

Qualitative Phytochemical Screening and In-vitro Antioxidant Evaluation of *Martynia Annua* Leaves Collected from Alwar District, Rajasthan, India

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Abstract

Medicinal plants are recognized as valuable reservoirs of structurally diverse bioactive compounds and continue to contribute significantly to drug discovery, traditional therapeutic systems, and modern pharmacological research. *Martynia annua* L. (family Martyniaceae), commonly known as “Devil’s Claw,” is widely distributed across tropical regions of India and has been traditionally employed for managing inflammatory disorders, microbial infections, and various skin-related conditions. Despite its ethnomedicinal relevance, comprehensive phytochemical and antioxidant evaluations of this species remain limited. The present study aimed to investigate the phytochemical composition and in-vitro antioxidant potential of the hydroalcoholic leaf extract of *M. annua* collected from the Alwar district of Rajasthan, India. Leaves were shade-dried, powdered, and extracted with a methanol–water solvent system (70:30, v/v). The crude extract was qualitatively screened using standard phytochemical assays, which confirmed the presence of alkaloids, flavonoids, phenols, tannins, saponins, and quinones, whereas glycosides were absent. Antioxidant efficacy was assessed using DPPH and ABTS radical-scavenging assays. The extract demonstrated 63.17% DPPH inhibition at a concentration of 1 mg/ml, with an IC₅₀ value of 0.79 mg/ml, indicating moderate antioxidant activity relative to ascorbic acid (IC₅₀ < 0.2 mg/ml). In the ABTS assay, the extract exhibited substantial radical-scavenging potential, achieving 76.89% inhibition within six minutes. These findings suggest that *M. annua* leaves possess noteworthy antioxidant constituents that may contribute to the plant’s traditional therapeutic applications. The study underscores the need for further quantitative, chromatographic, and *in-vivo* investigations to isolate specific bioactive molecules and elucidate their pharmacological properties.

Keywords: ABTS, Antioxidant, DPPH, *Martynia annua*, Phytochemicals.

Introduction:

Medicinal plants have long been recognized as invaluable reservoirs of bioactive compounds that form the cornerstone of traditional healthcare systems and continue to inspire modern pharmacological research

(Newman and Cragg, 2020; Atanasov et al., 2021; Meghwal et al., 2024). These plants produce a diverse range of secondary metabolites, such as phenolics, flavonoids, alkaloids, tannins, and terpenoids that exhibit antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties (Roy et al., 2022; Patel et al., 2024). Systematic screening of these phytoconstituents and assessment of their antioxidant potential are essential steps toward identifying species with pharmacological and nutraceutical relevance (Shi et al., 2022; Meghwal et al., 2025). *Martynia annua* L., an herbaceous medicinal plant belonging to the family Martyniaceae, is widely distributed across the tropical and subtropical regions of India, encompassing arid and semi-arid ecosystems (Kalaichelvi and Dhivya, 2016; Kaushik et al., 2021). This annual herbaceous plant is characterized by its sticky hairs and upright, branched stems. Its opposite leaves are broad, oval in shape, heart-shaped at the base, and possess slightly wavy or toothed edges. The fruits are woody and have two sharp hooks that curl back, holding the seeds inside (Sharma et al., 2024). Ethnomedicinally, *M. annua* has been traditionally used for the treatment of epilepsy, cervical tuberculosis, scabies, inflammation, and painful urination. Leaf juice is used as a gargle for sore throat, and its paste is applied for insect stings and wounds in domestic animals (Saini and Pareek, 2025). Such extensive ethnobotanical applications suggest the presence of pharmacologically active phytochemicals.

Despite widespread traditional use, limited studies have characterized the phytochemical composition and antioxidant potential of *M. annua* leaves from arid and semi-arid regions such as the Alwar district of Rajasthan. Since the synthesis and accumulation of secondary metabolites are strongly influenced by climatic, edaphic, and geographical factors, region-specific analyses are vital to understanding variation in phytochemical profiles and bioactivity (Pant et al., 2021; Rawat et al., 2025).

Therefore, the present study aims to perform qualitative phytochemical screening and *in-vitro* antioxidant evaluation of the hydroalcoholic leaf extract of *Martynia annua* collected from Alwar district, Rajasthan.

Materials and Methods

Chemicals

All chemicals and reagents used in the present investigation were of analytical grade to ensure the reliability and accuracy of experimental outcomes. These chemicals were sourced from reputed and certified suppliers in India, including HiMedia Laboratories (Mumbai), SRL Chemicals (Mumbai), and Merck India. The solvents used for extraction, such as methanol and distilled water, were of high purity and suitable for phytochemical and antioxidant assays.

Plant Material Collection

Leaves of *Martynia annua* L. were collected during the rainy season (July to August) from Duhar Chougn village, Alwar district, Rajasthan, India (27.4202292 N, 76.3082795 E) (Figure 1). The collected plant material was authenticated by experts at the Herbarium, Department of Botany, University of Rajasthan, Jaipur (RUBL No. 211763). The Leaves were thoroughly washed with distilled water to remove dust and impurities, then shade dried at ambient temperature for several days. The dried material was hand-crushed into a fine powder using a mortar and pestle and stored in airtight containers until extraction.

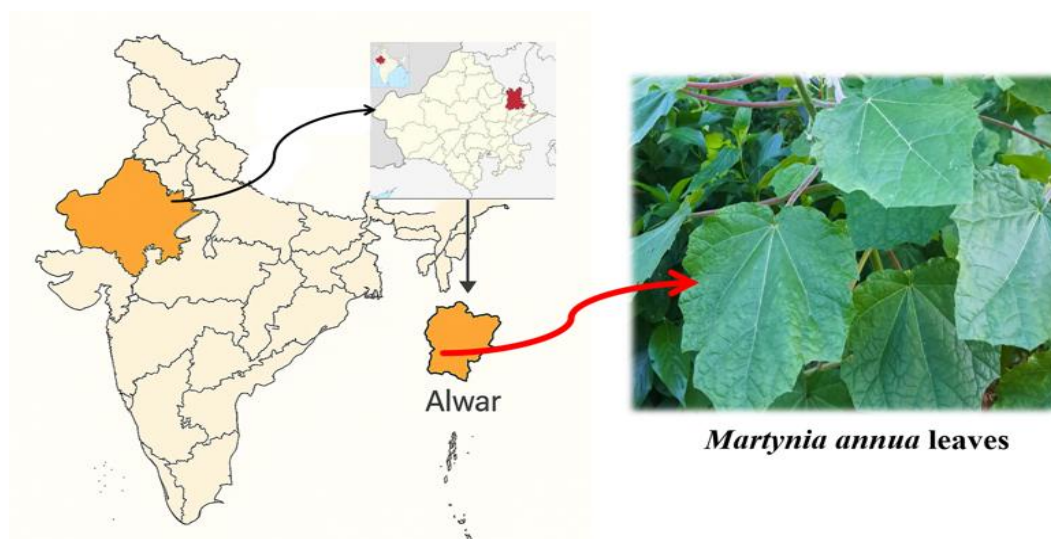


Figure:1. Collection site of *Martynia annua* L. leaves showing Alwar within Rajasthan, India, as the sampling location.

Preparation of Plant Extract

A total of 100 g of dried leaf powder was subjected to Soxhlet extraction using 1000 ml of a methanol–water solvent system (70:30, v/v). The extraction process was continued for 72 hours, or until the solvent in the siphon tube turned colourless, indicating exhaustive extraction of the phytoconstituents. The collected extract was filtered and concentrated to dryness in a hot air oven maintained at 30–40 °C to prevent degradation of thermolabile compounds. The dried crude extract was then weighed, transferred to airtight containers, and stored at 4°C until further analysis of its phytochemical composition and in-vitro antioxidant activity.

Phytochemical Analysis

Standard analytical techniques were employed to ascertain the presence of following phytochemical compounds in the leaves extract of *Martynia annua*.

Alkaloids: The Dragendorff's test qualitatively determines alkaloids. Two to three drops of Dragendorff's reagent (containing bismuth chloride and potassium iodide) were added to the extract and the formation of an orange-brown precipitate indicates alkaloid presence due to complex formation between alkaloid nitrogen and bismuth ions. Atropine was used as the positive control (Silva et al., 2017).

Flavonoids: The Shinoda test was used for preliminary detection of flavonoids. About 1–2 ml of plant extract was treated with magnesium ribbon and a few drops of concentrated HCl. The formation of pink to reddish orange colour indicated the presence of flavonoids, with quercetin used as the standard. This colour change results from the reduction of flavones, flavonols, and related compounds (Nanna et al., 2013).

Glycosides: The Keller–Killani test was used to test the presence of glycosides in the extract. 1.5 ml of glacial acetic acid, 2 drops of 5% FeCl₃, and concentrated H₂SO₄ were added sequentially to the extract. The emergence of a red or brown-red ring at the interface signifies the presence of cardiac glycosides. Digitoxin is used as the positive control for the study (Singh and Kumar, 2017).

Saponins: The foam test is a basic qualitative assay for identifying saponins. Approximately 0.5 mg of extract was mixed with 5 ml distilled water and shaken vigorously. Persistent foam formation lasting

several minutes indicates saponins, owing to their surfactant properties that reduce surface tension. Diosgenin was used as a positive control (Kashyap et al., 2023).

Quinones: To detect quinones, 10 mg of extract dissolved in isopropyl alcohol was treated with 2–3 drops of concentrated H₂SO₄. The appearance of a red, yellow, or orange coloration or a coloured ring at the interface confirms quinone presence. The reaction occurs as sulfuric acid dehydrates the compound, forming a coloured complex. 1,4-Benzoquinone was used as the standard (Maria et al., 2017).

Tannins: The Braymer's test was used to qualitatively identifies tannins in the extract. About 2 ml of extract was mixed with 10% alcoholic FeCl₃ solution. Formation of a blue or green colour indicates presence of tannins due to ferric-phenolate complex formation. Gallic acid was used as the reference compound (Uma et al., 2017).

Antioxidant Activity

DPPH Radical Scavenging Assay

The antioxidant potential of the extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described by Braca et al. (2001), with minor modifications. A reaction mixture containing 0.1 ml of plant extract and 3 ml of 0.1 mM DPPH solution prepared in methanol was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage inhibition of the DPPH radical was calculated according to the formula:

$$\% \text{ Inhibition} = 100 \times (A_b - A_a / A_b)$$

where *A_a* is the absorbance in the presence of the extract/sample and *A_b* is the absorbance of the control solution. The assay was performed at different concentrations, and inhibition percentages were plotted against concentration to determine the IC₅₀ values.

ABTS Radical Scavenging Activity

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay was carried out as per the method of Re et al. (1999), with slight adjustments. The ABTS radical cation (ABTS•⁺) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to incubate in the dark at room temperature for 12–16 hours. The resulting solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm. For the assay, 30 μL of the extract (1 mg/ml) was mixed with 3 ml of ABTS⁺ solution. The absorbance was recorded at 734 nm at 1-minute intervals up to 6 minutes. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100 \times (A_b - A_a / A_b)$$

where *A_a* is the absorbance in the presence of the extract/sample and *A_b* is the absorbance of the control solution.

Results

The qualitative phytochemical screening of *Martynia annua* leaf extract (Table 1) showed the presence of alkaloids, flavonoids, phenols, saponins, quinones, and tannins, whereas glycosides were found absent in the extract. These results indicate that the leaves contain diverse bioactive compounds that can be responsible for the plant's antioxidant and therapeutic potential.

Table: 1. Qualitative phytochemical screening of *Martynia annua* leaf extract

S.No.	Phytochemicals	Test Methods	Test Results
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1	Alkaloids	Dragendroff’s test	Present
2	Flavonoids	Shinoda’s test	Present
3	Glycosides	Keller-Killani test	Absent
4	Phenols	Ferric chloride test	Present
5	Saponins	Foam test	Present
6	Quinones	Sulphuric acid test	Present
7	Tannins	Ferric chloride test	Present

DPPH Radical Scavenging Activity

The antioxidant activity of the hydroalcoholic leaf extract of *Martynia annua* was evaluated using the DPPH assay, with ascorbic acid as the standard. Both samples showed a concentration-dependent increase in radical scavenging activity. The leaf extract exhibited moderate activity, with inhibition rising from 16.19% ± 0.87% at 0.2mg/ml to 63.17 ± 0.69% at 1mg/ml, compared to ascorbic acid which showed inhibition of 85.96 ± 0.005% at 0.2mg/ml and 96.86 ± 0.06% at 1mg/ml. The IC₅₀ value of the leaf extract was found to be 0.79 mg/ml indicated moderate antioxidant potential compared to the ascorbic acid (IC₅₀ < 0.2 mg/ml).

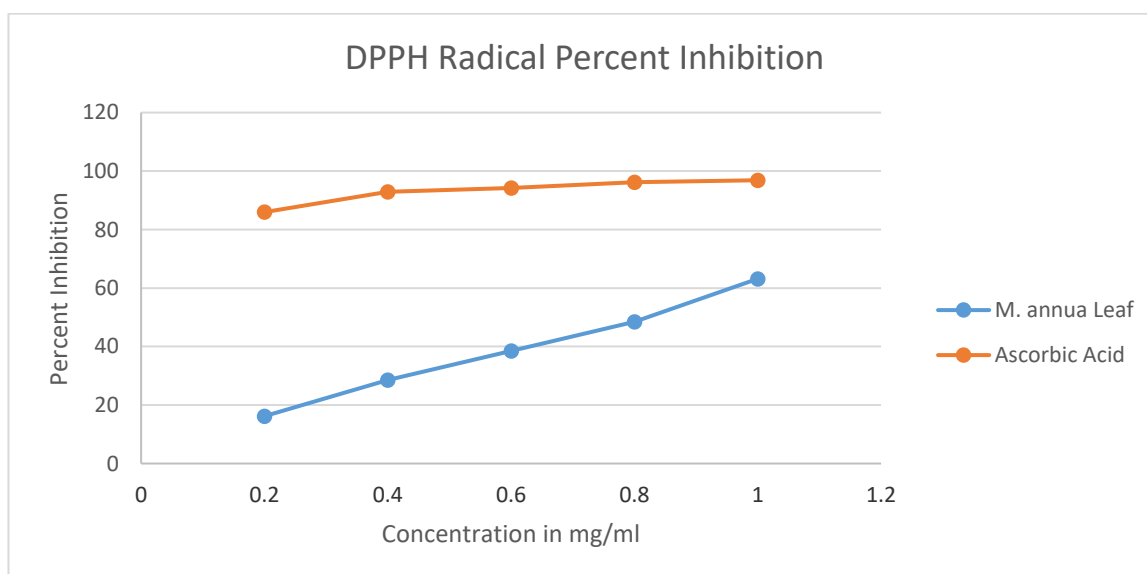


Figure 2. Radical scavenging activity expressed as % inhibition of DPPH by *M. annua* leaf extract at 1 mg/ml concentration compared with ascorbic acid.

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of the hydroalcoholic leaf extract of *Martynia annua* showed a gradual increase in antioxidant activity with time. Within 60 seconds, the extract exhibited 61.46% ± 0.12% radical scavenging activity (%RSA), indicating moderate antioxidant potential. This activity continued to rise steadily, reaching 76.89% ± 0.08% RSA after six minutes (360 sec), reflecting effective but slower radical neutralization compared to the ascorbic acid which displayed rapid and almost complete scavenging, with 98.00% ± 0.07% RSA at one minute and 98.18% ± 0.04% at six minutes, confirming its superior antioxidant efficiency.

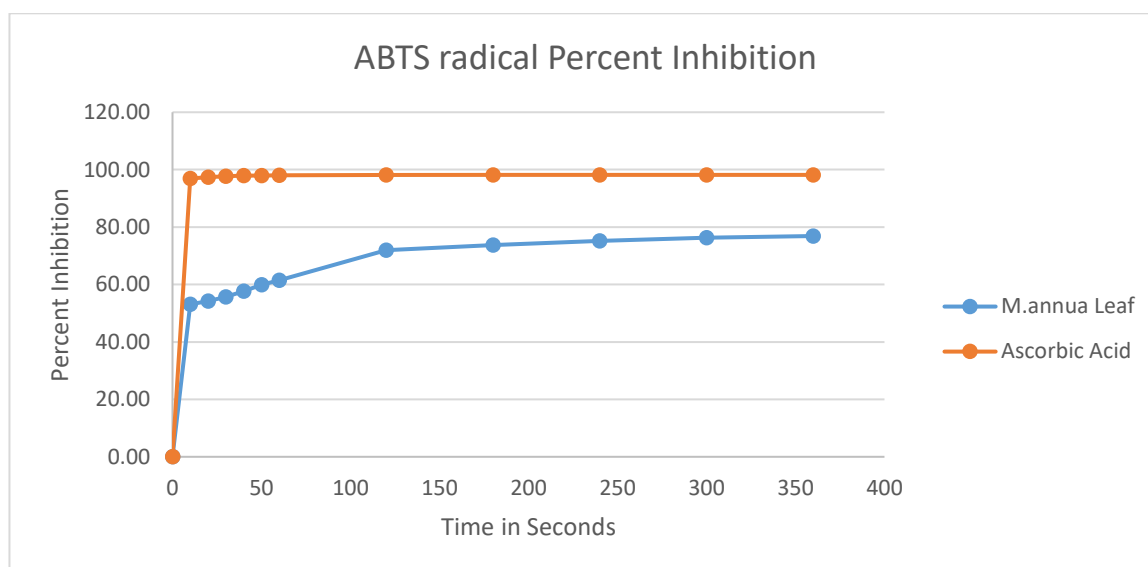


Figure 3. ABTS radical scavenging activity of *Martynia annua* leaf extract at 1mg/ml concentration compared with ascorbic acid over time. Values are expressed as percentage inhibition of ABTS radical.

Discussion

The present study demonstrated that the hydroalcoholic leaf extract of *Martynia annua* possesses notable phytochemical diversity and moderate antioxidant potential, supporting its traditional therapeutic uses. Qualitative screening confirmed the presence of alkaloids, flavonoids, phenols, saponins, quinones, and tannins in the hydroalcoholic leaf extract of *M. annua*. Whereas glycosides were found to be absent in the hydroalcoholic leaf extract. The findings of the present study align with recent comprehensive analyses of *M. annua* leaves and roots, which underscore its abundance of phenolic and flavonoid compounds linked to antioxidant and anti-inflammatory properties. (Kaushik et al., 2021; Palaramb et al., 2023; E-Habiba et al., 2025).

The antioxidant activity observed in the DPPH and ABTS assays supports the general trend reported for *Martynia annua*, which is recognized for moderate yet reliable radical-scavenging behaviour. This activity aligns with earlier findings that attribute the antioxidant potential of the species to its diverse array of secondary metabolites, particularly phenolics and flavonoids (Kalaichelvi and Dhivya, 2016; Kaushik et al., 2021). Numerous studies demonstrate that phenolic constituents contribute to antioxidant mechanisms by acting as reducing agents, hydrogen donors, and metal-ion chelators, while flavonoids neutralize free radicals through resonance stabilization and electron-transfer processes (Ayele et al., 2022; Shi et al., 2022; Khan et al., 2024). Additionally, the presence of tannins and quinones in many medicinal plants is known to enhance redox balance through synergistic interactions (Ouamnina et al., 2024). Taken together, the phytochemical composition of *M. annua* provides a strong mechanistic basis for its observed antioxidant behaviour and supports its inclusion among promising plant-based sources of natural antioxidants.

Environmental conditions prevalent in the semi-arid region such as Alwar, Rajasthan characterized by intense solar radiation, low humidity, and pronounced diurnal temperature fluctuations are known to influence plant metabolic pathways, particularly those involved in secondary metabolite synthesis (Qaderi et al., 2023). Under such abiotic stress, plants often enhance the production and accumulation of phenolic compounds as part of their adaptive defence response against oxidative damage. This physiological adjustment may help explain the moderate, yet consistent antioxidant activity observed in the present study.

Plants exposed to environmental stressors such as high temperature, low water availability, and intense sunlight typical of the collection region often activate defence mechanisms that lead to the enhanced synthesis of phenolics and other protective secondary metabolites. Such stress-induced biochemical responses could have contributed to the elevated levels of bioactive compounds detected in the *Martynia annua* leaf extract. Consequently, the environmental conditions of the region may have played a significant role in enriching the plant's antioxidant potential.

Conclusion

The study concludes that the hydroalcoholic leaf extract of *Martynia annua* contains a rich profile of secondary metabolites, including phenols, flavonoids, tannins, and other bioactive constituents, which collectively contribute to its biological activities. The extract exhibited moderate yet significant antioxidant potential, as demonstrated through DPPH and ABTS radical-scavenging assays. These results provide scientific validation for the plant's traditional medicinal applications and underscore its relevance as a natural source of antioxidant compounds. The findings further suggest that *M. annua* holds promising potential for development in nutraceutical, herbal, and pharmaceutical formulations, warranting more detailed phytochemical and in-vivo investigations.

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