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Evaluation of Antioxidant and Antibacterial Properties of Fruit and Flower Extracts of *Martynia annua* from Alwar District, Rajasthan, India

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ABSTRACT: Medicinal plants are a rich source of natural bioactive compounds with therapeutic applications in disease management and drug development. *Martynia annua* L. (Martyniaceae), commonly known as cat's claw, devil's claw, or Bichhuu, is an herbaceous annual plant widely used in traditional medicine and recognized for its pharmacological potential. This study aimed to comparatively evaluate the antioxidant and antibacterial activities of hydroalcoholic extracts from the fruit and flower parts of *M. annua* collected from Alwar District, Rajasthan, India. Plant materials were authenticated and extracted using a Soxhlet apparatus with a methanol–water (70:30 v/v) solvent system. Antioxidant potential was assessed through DPPH and ABTS radical scavenging assays, while antibacterial activity was determined using the disc diffusion method against *Corynebacterium xerosis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The fruit extract showed significant antioxidant activity in the DPPH assay, with 80.85% inhibition at 1 mg/ml and an IC_{50} of 0.53 mg/ml. The flower extract had weaker activity, with 47.16% inhibition at 1 mg/ml and an IC_{50} of 1.045 mg/ml. Whereas ascorbic acid showed the highest activity, with 96.82% inhibition and an IC_{50} of less than 0.2 mg/ml. The ABTS assay confirmed the fruit extract's higher activity, with 87.99% inhibition compared to the flower's 48.92% and ascorbic acid's 98.05%. Antibacterial testing revealed moderate, concentration dependent inhibition by both extracts, with the fruit extract showing zones up to 16.0 mm and the flower extract up to 16.33 mm, particularly against *P. aeruginosa* and *B. subtilis*. These results highlight the fruit extract of *M. annua* as a promising source of natural antioxidants and antibacterial agents, supporting its ethnomedicinal relevance and potential pharmaceutical applications.

KEYWORDS: Antibacterial; Antioxidant; *Martynia annua*; Medicinal plants.

I. INTRODUCTION

Medicinal plants have been an indispensable part of human healthcare for centuries, serving as a crucial source of bioactive compounds that help in the prevention and treatment of various diseases. Different plant parts, including fruits, flowers, leaves, stems, seeds, and roots, are rich reservoirs of secondary metabolites such as phenolics, flavonoids, alkaloids, and tannins. These compounds are responsible for a wide range of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Kaushik et al., 2021). Traditional medicine systems across the globe, from Ayurveda and Unani to Chinese and African medicinal practices, continue to utilize plant-based therapies to meet primary healthcare needs, especially in rural and tribal regions. These remedies are not only culturally accepted but also represent an untapped repository of pharmacologically active molecules, many of which have been scientifically validated and adapted into modern therapeutic formulations (Rizvi et al., 2022; Chaachouay et al., 2024).

One such promising plant species is *Martynia annua* L., belonging to the family Martyniaceae. Commonly referred to as cat's claw, devil's claw, or bichhuu, *M. annua* is an herbaceous annual plant native to tropical America. Over time, it has naturalized in various parts of Asia, including India, where it is found in semi-arid and arid regions (Sharma et al., 2024). Ethnomedicinal surveys from different parts of the country have documented its extensive therapeutic applications. In the tribal pockets of the Satpura Plateau in Madhya Pradesh, the root paste of *M. annua* is traditionally

applied to manage cancer and rheumatism (Gupta et al., 2018). The leaves are known for antiseptic and antiepileptic properties and are applied to tubercular swellings, whereas the fruits are recognized for their antioxidant, anti-inflammatory, and sedative effects. The seeds and seed oil are widely used for treating skin infections, abscesses, and even hair disorders (Duraismy et al., 2022). More recently, attention has turned to the flowers of *M. annua*, which have been shown to contain important secondary metabolites with therapeutic potential, further broadening the plant's medicinal relevance (Kumar et al., 2025).

One of the critical mechanisms underlying many chronic diseases is oxidative stress, which arises from an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defense systems. Excessive ROS and free radicals can damage lipids, proteins, and DNA, thereby contributing to the onset and progression of conditions such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders like Alzheimer's and Parkinson's disease (Sharifi et al., 2020; Leyane et al., 2022). Antioxidants play a vital role in neutralizing these reactive species and preventing cellular damage. Among natural sources of antioxidants, plant derived compounds such as phenolic acids, flavonoids, vitamins, carotenoids, and anthocyanins are particularly valued due to their effectiveness and safety profiles. The evaluation of antioxidant potential through in vitro assays is a common practice, with methods such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay being widely used to measure free radical scavenging capacity (Thangapushbam et al., 2024).

In addition to oxidative stress, bacterial infections remain a significant global health challenge, especially with the increasing prevalence of antimicrobial resistance. Conventional antibiotics are becoming less effective against pathogenic bacteria, urging the search for alternative therapeutic agents. In this context, plant-based extracts have emerged as promising candidates due to their diverse array of bioactive secondary metabolites. These natural products often exhibit broad spectrum antibacterial properties, targeting a variety of pathogens with different mechanisms of action. Recent studies on *M. annua* have confirmed its antibacterial potential, particularly in extracts derived from leaves and flowers (Duraismy et al., 2022; Kumar et al., 2025), suggesting that this species could serve as a source of novel antibacterial agents.

Despite the growing body of research on *M. annua*, most studies have been conducted in regions with distinct ecological conditions, such as tropical or subtropical climates. Arid and semi-arid areas, like Rajasthan, pose unique environmental challenges, including high temperatures, low rainfall, and poor soil quality, which can significantly influence the phytochemical composition and biological activity of plants. These stress factors often induce the production of secondary metabolites as part of the plant's adaptive response, potentially enhancing their therapeutic properties. However, there is limited scientific evidence on the antioxidant and antibacterial activities of *M. annua* from such regions, and comprehensive studies are lacking.

Therefore, the present study focuses on evaluating the antioxidant and antibacterial activities of methanolic extracts prepared from the fruits and flowers of *Martynia annua* collected from Alwar District, Rajasthan. The study aims to fill the knowledge gap by investigating how environmental stress factors in arid regions may affect the bioactivity of the plant's extracts. Antioxidant activity was assessed using DPPH and ABTS assays, while antibacterial efficacy was tested through the disc diffusion method against selected bacterial strains, including *Corynebacterium xerosis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Standard compounds, ascorbic acid and streptomycin, were used as references to validate the results.

By providing scientific data from an arid region, this research contributes to understanding the therapeutic potential of *M. annua* in diverse ecological settings and supports its ethnomedicinal applications. The findings are expected to aid in the development of natural antioxidant and antibacterial formulations that could serve as complementary or alternative remedies in regions where conventional healthcare resources are limited.

II. MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

Fruits 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. The fruits were thoroughly washed with distilled water to remove dust and impurities, then shade dried at ambient temperature for several days. The dried material was finely powdered using a mechanical grinder and stored in airtight containers until extraction.

For extraction, 100 g of the fruit powder was subjected to Soxhlet extraction using 1000 ml of methanol–water (70:30 v/v) as the solvent system. The extraction process was continued for 72 hours or until the solvent in the siphon tube became colorless, indicating exhaustive extraction. The obtained extract was concentrated using a hot air oven at 30–40 °C until dryness and subsequently stored at 4 °C in sealed containers for further evaluation of antioxidant and antimicrobial activities.



Figure 1. Flowers and fruits of *Martynia annua* collected from the Alwar district of Rajasthan, India.

Antioxidant Activity

DPPH Radical Scavenging Assay

The antioxidant potential of the extracts 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_b is the absorbance of the control (without extract), and A_a is the absorbance of the test sample. The assay was performed at different concentrations, and inhibition percentages were plotted against concentration to determine the IC_{50} values.

ABTS Radical Scavenging Assay

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 ($ABTS^{\bullet+}$) 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 μ L of the extract (1 mg/ml) was mixed with 3 ml of $ABTS^{\bullet+}$ 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_b is the absorbance of the control solution, and A_a is the absorbance in the presence of the extract.

Antimicrobial Activity

The antibacterial activity of fruit and flower extracts of *M. annua* was evaluated using the standard agar disc diffusion method (Gould and Bowie, 1952). Sterile Whatman filter paper discs (6 mm diameter) were oven sterilized at 140 °C for one hour before being impregnated with the test extracts. The discs were air dried at room temperature to remove any residual solvent. Nutrient agar plates were uniformly prepared with a consistent thickness and inoculated with bacterial cultures: *Corynebacterium xerosis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The extract impregnated discs were carefully placed on the inoculated agar surface. A standard antibiotic disc containing Streptomycin served as the positive control on each plate. The plates were incubated at 37 °C for 20–24 hours. After incubation, the zones of inhibition around each disc, including the 6 mm diameter of the disc, were measured using a ruler. Each experiment was performed in triplicate, and the mean values were recorded. The antibacterial activity was further quantified by calculating the Activity Index (A.I.) using the following formula:

$$A.I. = \frac{\text{Inhibition Zone of Sample (mm)}}{\text{Inhibition Zone of Standard (mm)}}$$

This allowed for a comparative evaluation of the extracts relative to the reference antibiotic.

III. RESULTS

DPPH Radical Scavenging Activity

The antioxidant potential of *Martynia annua* fruit and flower extracts was evaluated using the DPPH radical scavenging assay. The results demonstrated that the fruit extract exhibited considerable free radical scavenging activity across the tested concentration range (Figure 2). Specifically, the % inhibition values ranged from 27.12% at 0.2 mg/ml to 80.85% at 1.0 mg/ml, indicating a concentration dependent increase in antioxidant capacity (Figure 3). The IC_{50} value, calculated through linear interpolation, was determined to be 0.53 mg/ml, which reflects a moderate antioxidant effect. In contrast, the flower extract displayed comparatively weaker activity, with % inhibition values ranging from 17.79% at 0.2 mg/ml to 47.16% at 1.0 mg/ml and IC_{50} value was found to be 1.045 mg/ml, suggesting a substantially lower antioxidant potential than the fruit extract (Figure 4). For comparison, ascorbic acid was used as a standard antioxidant and demonstrated superior radical scavenging activity % inhibition values ranged from 85.78% at 0.2 mg/ml to 96.82% at 1.0 mg/ml, with an IC_{50} value below 0.2 mg/ml (Figure 5).

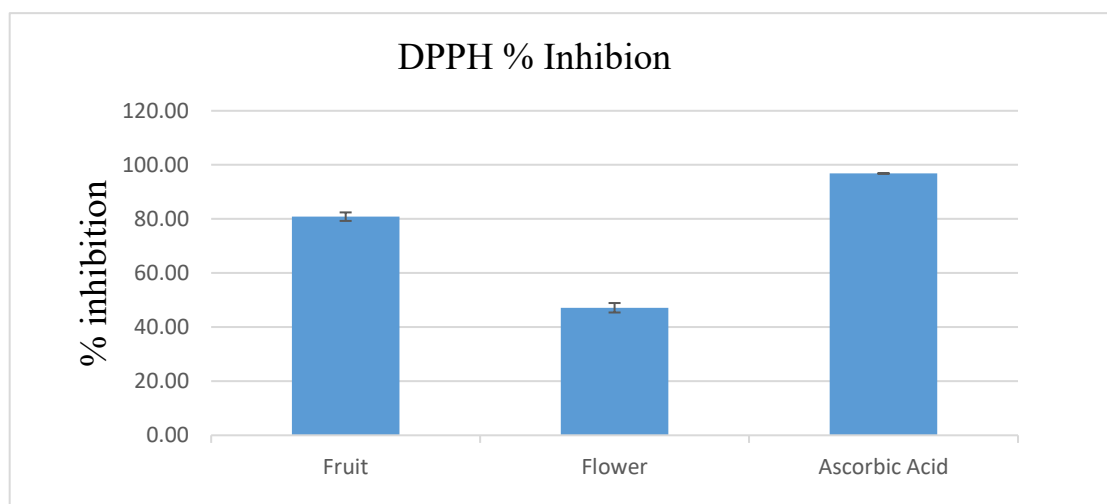


Figure 2. DPPH % inhibition of *Martynia annua* fruit and flower extracts compared with ascorbic acid

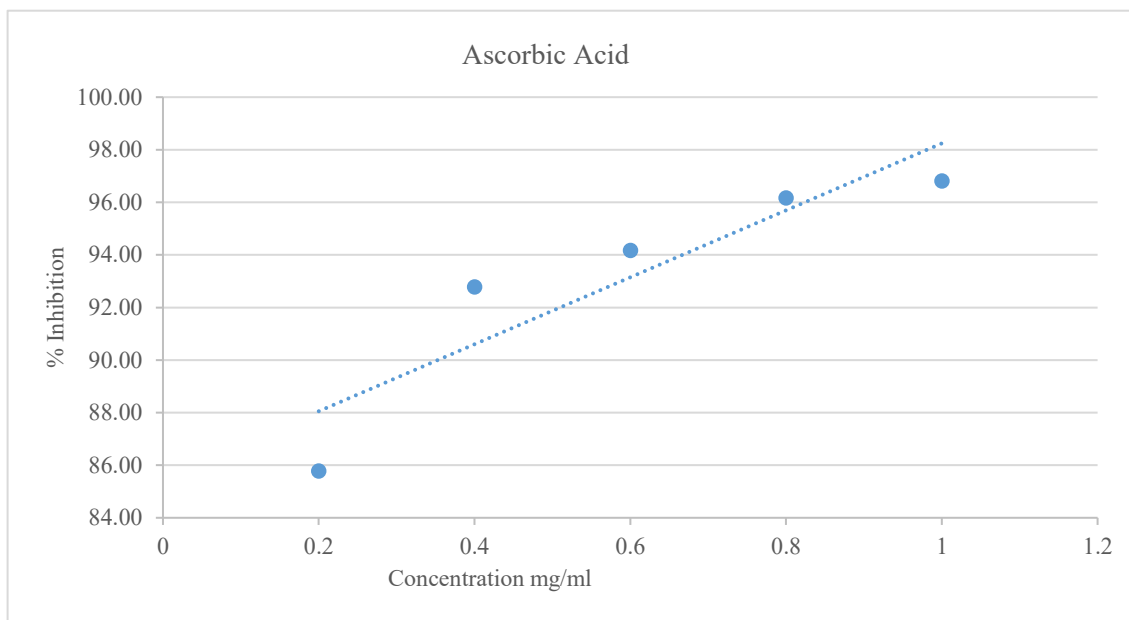


Figure 3. Linear regression analysis of DPPH radical scavenging activity (%RSA) of Martynia annua fruit extract at different concentrations (mg/ml), showing IC₅₀ estimation

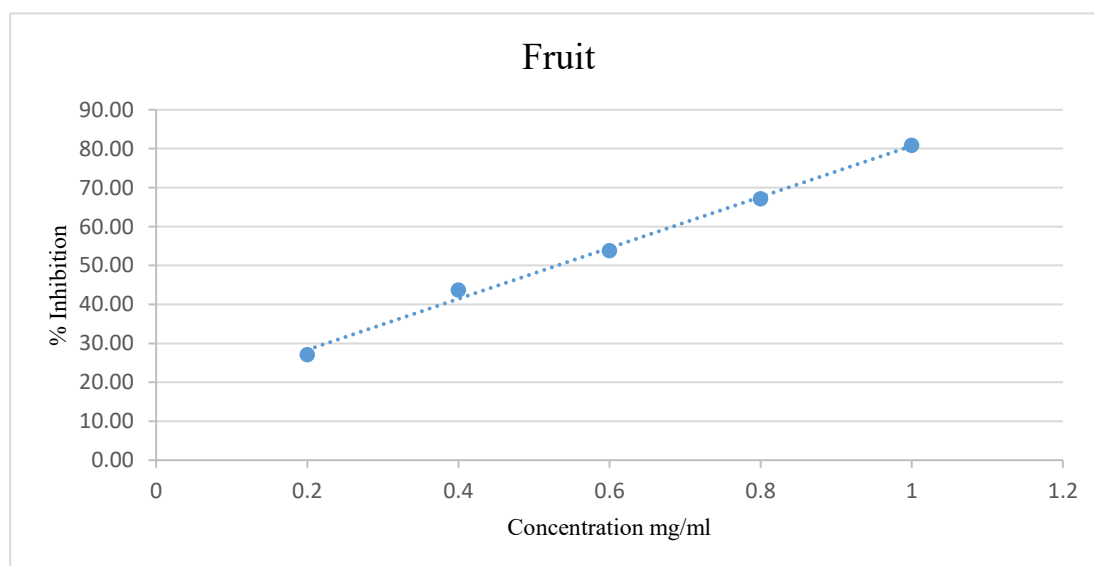


Figure 4. Linear regression analysis of DPPH radical scavenging activity (%RSA) of Martynia annua flower extract at different concentrations (mg/ml), showing IC₅₀ estimation.

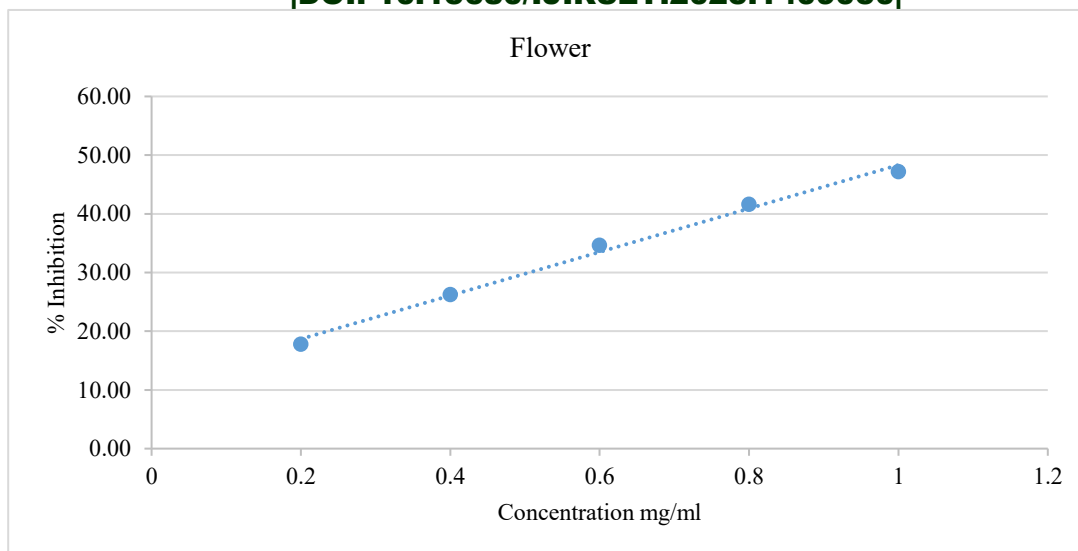


Figure 5. Linear regression analysis of DPPH radical scavenging activity (%RSA) of ascorbic acid at different concentrations (mg/ml), used as the standard for IC₅₀ comparison.

ABTS Radical Scavenging Activity

The ABTS assay further substantiated the differences in antioxidant capacities between the fruit and flower extracts. Ascorbic acid, serving as the positive control, exhibited nearly complete radical scavenging activity, achieving 96.96% inhibition after 1 minute and 98.05% after 6 minutes at a concentration of 1.0 mg/ml. This validated its role as a benchmark for antioxidant activity. Among the plant extracts, the fruit extract demonstrated substantially greater activity than the flower extract. At 1.0 mg/ml, the fruit extract achieved 80.92% inhibition after 1 minute, which further increased to 87.99% after 6 minutes. These results indicate that the fruit extract approaches the antioxidant efficiency of ascorbic acid at higher concentrations. On the other hand, the flower extract showed significantly lower activity at 1.0 mg/ml, the inhibition percentage was only 32.60% after 1 minute and 48.92% after 6 minutes further validating its limited antioxidant effect in comparison with the fruit extract. Overall, the ABTS assay results confirmed that the fruit extract of *M. annua* possesses a considerably higher antioxidant capacity than the flower extract, identifying it as a more promising source of natural antioxidants (Figure 6).

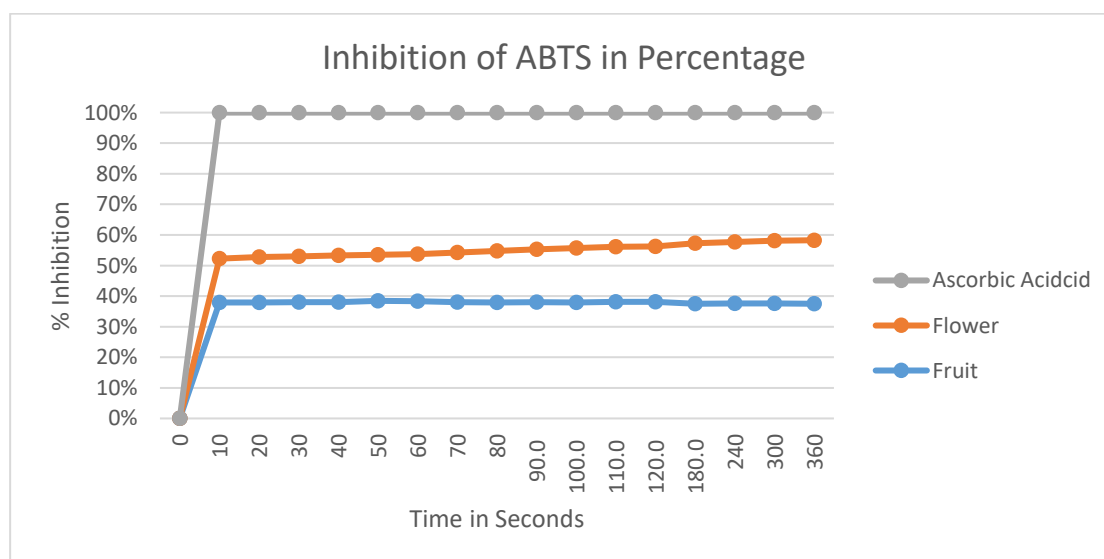


Figure 6. ABTS % inhibition of *Martynia annua* fruit and flower extracts compared with ascorbic acid over time

Antimicrobial Activity

The antibacterial efficacy of *Martynia annua* fruit and flower extracts was evaluated against four bacterial strains *Corynebacterium xerosis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* using the disc diffusion method, with Streptomycin as the standard antibiotic control. The standard drug consistently exhibited strong inhibition zones ranging from 26.0 mm to 29.66 mm, confirming the sensitivity of the bacterial strains used in the study (Figure 7). Both fruit and flower extracts displayed moderate antibacterial activity, which increased with higher concentrations of the extracts. The fruit extract exhibited inhibition zones of 9.66 mm at 500 µg/disc and 14.33 mm at 1000 µg/disc against *C. xerosis*. Against *Proteus mirabilis*, the zones of inhibition ranged from 7.0 mm to 15.66 mm at the corresponding concentrations. Notably, stronger antibacterial effects were observed against *Pseudomonas aeruginosa* and *Bacillus subtilis*, where inhibition zones varied between 10.66–15.0 mm and 10.66–16.0 mm, respectively (Table 1). These findings suggest that the fruit extract is more effective against Gram negative and Gram-positive strains at higher doses.

The flower extract also demonstrated increasing antibacterial activity with concentration. Against *C. xerosis*, inhibition zones ranged from 9.33 mm at 500 µg/disc to 14.0 mm at 1000 µg/disc, while *P. mirabilis* exhibited zones from 10.66 mm to 14.66 mm. The flower extract showed greater activity against *P. aeruginosa* (11.0–15.33 mm) and *B. subtilis* (9.33–16.33 mm), indicating the relatively higher susceptibility of these strains to the extract (Table 2). Despite showing moderate antibacterial potential, both fruit and flower extracts were less potent than the standard antibiotic. Nevertheless, the results reveal that the extracts, particularly those from the fruit and flower of *M. annua*, possess appreciable inhibitory effects against a range of bacterial strains and may serve as potential sources of bioactive compounds for further therapeutic exploration (Figure 8).

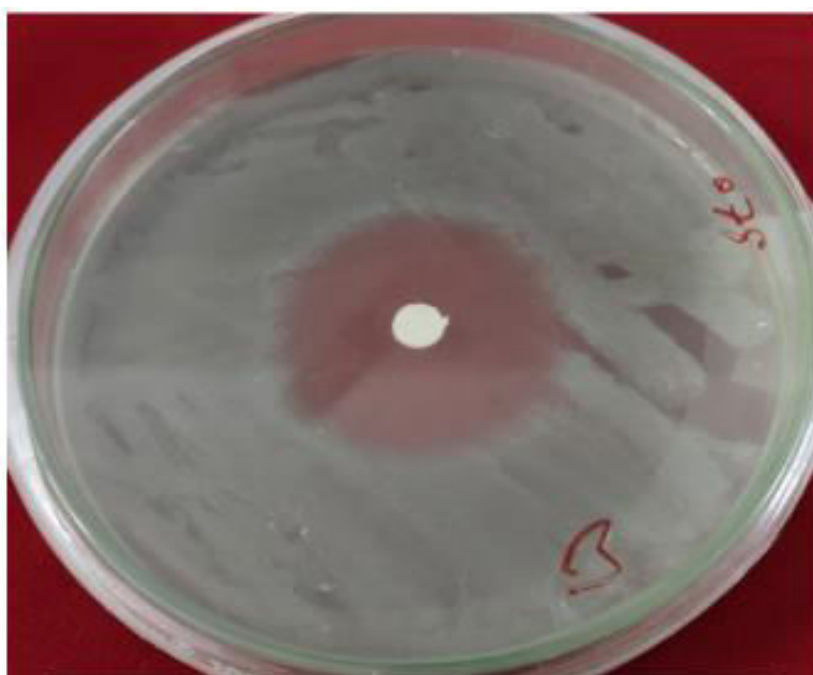


Figure 7. Antibacterial activity of standard streptomycin showing zone of inhibition by disc diffusion method.

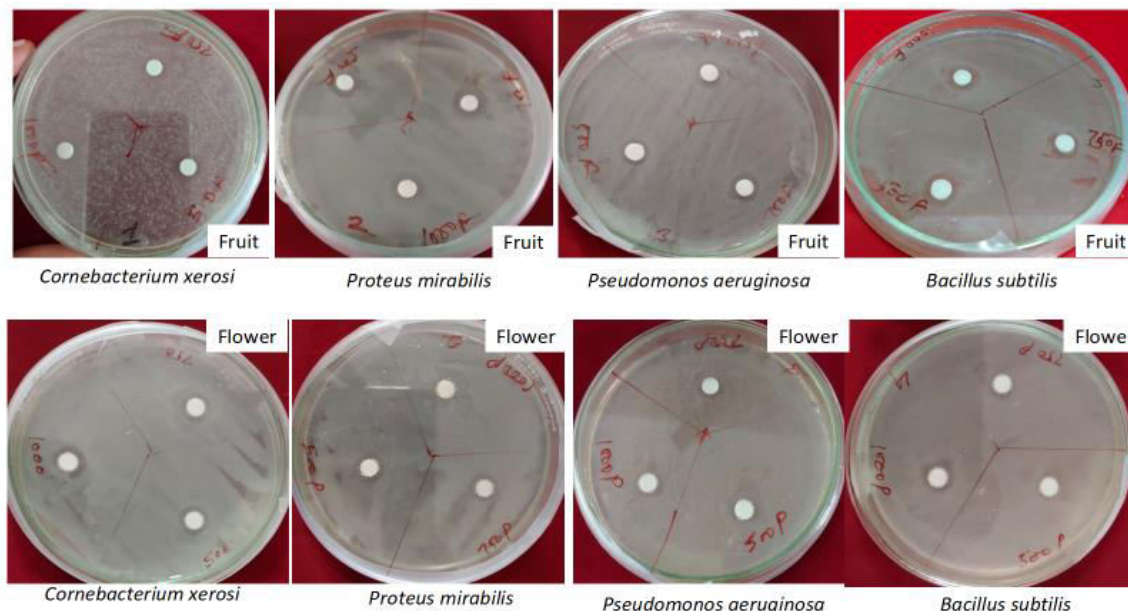


Figure 8. Antibacterial activity of Martynia annua fruit and flower extracts against Corynebacterium xerosis, Proteus mirabilis, Pseudomonas aeruginosa, and Bacillus subtilis by disc diffusion method.

Table 1: Antibacterial activity of the fruit extract of M. annua by disc diffusion method

Test Bacteria	Inhibition zone (mm) of Standard (Streptomycin)	Fruit extract of M. annua					
		500mg/ml		750mg/ml		1000mg/ml	
		I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.
Cornebacterium xerosis	27.66	9.66	0.34	13.66	0.49	14.33	0.51
Proteus mirabilis	29.66	7.0	0.25	14.0	0.47	15.66	0.52
Pseudomonos aeruginosa	26	10.66	0.41	13.33	0.51	15.0	0.57
Bacillus subtilis	29	10.66	0.36	13.0	0.44	16.0	0.55

Table 2: Antibacterial activity of the flower extract of M. annua by disc diffusion method

Test Bacteria	Inhibition zone (mm) of Standard (Streptomycin)	Fruit extract of M. annua					
		500mg/ml		750mg/ml		1000mg/ml	
		I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.
Cornebacterium xerosis	27.66	9.33	0.34	13	0.46	14	0.50
Proteus mirabilis	29.66	10.66	0.35	13	0.43	14.66	0.49
Pseudomonos aeruginosa	26	11	0.42	13	0.50	15.33	0.58
Bacillus subtilis	29	9.33	0.32	14	0.48	16.66	0.56

IV. DISCUSSION

The present study systematically evaluated and compared the antioxidant and antibacterial properties of fruit and flower extracts of Martynia annua collected from the arid region of Alwar District, Rajasthan, India. The findings demonstrate significant variation in the bioactivity between the two plant parts, highlighting the importance of plant part specificity in pharmacological assessments and reinforcing the ethnomedicinal relevance of M. annua.

The antioxidant potential of the fruit extract was markedly higher than that of the flower extract in both the DPPH and ABTS assays. The fruit extract exhibited a concentration dependent radical scavenging capacity, in the DPPH assay. Similarly, in the ABTS assay, the fruit extract achieved up to 87.99% inhibition at 1.0 mg/ml, with IC₅₀ values of 0.48 mg/ml (1 min) and 0.19 mg/ml (6 min), signifying substantial radical scavenging efficiency. These results are in alignment with prior studies, such as those by Santram et al. (2011), which reported that *M. annua* fruits are rich in phenolic acids and flavonoids bioactive compounds that are widely acknowledged for their antioxidant mechanisms, including free radical neutralization and prevention of lipid peroxidation.

In contrast, the flower extract displayed relatively weaker activity, with % inhibition values ranging from 18.03% to 47.21% in the DPPH assay and failing to reach 50% inhibition, resulting in an IC₅₀ value greater than 1.045 mg/ml. Likewise, in the ABTS assay, inhibition values ranged between 32.60% and 48.92%, with an estimated IC₅₀ exceeding 1.0 mg/ml. These results suggest that the flower extract possesses a lower concentration of antioxidant metabolites, such as phenolics and flavonoids, which may limit its free radical scavenging potential. Similar outcomes have been observed in studies by Palaramb et al. (2023), where flower and leaf extracts demonstrated lower antioxidant activity relative to fruit extracts, underscoring the variability of phytochemical content across plant organs.

The superior antioxidant potential of the fruit extract positions it as a promising candidate for further exploration as a natural source of antioxidants, potentially useful in combating oxidative stress related disorders such as cancer, cardiovascular diseases, and neurodegeneration. Nevertheless, the comparatively modest activity observed in the flower extract highlights the need for targeted investigations into its phytochemical profile and possible synergistic interactions among its constituents.

The antibacterial assessment revealed that both fruit and flower extracts possess moderate inhibitory effects against *Corynebacterium xerosis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, with activity increasing at higher concentrations. While neither extract matched the efficacy of the standard antibiotic Streptomycin—which consistently produced inhibition zones between 26.0 mm and 29.66 mm—they nonetheless exhibited appreciable antibacterial potential, particularly against *P. aeruginosa* and *B. subtilis*. The fruit extract demonstrated inhibition zones of up to 16.0 mm at 1000 µg/disc against *B. subtilis*, whereas the flower extract showed zones reaching 16.33 mm, indicating comparable susceptibility in some bacterial strains. The enhanced activity against Gram negative *P. aeruginosa* and Gram-positive *B. subtilis* may be attributed to mechanisms such as cell wall disruption, enzyme inhibition, or interference with metabolic pathways—mechanisms previously suggested in studies by Santram et al. (2011). Recent reports further corroborate these findings. Duraisamy et al. (2022) observed significant antibacterial activity of ethanol leaf extracts and chitosan-based formulations of *M. annua*, including against *P. aeruginosa*. Similarly, Kumar et al. (2025) identified tannins, phenolics, and saponins in flower extracts exhibiting measurable antibacterial effects against *Escherichia coli*, reinforcing the broad-spectrum potential of this plant species.

Despite the moderate activity demonstrated, these results underscore the therapeutic promise of *M. annua* as an alternative or complementary antimicrobial agent, particularly considering the escalating issue of antibiotic resistance. The variation in antibacterial efficacy between the fruit and flower extracts likely reflects differences in secondary metabolite composition, further emphasizing the importance of detailed phytochemical analyses. The findings of this study substantiate the traditional use of *M. annua* in ethnomedicine and highlight its pharmacological potential as a source of natural antioxidants and antimicrobial agents. The superior activity of the fruit extract aligns with historical and contemporary reports and suggests its utility in developing plant based therapeutic formulations aimed at mitigating oxidative stress and bacterial infections.

However, this study is limited to in vitro evaluations, which, while informative, do not fully reflect the complex interactions in biological systems. Therefore, future investigations should focus on in vivo studies to assess bioavailability, metabolic pathways, and toxicity profiles. Additionally, comprehensive phytochemical profiling using advanced techniques such as HPLC, GC-MS, or LC-MS would further elucidate the specific constituents responsible for the observed activities.

This study compared the antioxidant and antibacterial properties of fruit and flower extracts from *Martynia annua*. The fruit extract demonstrated significantly higher antioxidant activity, likely due to its higher concentration of phenolic

acids and flavonoids. Both extracts showed moderate antibacterial activity against several bacterial strains, with the fruit extract exhibiting slightly higher efficacy.

V. CONCLUSION

This study provides scientific evidence supporting the traditional use of *Martynia annua* as a valuable medicinal plant with antioxidant and antibacterial properties. Fruit extract has significantly higher free radical scavenging activity than flower extract, likely due to its higher content of phenolic and flavonoid compounds. Both extracts exhibit concentration dependent inhibition against tested bacterial strains, with notable activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Although less potent than the standard antibiotic, these extracts hold promise as natural antimicrobial agents, especially in the context of rising antibiotic resistance. Further in vivo studies, chemical characterization, and safety assessments are essential to explore its therapeutic potential and support its development into effective plant-based formulations. These findings contribute to the growing body of research on ethnomedicinal plants and their role in modern medicine.

Author Contribution Statement:

Mr. Kamlesh Kumar Sharma conducted the experiments, analyzed the results, and contributed to manuscript preparation. Mr. Mahendra Kumar Jeengar assisted in performing the experiments and contributed to manuscript preparation. Dr. Priyadarshi Meena and Dr. Dev Dutt Patel conceptualized and designed the experimental plan reviewed the results and critically revised the manuscript.

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Approval:

This study is part of the PhD thesis and approved by the departmental research committee (DRC), Department of Zoology, University of Rajasthan, Jaipur.

Conflict of Interest:

Authors declare no conflict of interest.

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