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IDENTIFICATION OF PHYTOCONSTITUENTS IN THE LEAF EXTRACT OF *MARTYNIA ANNUA* USING VARIOUS PHYTOCHEMICAL METHODS

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ABSTRACT
The present study was conducted to determine the phytochemical constituents in the leaves of *Martynia annua*. Fresh, healthy leaves were collected in Jaipur, Rajasthan. The leaves were shade-dried, powdered, and extracted with distilled water by sonication at 40°C for 10 minutes. The aqueous extract was screened for different primary and secondary metabolites using standard qualitative tests. The results showed the presence of carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins. Alkaloids were not detected in the extract. The positive results were confirmed by characteristic color changes or precipitate formation in the respective tests. The presence of several classes of phytochemicals indicates that the leaves of *M. annua* contain a diverse range of bioactive constituents. These findings provide useful information on the phytochemical profile of M. annua leaves and offer a basis for further studies of their biological and medicinal potential.

KEYWORDS: *Martynia annua*, leaves, extraction, phytochemicals.

1. INTRODUCTION

Plants are rich sources of diverse bioactive constituents, including phenols, flavonoids, terpenoids, alkaloids, and saponins, which can contribute significantly to the biological and functional properties of plant-derived materials (Saraf et al., 2026). Phytochemical screening is a simple and useful method for detecting these compounds in plant extracts. It also provides basic information for further studies on the biological properties of plants.

Martynia annua L. belongs to the family Martyniaceae. It is an annual herb and is commonly found in India and other tropical regions. The plant is also known as Kakanasika and Devil's Claw. It is traditionally used for several purposes, and different parts of the plant have been used in traditional medicine (Saini et al., 2025; Patil et al., 2026).

Previous studies have reported several phytochemicals in *M. annua*. These include flavonoids, phenolic compounds, terpenoids, glycosides, tannins, and other secondary metabolites. Some individual compounds reported from the plant include luteolin, apigenin, chlorogenic acid, sinapic acid, and 4-hydroxybenzoic acid. Leaves have also been reported to contain a diverse range of phytoconstituents (Patil et al., 2026; Raju et al., 2023).

The phytochemical composition of *M. annua* may vary depending on the plant part and the extraction method. Recent studies have also reported different classes of phytochemicals in aqueous and other solvent extracts of the leaves. These findings support the need for further investigation of the leaf extract using simple and standard phytochemical tests (Sharma *et al.,* 2024).

The study of plant phytochemicals is also important because these compounds may contribute to different biological activities. Previous reviews have reported antioxidant, antibacterial, anti-inflammatory, wound-healing, and other activities for extracts or compounds obtained from *M. annua*. These reports provide a basis for further investigation of its chemical composition and biological potential (Malik & Katare, 2020; Patil et al., 2026).

Although *M. annua* has been studied for its medicinal and phytochemical properties, the composition of an extract can differ with geographical location, plant material, and extraction conditions. The present study was therefore carried out to investigate the primary and secondary metabolites present in the aqueous leaf extract of *M. annua* collected from Jaipur, Rajasthan. Qualitative tests were used to determine the presence of carbohydrates, proteins, flavonoids, alkaloids, terpenoids, glycosides, saponins, and phenols. The study provides basic information on the phytochemical profile of *M. annua* leaves and can serve as a basis for further biological and pharmacological investigations.

2. MATERIALS AND METHODS

2.1 Plant material collection

Fresh and healthy leaves of *Martynia annua* L. were collected from Delhi road, Amber, Jaipur, Rajasthan, India.

Freshly collected leaves were thoroughly washed under running tap water to remove dust and other adhering surface contaminants. The leaves were then soaked in sterile 0.9% (w/v) saline solution and repeatedly washed with deionized distilled water to remove residual impurities. The excess water was removed by gently blotting the leaves, followed by shade drying at room temperature for 8–10 days (Anuradha et al., 2015; Kamaraj et al., 2020). To assess moisture loss and determine the dry weight of the plant material, 5 g of fresh leaves were weighed and placed in a laboratory cabinet dryer set to 40–50°C. The samples were weighed at regular intervals until a constant weight was attained (Anuradha et al., 2015). The completely dried leaves were then pulverized using a laboratory grinder (Gupta et al., 2024) and passed through a 100-mesh sieve to obtain a finely powdered and homogeneous plant material (Suresh et al., 2015). The prepared powder was stored in airtight containers until further experimental use.

2.2 Preparation of aqueous leaf extract

Aqueous extract of *Martynia annua* was prepared using the previously dried and powdered leaves. Briefly, 10 g of leaf powder was accurately weighed and dispersed in 100 mL of distilled water. The resulting mixture was ultrasonicated at 40°C for 10 min to enhance the release and extraction of phytochemicals and other bioactive constituents into the aqueous phase (Shahzad et al., 2022). Following sonication, the extract was cooled to room temperature and filtered through Whatman No. 42 filter paper to eliminate insoluble plant debris (Ganaie et al., 2014). The clear filtrate obtained from the plant was collected separately and maintained at 4°C until further use. The extract was analyzed for the presence of phytochemical groups, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides, and other relevant secondary metabolites, using standard qualitative procedures.

2.3 Qualitative determination of primary and secondary metabolites

2.3.1 Carbohydrate test: Fehling's test

2 mL of Fehling's solution was added to 0.5 mg of extract, and the mixture was boiled in a water bath. The presence of red color indicated the presence of reducing sugars, while the appearance of blue color indicated their absence.

2.3.2 Protein test: Ninhydrin test

0.5 mg of extract was taken, 2 drops of freshly prepared 0.2% ninhydrin reagent were added, and the mixture was heated. The appearance of pink/ purple/yellow color indicated the presence of proteins, peptides, or amino acids.

2.3.3 Flavonoids- Lead acetate test

1 ml of extract was taken, and a few drops of 10% lead acetate solution were added. The appearance of a yellow-colored precipitate indicates the presence of a flavonoid.

2.3.4 Alkaloids: Mayer's test

Mayer's reagent is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) in water (100.0 ml). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution) to give a precipitate.

2.3.5 Terpenoid test: Salkowski test

1 mL of chloroform was added to 2.5 mL of plant extract. Then 1.5ml conc. H_2SO_4 was gently poured along the wall of the test tube to produce a layer. The presence of terpenoids was indicated by a reddish-brown coloring of the interface.

2.3.6 Glycosides: NaOH test

0.5 mg extract was dissolved in 1 ml of water, and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides

2.3.7 Saponins: Honeycomb test

0.5 mg of extract was taken in a test tube, and a few drops of 5% sodium bicarbonate solution were added. The mixture was vigorously shaken and left for 3 min. The formation of honeycomb-like froth indicates the presence of saponins.

2.3.8 Phenols: FeCl3 test

Phenol is a hydroxyl group (-OH) on an aromatic ring, or simply the hydroxy derivatives of aromatic compounds are known as phenols. Phenols are weaker acids than carboxylic acids. It undergoes a substitution reaction easily. Phenol is one of the most versatile and important industrial organic chemicals.

An aqueous solution of phenol reacts with a freshly prepared ferric chloride solution to give a colored complex. Most phenols give dark colored solutions.

The chemical reaction is given below.

$6C_6H_5OH + FeCl_3 \rightarrow Fe(OC_6H_5)_3$– (violet colour complex) + 3HCl

3. RESULTS

The aqueous leaf extract of *Martynia annua* was tested for eight different primary and secondary metabolites. The tests included carbohydrates, saponins, flavonoids, glycosides, alkaloids, terpenoids, phenols, and proteins. The results were based on the color change, precipitate formation, or foam formation observed during each test.

3.1 Carbohydrates

The presence of carbohydrates was tested using Fehling's test. The aqueous leaf extract was mixed with Fehling's solution and heated in a water bath. A red precipitate was observed after the reaction. The formation of this red

precipitate gave a positive result for reducing sugars. Thus, carbohydrates were detected in the aqueous leaf extract of *M. annua*. This result indicates that the leaves contain detectable levels of carbohydrate compounds.

3.2 Saponins

Saponins were tested using the honeycomb test. The extract was mixed with 5% sodium bicarbonate solution and shaken vigorously. Foam formation was observed after shaking and keeping the mixture for a few minutes. The formation of stable foam was considered a positive result for saponins. Therefore, saponins were detected in the aqueous leaf extract of *M. annua*.

3.3 Flavonoids

The presence of flavonoids was examined using the lead acetate test. After adding lead acetate solution to the plant extract, a transparent to pale yellow precipitate was observed. This observation was considered positive for flavonoids. The result confirms that flavonoid compounds were present in the aqueous leaf extract of *M. annua*.

3.4 Glycosides

Glycosides were tested using the NaOH test. The extract was treated with aqueous sodium hydroxide solution. A red color developed after the addition of the reagent. The appearance of this color was recorded as a positive reaction for glycosides. Thus, glycosides were detected in the leaf extract of *M. annua*.

3.5 Alkaloids

The presence of alkaloids was tested using Mayer's test. Mayer's reagent is commonly used to detect alkaloidal compounds by forming a precipitate. In the present study, no precipitate was observed after the addition of the reagent. The test was therefore recorded as negative. This indicates that alkaloids were not detected in the aqueous leaf extract under the conditions used in this study.

3.6 Terpenoids

Terpenoids were tested using the Salkowski test. The plant extract was mixed with chloroform, then carefully treated with concentrated sulphuric acid. A transparent yellow color was observed during the test. This observation was recorded as a positive result for terpenoids. Therefore, terpenoid compounds were detected in the aqueous leaf extract of *M. annua*.

3.7 Phenols

The presence of phenolic compounds was examined using the ferric chloride ($FeCl_3$) test. After adding ferric chloride to the extract, a yellowish-green color was observed. This color change was considered a positive reaction for phenolic compounds. The result indicates that phenolic compounds were present in the aqueous leaf extract of *M. annua*.

3.8 Proteins

Proteins were detected using the ninhydrin test. The plant extract was treated with freshly prepared ninhydrin reagent and heated. A creamish-yellow color was observed. This reaction was recorded as positive for proteins. Thus, protein-related compounds were detected in the aqueous leaf extract of *M. annua*.

Table 1: Qualitative phytochemical determination of leaf extract of *Martynia annua*

S.No.	Test Name	Observation	Result
1	Carbohydrate Test (Fehling's Test)	Red Ppt.	Positive
2	Saponin's Test	Foam formed	Positive
3	Flavanoid's Test (Lead Acetate Test)	Transparent/ Pale Yellow Ppt.	Positive
4	Glycoside Test	Red Colour	Positive
5	Alkaloid's Test (Dragondroff's Test)	No Ppt.	Negative
6	Terpenoid's Test	Transparent yellow Colour	Positive
7	Phenol Test by $FeCl_3$	Yellowish Green Color	Positive
8	Protein Test	Creamish Yellow Color	Positive

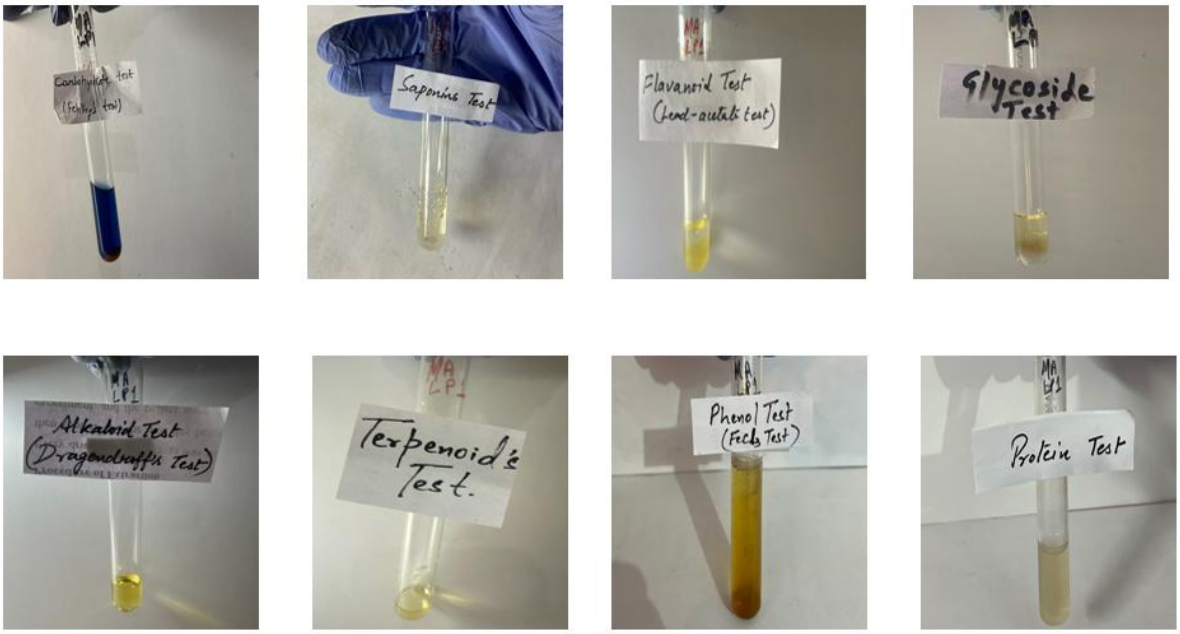


Figure 1: Qualitative phytochemical analysis of the leaf extract of *Martynia annua.*

4. DISCUSSION

The present study showed that the aqueous leaf extract of *Martynia annua* contains several important phytochemical groups. Carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins were detected, while alkaloids were not detected. Previous studies have also reported a diverse phytochemical composition in *M. annua*. Flavonoids, phenolic compounds, glycosides, saponins, terpenoids, and other secondary metabolites have been reported from different parts and extracts of the plant (Patil et al., 2026; Aakanksha et al., 2021).

The positive result for carbohydrates indicates the presence of carbohydrate compounds in the leaves. Carbohydrates are important primary metabolites in plants and are involved in energy storage and metabolism. A recent study on *M. annua* leaf extracts also reported the presence of carbohydrates during preliminary phytochemical screening (Aakanksha et al., 2021).

Saponins were detected by the formation of foam. Saponins are commonly reported secondary metabolites in medicinal plants. Previous phytochemical studies of *M. annua* have also reported saponins in leaf and other plant extracts (Patil et al., 2026; Aakanksha et al., 2021). Their presence supports the need for further investigation of the biological properties of the leaf extract.

The positive reaction for flavonoids indicates that flavonoid compounds were present in the aqueous leaf extract. Flavonoids are important plant metabolites and are widely studied for their antioxidant properties. Previous studies have reported the presence of flavonoids in M. annua and suggested that the plant contains several biologically active phytoconstituents (Saini et al., 2025; Patil et al., 2026). A recent study also found flavonoids in different *M. annua* leaf extracts and reported measurable total flavonoid content (Aakanksha et al., 2021).

Glycosides were also detected in the present study. The positive reaction indicates the presence of glycosidic compounds in the aqueous leaf extract. Glycosides have previously been reported from *M. annua* and are included among the phytochemical groups identified in different extracts of the plant (Patil et al., 2026; Aakanksha et al., 2021). The detection of glycosides adds to the chemical diversity of the leaf extract.

The terpenoid test was positive in the present study. Terpenoids are an important class of plant secondary metabolites. Previous reviews of *M. annua* have reported terpenoids as one of the major phytochemical groups present in the plant (Patil et al., 2026). Terpenoids have also been detected in recent phytochemical screening of *M. annua* leaf extracts (Aakanksha et al., 2021). This agreement supports the presence of terpenoid constituents in the leaves.

Phenolic compounds were detected by the ferric chloride test. Phenolic compounds are widely distributed plant metabolites and are frequently associated with antioxidant activity. Previous studies have reported the presence of phenolic compounds in *M. annua* and investigated their potential contribution to the plant's biological activity (Saini et al., 2025; Patil et al., 2026). Aakanksha et al. (2021) also reported measurable total phenolic content in *M. annua* leaf extracts.

The protein test gave a positive result in the present study. Proteins are essential plant biomolecules involved in growth, metabolism, and cellular functions. Proteins have also been identified among the phytochemical constituents detected in recent screenings of *M. annua* leaf extracts (Aakanksha et al., 2021). The positive protein reaction in the present aqueous extract is therefore consistent with previous screening results.

In contrast, alkaloids were not detected in the present aqueous leaf extract. This result may be related to the solvent and extraction conditions used in the study. Phytochemical composition can vary with the plant part, geographical

location, solvent, extraction method, and experimental conditions. This is particularly relevant for *M. annua* because previous studies have reported alkaloids in some extracts of the plant, and their presence can vary among extracts (Patil et al., 2026; Aakanksha et al., 2021). Thus, the negative result in the present study should be interpreted as alkaloids not detected under the conditions used, rather than as proof that alkaloids are completely absent from the leaves.

The phytochemical profile obtained in the present study is generally consistent with the reported chemical diversity of *M. annua*. Previous reviews have described flavonoids, phenolics, terpenoids, glycosides, saponins, and other phytoconstituents from different parts of the plant (Saini et al., 2025; Patil et al., 2026). Recent work on leaf extracts has also reported a broad range of phytochemicals and demonstrated antioxidant and antibacterial activities, particularly in ethanolic extracts (Aakanksha et al., 2021).

The differences between the present results and some earlier reports may be due to differences in the extraction solvent. In the present study, only an aqueous extract was used. Previous studies have used water, ethanol, methanol, petroleum ether, and other solvents. Different solvents extract different classes of compounds. Plant material, geographical origin, maturity, and extraction conditions can also affect the phytochemical profile (Patil et al., 2026; Malik & Katare, 2020).

The present findings provide a preliminary phytochemical profile of *M. annua* leaves collected from Delhi road, Amber, Jaipur, Rajasthan, India. The presence of several phytochemical groups supports further investigation using quantitative assays and chromatographic techniques. Further studies, such as total phenolic and flavonoid estimation, antioxidant assays, GC-MS, HPLC, and isolation of individual compounds, can provide more detailed information about the chemical composition of the leaves.

5. CONCLUSION

The present study showed that the aqueous leaf extract of *Martynia annua* contains a wide range of phytochemical constituents. Carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins were detected in the extract. Alkaloids were not detected under the conditions used in the present study. The presence of several important phytochemical groups indicates that *M. annua* leaves have a diverse chemical composition. These findings provide a preliminary basis for further studies of the plant's biological properties. Quantitative phytochemical analysis, antioxidant and antimicrobial assays, and compound identification using GC-MS or HPLC can provide further information about the active constituents of the leaves.

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Author contributions:

Arindam Saraf: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, and Writing – original draft.

Syed Burhan Geelani: Investigation, Data curation, Formal analysis, and Resources.

Prasanta Das: Supervision, Validation, and Writing – review and editing.

Anuradha Jayaraman: Supervision, Validation, and Writing – review and editing.

Conflict of interest

The authors declare that they have no conflict of interest concerning the research, authorship, or publication of this article.

Data Availability Statement

All data generated and analyzed during this study are included in this published article and its supplementary information.

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