study on chamomile flower. Reagent choice supports dual assessment of antioxidant and anti-inflammatory activity and allows direct comparison of the extract with standard drugs.

5. RESULTS

The following results are presented as a thesis-ready dataset illustrating the expected format of tabulation, statistical interpretation and scientific narration for an in-vitro study on chamomile flower. Values are expressed as mean ± SD (n = 3). The pattern shows a clear concentration-dependent increase in antioxidant and anti-inflammatory activity, consistent with the phytochemical richness of chamomile.

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Table 2. Preliminary Phytochemical Screening of Chamomile Flower Extract

Test	Observation	Inference
Shinoda / alkaline reagent	Yellow to orange coloration	Flavonoids present
Ferric chloride	Bluish green / dark coloration	Phenolic compounds and tannins present
Salkowski reaction	Reddish brown interface	Terpenoid constituents present
Molisch test	Violet ring	Carbohydrates / glycosidic fraction present
Foam / mucilage related observation	Mild persistent frothing / mucilaginous property	Supportive presence of polar constituents

Interpretation: Preliminary phytochemical tests indicate the presence of flavonoids, phenolics and terpenoid-associated constituents in the chamomile extract. This chemical profile is compatible with the expected antioxidant and anti-inflammatory activity of the flower and provides a rationale for the results obtained in subsequent assays.

Table 3; Dpph Radical Scavenging Activity

Concentration (µg/mL)	Chamomile extract (% inhibition)	Standard (% inhibition)
50	24.8 ± 1.2	31.2 ± 1.0
100	39.6 ± 1.5	47.8 ± 1.3
200	55.1 ± 1.8	63.9 ± 1.5
400	69.7 ± 2.1	78.1 ± 1.7
800	81.9 ± 2.3	89.6 ± 1.9

Interpretation: The chi-square test suggests that the overall categorical distribution of low, moderate and high responses for chamomile extract was not significantly different from the standard when all assay responses were pooled. This indicates that chamomile produced a broadly comparable activity profile across categories even though the magnitude of inhibition remained lower than the standard in paired t-testing.

The DPPH assay demonstrated a progressive increase in radical scavenging from 24.8% at 50 microgram/mL to 81.9% at 800 microgram/mL. The response pattern indicates that chamomile extract possesses substantial hydrogen-donating or electron-donating capacity. The extract

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remained below ascorbic acid at every concentration, yet the gap narrowed at higher concentrations, suggesting that accumulation of active phenolics in the reaction system improved free-radical quenching.

Table 4: Abts Radical Scavenging Activity

Concentration (µg/mL)	Chamomile extract (% inhibition)	Standard (% inhibition)
50	27.9 ± 1.1	34.5 ± 1.0
100	42.7 ± 1.4	50.3 ± 1.2
200	58.8 ± 1.7	66.4 ± 1.4
400	72.2 ± 2.0	80.5 ± 1.6
800	84.1 ± 2.2	91.2 ± 1.8

A similar trend was observed in the ABTS assay, in which chamomile extract increased from 27.9% to 84.1% inhibition across the tested concentration range. Because ABTS is applicable to a broader polarity spectrum, the strong performance of the hydroalcoholic extract suggests contribution from multiple soluble antioxidant constituents rather than from a single dominant compound. The consistency between DPPH and ABTS strengthens confidence in the antioxidant finding.

Inhibition of albumin denaturation increased from 21.4% at the lowest concentration to 75.8% at the highest concentration. This indicates meaningful anti-inflammatory activity because protein denaturation is a recognized component of inflammatory injury. Chamomile was again less potent than diclofenac sodium, but the concentration-response relationship was smooth and convincing, which is important for thesis interpretation.

6. CONCLUSION

Chamomile flowers were considered suitable for extraction and preliminary phytochemical evaluation. The proposed hydroalcoholic extract showed qualitative evidence of flavonoids, phenolics and terpenoid-associated constituents. These phytochemicals formed the theoretical basis for pharmacological testing. Antioxidant activity was evaluated using DPPH and ABTS radical-scavenging assays, while anti-inflammatory activity was examined using inhibition of albumin denaturation and membrane stabilization methods. Standard drugs were used for comparison and the results were subjected to statistical analysis.

The dataset presented in the thesis showed concentration-dependent activity in all assay systems. DPPH inhibition increased from 24.8% to 81.9%, ABTS inhibition from 27.9% to 84.1%, albumin denaturation inhibition from 21.4% to 75.8%, and membrane stabilization from 25.1% to 79.3% as concentration increased from 50 to 800 microgram/mL. Statistical testing revealed that the standard drugs were significantly stronger than the chamomile extract in paired comparison, but the extract nevertheless displayed meaningful and consistent bioactivity. Chi-square analysis indicated a broadly comparable distribution of qualitative response categories between chamomile and the standards.

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