



Biomarker and Evaluation of Antioxidant Property in *Chrysanthemum Morifolium*

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ABSTRACT

The blooming plant *Chrysanthemum morifolium* (Asteraceae) is prized for its rich phytochemical composition and health-promoting qualities. It has long been utilized for both decorative and therapeutic uses. The flowers have a variety of bioactive substances, such as flavonoids (such luteolin and quercetin) and phenolic acids (like chlorogenic acid), which are important sources of antioxidant activity. Higher total phenolic content is closely correlated with stronger antioxidant ability. These phenolics and flavonoids have been demonstrated to scavenge free radicals and reduce oxidative stress in a variety of in vitro experiments, including DPPH and ABTS. Plant extracts are frequently made via maceration, a straightforward extraction method that involves soaking dried flower powder in a solvent (such as ethanol) at room temperature for a long time in order to assess antioxidant activity. Maceration makes it easier for phytochemicals to diffuse into the solvent; the yield of antioxidant compounds is influenced by the polarity of the solvent and the duration of extraction. When compared to water extraction, ethanol maceration frequently produces increased flavonoid and phenolic levels, which boost antioxidant activity. All things considered, *C. morifolium* flower extracts produced by optimal maceration exhibit encouraging inherent antioxidant qualities.

Keywords: Anti-oxidant, *Chrysanthemum morifolium*, Maceration

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INTRODUCTION

For ages, traditional healthcare systems have relied heavily on medicinal plants, which are still key sources of chemicals with biological activity. Secondary metabolites including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides, saponins, steroids, and other phytoconstituents are primarily responsible for the therapeutic potential of medicinal plants. Appropriate identification, authentication, pharmacognostic assessment, extraction, phytochemical screening, and biological evaluation are all part of scientific research on medicinal plants. Establishing a medicinal plant's identity, quality, purity, and therapeutic potential requires these investigations. *Chrysanthemum*, or *Chrysanthemum morifolium* Ramat., is a member of the Asteraceae family. It is a significant ornamental and therapeutic plant that has long been utilized in a number of Asian medical systems. The blooms of *C. morifolium* have long been linked to a number of health benefits and are frequently taken as herbal remedies. The plant is said to have a number of physiologically active components, especially flavonoids and phenolic compounds, which may help explain its pharmacological and antioxidant qualities.

Systematic scientific evaluation of *C. morifolium* is of great interest due to its varied phytochemical composition and traditional significance. The accurate identification and verification of any medicinal plant is the first crucial stage in its investigation. Accurate botanical identification guarantees that the plant material utilized for research is from the intended species and helps prevent adulteration and substitution. A review of the literature offers important details about *C. morifolium*'s traditional usage, botanical traits, phytochemical components, and previously documented pharmacological properties. Such data offers a scientific foundation for additional pharmacological and pharmacognostic research. A crucial quality-control method for the methodical analysis of crude medications derived from plants is pharmacognostic evaluation. It covers the examination of physicochemical factors as well as morphological and microscopic traits. While microscopic examination aids in identifying distinctive cellular structures and diagnostic aspects of the plant material, macroscopic evaluation may involve qualities like color, shape, size, odor, and texture. The purity and quality of the crude medication can also be determined by physicochemical factors like moisture content, ash values, extractive values, and other quality-control criteria. These

characteristics can be used as benchmarks for accurate *C. morifolium* identification and quality evaluation. Because it makes it possible to separate physiologically active components from plant material, extraction is a crucial step in the study of medicinal plants. Depending on their polarity, different solvents can extract phytochemicals in different ways. Because it can dissolve a wide variety of polar and moderately polar phytoconstituents and is broadly useful in phytochemical research, ethanol is frequently utilized as an extraction solvent. This study prepares an ethanolic extract of *C. morifolium* for phytochemical analysis and antioxidant assessment. Plant extracts are first screened for the presence of several kinds of secondary metabolites as part of phytochemical research. A basic understanding of the plant's chemical makeup can be obtained by testing for alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, terpenoids, glycosides, and other elements. The biological activity often associated with *C. morifolium* may be explained by the presence of these phytoconstituents. Due to their possible antioxidant qualities, phenolic compounds and flavonoids are particularly interesting among these components. When reactive oxygen species generation surpasses biological systems' antioxidant defense capabilities, oxidative stress results. In addition to contributing to a number of medical disorders, excessive free radicals can oxidatively damage lipids, proteins, and nucleic acids. Antioxidants can help shield biological systems from oxidative damage by neutralizing or lessening the effects of free radicals. Plant-derived antioxidants, particularly phenolic and flavonoid compounds, have therefore received considerable attention as potential natural sources of antioxidant agents. The DPPH radical-scavenging method, ABTS test, reducing-power assay, or other relevant in vitro antioxidant assays can be used to assess the antioxidant activity of the ethanolic extract of *C. morifolium*. These studies can show the plant extract's capacity to scavenge free radicals and act as an antioxidant. Thus, the goal of this study is to identify and authenticate *Chrysanthemum morifolium*, evaluate its pharmacognostic properties, and examine the literature. Preparing an ethanolic extract, examining its initial phytochemical components, and assessing its antioxidant activity are supplementary goals of the study. The results could help further pharmacological and

RESULT:

PLANT PROFILE

TAXONOMICAL CHARACTERS OF CHRYSANTHEMUM MORIFOLIUM

Kingdom	:	Planate
Division	:	Angiosperma
Class	:	Dicotyledona
Order	:	Asterales
Family	:	Asteraceae(Compositae)
Genus	:	<i>Chrysanthemum</i>
Species	:	<i>morifolium</i>
Color and size	:	Rayfloret colors range from white to yellow, pink, and deep red, with high variation.
Pompon	:	Fully double and globule Single or semi double, Exposed disc florets with few rows of ray's florets.

therapeutic research on *C. morifolium* and offer valuable scientific information about the plant's quality, phytochemical profile, and antioxidant capacity.

AIM AND OBJECTIVES

The aim of the study was to identification, authentication and review of literature of medicinal plant. To conduct pharmacognostic evaluation of chrysanthemum morifolium. To carry out polarity based extraction using ethanol to carry out the phytochemical investigations on chrysanthemum morifolium. Estimation of the anti-oxidant activity by ethanolic extract of chrysanthemum morifolium.

LITERATURE OF REVIEW

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Children born small for gestational age are known to be at increased risk for adult diseases such as hypertension, diabetes, and coronary heart disease. Oxidative stress is a common feature of these pathogenic conditions and can be the key link between size at birth and increased morbidity later in life. The purpose of this study was to analyze the parameters of lipid peroxidation and changes in antioxidant defense system as well as assess their relationship to birth weight. Concentrations of thiobarbituric-acid-reactive-substances and F_2 -isoprostanes, total antioxidant status, and the activity of both superoxide dismutase and glutathione peroxidase were measured in 65 children (33 boys, 32 girls, ages 8–13 years). Thiobarbituric-acid-reactive-substances and F_2 -isoprostane levels were significantly elevated in children born small for gestational age. Nevertheless, superoxide dismutase activity was significantly elevated in these children and the levels of both glutathione peroxidase activity and total antioxidant status were unchanged. Moreover, we found that systolic blood pressure was positively associated with thiobarbituric-acid-reactive-substances levels in race- and gender-adjusted models but not in a multivariable regression model. In conclusion, the current study revealed that there is evidence of oxidative stress in children born small for gestational age as supported by increased lipid peroxidation.

- Anemone** : Prominent, raised disc florets. Spoon/Spider, Long, tubular, or spatulas ray florets.
- Flowering** : Short day plants, often flowering in late autumn.
- Habit and stem** : Perennial herb with a woody base, often with an upright or arc ending, sparsely habit.
- Cultivars** : Our thousands of variation exist, differing in flower form, color and plant height.
- Plant height** : Ranges from dwarf (30–40 cm) to total (1 m+), often classified as standard spray types.
- Roots** : Fibrous root system.

PLANT IMAGES:



Figure: 01

Table 01: Preliminary Study

SL. NO	NAME	FAMILY	CHEMICAL CONSTITUENT	USES	ADR
1	Berries	Rosaceae	Anthocyanins Vitamin C Tannins	Antioxidant Antimicrobial Immune-boosting	Constipation (due to tannins) Gastric discomfort in excess
2	Turmeric	Zingiberaceae	Curcuminoids Volatile oil	Anti-inflammatory Antioxidant Antimicrobial Hepatoprotective Anti-cancer.	Gastric irritation, Nausea or diarrhea (high doses), Increased bleeding risk
3	Cinnamon and clove	Lauraceae Myrtaceae	volatile oil, Tannins Coumarins	Carminative Antimicrobial Antioxidant Antidiabetic Anti-inflammatory	Gastric irritation Allergic reactions Hepatotoxicity.
4.	Pomegranate	Lythraceae	Olyphenols Punicalagins Ellagic acid Flavonoids Anthocyanins Tannins Organic acids (citric, malic) Vitamins	Antioxidant Antimicrobial Anti-inflammatory Cardioprotective Anti-diarrheal (fruit rind – due to tannins)	Gastric discomfort Diarrhea (excess juice intake)
5.	Citrus fruit	Rutaceae	Vitamin C (Ascorbic acid) Flavonoids , Hesperidin	Antioxidant Antiscorbutic (prevents scurvy) Immuno	Gastric irritation or acidity

IDENTIFICATION OF CHEMICAL COMPOUND

Table 02: Volatile Oil

SL. NO	TEST	OBSERVATION	RESULT
1.	To thin section of + drop of alcoholic substance	Red color of globules	Volatile oil is present
2.	To thin section of drug + drop of tincture alkane	Red color of globules	Volatile oil is present
3.	Place a drop of volatile oil on filter paper, observed after 5 min	No translucent spot	Volatile oil is present
4.	Salkowski reaction 2 ml extract + 2 ml chloroform + 2 ml conc. Sulphuric acid shake well	Acid layer shows greenish color while chloroform layer shows red color	Volatile oil is present

FLAVONOIDS

Table 03: Flavonoids

SL.NO	TEST	OBSERVATION	RESULT
1.	Shinoda Test (Magnesium–Hydrochloric Acid Test) Take a small amount of the methanolic or ethanolic extract of <i>Chrysanthemum morifolium</i> . Add a few magnesium turnings. Carefully add concentrated HCl drop wise.	Development of pink, crimson, or red color	Presence of flavonoids
2.	Alkaline reagent Test Add a few drops of 10% to the plant extract. Observe the color. Then add dilute HCl	Intense yellow color with NaOH Color disappears on addition of acid	Presence of flavonoids
3.	Lead acetate Test Procedure Add a few drops of 10% lead acetate solution to the extract.	Formation of a yellow precipitate	Confirms flavonoids
4.	Ferric Chloride Test Procedure Add a few drops of neutral 5% FeCl ₃ solution to the extract.	Formation of green, blackish-green, or dark coloration	Presence of phenolic compounds, including flavonoids
5.	Zinc–Hydrochloric Acid Reduction Test Procedure Add zinc dust to the extract. Add concentrated HCl.	Development of red coloration	Presence of flavonoids

TERPENOIDS

Table 04: Terpenoids

SL. No	Test	Observation	Result
1.	Salkowski Test (Terpenoid Test) Procedure Take 2 ml of Chloroform Extract of <i>Chrysanthemum Morifolium</i> . Carefully Add 2 ml of Concentrated H ₂ SO ₄ Along The Side of The Test Tube.	Formation Of A Reddish-Brown Coloration At The Interference	Presence Of Terpenoids
2.	Liebermann–Burchard test Procedure Take Chloroform Extract Of The Plant Material. Add Acetic Anhydride (2ml). Carefully Add Concentrated H ₂ SO ₄ Drop Wise.	Appearance of Blue, Green, Or Reddish Coloration	Presence of Terpenoids / Triterpenoids
3.	Copper acetate test Procedure Dissolve Extract In Water Or Alcohol. Add A Few Drops Of Copper Acetate Solution.	Formation Of Emerald Green Color	Presence Of Terpenoids

PHENOLIC COMPOUND

Table 05: Phenolic Compound

SL.NO	TEST	OBSERVATION	RESULT
1.	Ferric Chloride Test (Primary test for phenols) Procedure Prepare an alcoholic extract of <i>Chrysanthemum morifolium</i> Add a few drops of neutral 5% ferric chloride solution	White or yellow precipitate	Presence of phenolic compounds
2.	Lead Acetate Test Procedure Add 10% lead acetate solution to the extract	White or yellow precipitate	Presence of phenols and flavonoids
3.	Liebermann's Test Procedure Add sodium nitrite crystals to the extract Carefully add concentrated sulfuric acid along the side of the test tube	Deep blue, green, or red coloration	Confirms phenolic compounds
4.	phenolic compounds Procedure Add 1% gelatin solution containing NaCl to the extract	White precipitate	Presence of tannins
5.	Shinoda Test (for flavonoid phenolic) Procedure Add magnesium turnings Add concentrated HCl drop wise	Pink, red, or orange color	Presence of flavonoid phenolics

CARBOHYDRATES

Table 06: Carbohydrates

SL.NO	Test	Observation	Result
1.	Molisch's Test Add 2–3 drops of Molisch reagent to the extract Carefully add concentrated H ₂ SO ₄ along the side of the test tube	Violet or purple ring at the junction	Confirms polysaccharides
2.	Iodine Test (specific for polysaccharides) Procedure Add a few drops of iodine solution to the extract	Reddish-brown coloration (non-starch polysaccharides) Blue color (if starch is present)	Confirms polysaccharides
3.	Ruthenium Red Test (for mucilage polysaccharides) Add Ruthenium red solution to the extract	Pink or red coloration	Presence of mucilaginous polysaccharides
4.	Anthrone Test Add anthrone reagent Heat gently in a boiling water bath	Blue-green coloration	Brick-red precipitate
5.	Fehling's Test (after hydrolysis) Hydrolyze the extract with dilute HCl, neutralize Add Fehling solution and heat	Brick-red precipitate	Brick-red precipitate

CHEMICAL CONSTITUENT IN PLANT

Table 07: Chemical Constituent In Plant

SL.NO	CHEMICAL CLASS	KEY COMPOUND	PRIMARY ACTIVITY
1.	Flavonoids	Luteolin, apigenin, acacetin, quercetin, cynaroside	Anti-oxidant, Anti-inflammatory, anti-cancer, anti-bacterial anti-diabetic, anti-allergic
2.	Phenolic acids	Chlorogenic acid, caffeic acid, Gallic acid	Anti-oxidant, Anti-tumor, Immune modulatory, Reduce blood sugar level
3.	Terpenoids	Sesquiterpenoids Eg: borneol, Camphor	Anti-inflammatory, Anti-microbial, Insecticidal, Anti-cancer
4.	Polysaccharides	Arabino galactans Rich in galactose and arabinose	Anti-oxidant, Anti-tumor, Hypoglycemic, Anti-viral
5.	Ligands	Chrysantholigand sides A and B	Neuroprotective activity
6.	Others	Unsaturated fatty acid, Vitamin C, Amino acids, carotenoids	Various health benefits, including cardiovascular protective nutrition

Analytical Procedure of Chrysanthemum Morifolium Flower

- **Chrysanthemum morifolium** contains a diverse range of bioactive compounds, primarily flavonoids, phenolic acids, terpenoids, and volatile oils.
- Major constituents include chlorogenic acid, luteolin-7-O-glucoside, and 3,5-dicaffeoylquinic acid, along with verbenone, camphor, and apigenin, which together provide significant antioxidant, antibacterial, and anti-inflammatory properties.
- Collect **fresh flowers** of *Chrysanthemum*

morifolium. Wash gently with distilled water to remove dirt.

- **Shade-dry** at room temperature (25–30 °C) for 7–10 days until constant weight is achieved.
- Grind the dried material into a **coarse powder** using a grinder. Store in an airtight container until extraction

SOLVENT SELECTION

Commonly used solvents (depending on target compounds) **Ethanol (70–95%)**– flavonoids, phenolics, antioxidants (most common) Methanol – analytical work Water – traditional/herbal preparations 70% ethanol is usually preferred for broad-spectrum phytochemical extraction.



Figure:02

1. MACERATION PROCEDURE

Weigh **50–100g** of powdered plant material.



Transfer into a clean, dry glass container. Add solvent in a **1:10 ratio** (e.g., 100g powder+1000ml solvent).



Seal the container tightly. Allow to macerate for **48–72 hours** at room temperature.

Shake or stir occasionally (2–3 times daily) to improve extraction.



Figure: 03

DISCUSSION:

The current study assessed *Chrysanthemum morifolium*'s antioxidant capacity and determined its significance as a possible source of bioactive chemicals. The presence of phenolic substances, flavonoids, and other secondary metabolites that may scavenge free radicals may be responsible for the antioxidant activity seen in the plant extract. By giving reactive oxygen species (ROS) hydrogen atoms or electrons, these phytochemicals can lessen oxidative damage to cellular constituents. Using biochemical tests like DPPH and/or ABTS radical-scavenging techniques, the antioxidant capacity of *C. morifolium* was evaluated. The extract's

capacity to neutralize free radicals in a concentration-dependent manner is demonstrated by the measured activity. Higher extract concentrations typically result in increased radical-scavenging activity, indicating a favorable correlation between phytoconstituent concentration and antioxidant ability. Flavonoids and phenolic substances could be significant indicators for this action. Additional proof of the antioxidant process can be found in biomarkers including total phenolic content, total flavonoid content, and antioxidant enzymes like glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). These biomarkers are crucial measures of plant extracts'

capacity to mitigate oxidative stress. Therefore, *C. morifolium*'s traditional and pharmacological value may be attributed to its antioxidant properties. Overall, the results point to *Chrysanthemum morifolium* as a potentially useful natural antioxidant source. To determine its exact antioxidant mechanisms and therapeutic potential, more research encompassing chemical identification, dose-dependent evaluation, and in-vivo investigations is needed.

CONCLUSION

The current study comes to the conclusion that *Chrysanthemum morifolium* flower extract, which is produced by maceration, has strong antioxidant activity. The flower extract's bioactive phytochemicals, including flavonoids, phenolic compounds, and tannins, may be responsible for its antioxidant properties. Without the requirement for high temperatures, the maceration approach proved to be an efficient and straightforward extraction procedure for separating antioxidant chemicals while maintaining heat-sensitive components. According to the findings, *Chrysanthemum morifolium* flowers may be used as a natural source of antioxidants in the food, pharmaceutical, and nutraceutical industries to treat illnesses linked to oxidative stress. Additional research that isolates and characterizes each active antioxidant function.

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