Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 9, No.3, September : 2024

"ANTI-INFLAMMATORY ACTIVITY OF *MARTYNIA ANNUA* SEED EXTRACT IN CARRAGEENAN INDUCE PAW EDEMA"

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ABSTRACT
The study investigates the phytochemical composition and biological activities of the methanolic extract of *Martynia annua*. Phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenols, proteins, and amino acids. Quantitative assessments indicated significant total phenolic content (TPC) is 54mg/gm and total flavonoid content (TFC) is 17.66mg/gm, benchmarked against gallic acid and rutin standards, respectively. The extract demonstrated a 60.12% DPPH radical scavenging activity with an IC50 value of 50.47 µg/ml, in comparison to ascorbic acid, which exhibited 85.28% inhibition and an IC50 value of 22.98 µg/ml. Acute toxicity tests revealed no adverse effects at doses up to 2000 mg/kg body weight, justifying the use of 100 mg/kg and 200 mg/kg dosages for further evaluations. In the protein denaturation assay, the extract showed an IC50 value of 66.74 µg/ml, indicating anti-inflammatory properties. Furthermore, in a carrageenan-induced paw edema model in rats, the extract significantly reduced paw thickness, with maximum inhibition observed at 42% for a 200 mg/kg dose. These findings suggest that *Martynia annua* has potent antioxidant and anti-inflammatory properties, highlighting its potential for medicinal applications.
Keywords: Quantitative Assessments, *Martynia Annua*, Anti-Inflammatory Activity, Carrageenan-Induced, DPPH.

1. INTRODUCTION
Traditional medicinal herbs: Traditional medicinal herbs have served as a potential source of alternative medicine and different healthcare products. Knowledge of herbal medicines has derived from rich traditions of ancient civilizations and scientific heritage. From ancient time Indian, Chinese, Egyptian, Greek, Roman and Syrian medicinal system documented the use of different plant based medicine for different diseases **(Kamboj 2000).** Folk medicine and their use against diseases in different cultures is a vast traditional knowledge; which is based on the necessities, instinct, observation, trial and error and long experience of ancient/tribal people. Indigenous or herbal medicines confer considerable economic benefits to most rural and poor people. WHO noted that about 25% of modern medicines are descended from plants sources used traditionally and research on traditional medicinal herbal plant leads discovery of 75% of herbal drugs. From this research, it is clear that plant extracts have anti-inflammatory properties that influence various stages of the inflammatory process by blocking the production of cytokines and eicosanoids; they also prevent the inflammatory reaction cascade from starting, which lessens flare-ups, itching, and excessive exfoliation. **(Verma and Singh 2008).**
Inflammation: Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release **(Chertov et al., 2000).**

Types of inflammation

Acute inflammation: Acute inflammation starts after a specific injury that will cause soluble mediators like cytokines, acute phase proteins, and chemokines to promote the migration of neutrophils and macrophages to the area of inflammation **(Germolec *et al*., 2018)**

Chronic inflammation: It is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. The classic signs of inflammation are local redness, swelling, pain, heat and loss of function **(Hughes *et al*., 2012)**

Phases of Inflammation

- **Acute phase** - temporary local vasodilation and increased capillary permeability
- **Delayed, sub-acute phase** - infiltration of leukocytes and phagocytic cells
- **Chronic proliferative phase** - tissue deterioration and fibrosis

2. MATERIAL AND METHODS

2.1 Chemicals

Ammonia, sodium hydroxide, nitroprusside, and glacial acetic acid were obtained from Merck, a reputable supplier of analytical reagents. Research lab provided the petroleum ether, and Fizmerck provided the concentrated sulfuric acid. Molychem provided the ethanol, while Clorofiltind supplied the concentrated hydrochloric acid and 95% alcohol, along with chloroform. Himedia supplied the magnesium, and Rankem provided the 1% copper sulphate solution.

2.2 Plant collection

The medicinal plant *Martynia annua* (300 gm) was collected. After cleaning, plant part (Seed) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Authentication of selected traditional plant - Medicinal plant *Martynia annua* was authenticated by a plant taxonomist in order to confirm its identity and purity. Plant Authentication no:- **AC/060/24.**

2.3 Extraction

In present study, *Martynia annua* seed was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Martynia annua* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with methanol solvent. For each solvent, soxhlation was continued till no visual colour change will observed in siphon tube and completion of extraction were confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al*., 2002).**

2.4 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used **(Kokate *et al*., 2000).**

2.5 Quantitative Phytochemical Estimation

2.5.1 Total Phenolic Content (TPC)

The total phenolic content of *Martynia annua* extract was determined using the Folin-Ciocalteu Assay. The *Martynia annua* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs

before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically (**Tangco *et al*., 2015)**.

2.5.2 Total flavonoid Content (TFC)

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Martynia annua* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy *et al*., 2009).**

2.6 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of *Martynia annua* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared. Different concentration of *Martynia annua* extracts/standard (20 – 100µg/ml) was prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm **(Athavale *et al*., 2012).**

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

2.7 Acute Toxicity Study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight (**Guideline Document on 1996**).

2.8 Evaluation of *in vitro* anti-inflammatory activity of plant extract: -

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of plant extract so that final concentrations become 20, 40, 60, 80 and 100 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ^{o}C in Incubator (Universal) for 15 min and then heated at 70 ^{o}C for 5 min. After cooling, their absorbance was measured at 660 nm (Shimadzu 1700) by using vehicle as blank. Diclofenac sodium at the final concentration of (20, 40, 60, 80 and 100µg/ mL) was used as reference drug and treated similarly for determination of absorbance and viscosity **(Chandra *et al*., 2012)**.

The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 × (Vt / Vc - 1)

2.9 *In vivo* study

2.9.1 Animals

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). CPCSEA Approval No:- **PBRI/IAEC/21-03-24/027**. The animals used of either sex were of approx 200-250g body weight in range. Before the experiment for the acclimatization, the animals were kept in groups of six in individual cages with temperature controls set at 22 ± 2°C. After that every animal received a consistent supply of water and a standard meal (golden feed, New Delhi).

2.9.2 Carrageenan-Induced Edema in Rats

Four Groups of six animals were used. Paw swelling was induced by sub plantar injection of 0.1 mL 1% carrageenan in saline into the right hind paw. The *Martynia annua* extracts at dose of 100 and 200 mg/kg were administered orally 30 min before carrageenan injection. Ibuprofen (50 mg/kg) was used as reference drug. Control group received the vehicle only (10 mL/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a vernier calliper at time 1, 2, and 3 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined, and the percent inhibition of edema was calculated in comparison with the control animals.

2.9.3 Experimental setup

Thirty minutes after drug or test compound (extracts) administration, 0.1 mL of 1% carrageenan in distilled water was injected into the sub plantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of vernier calliper at 1, 2, and 3 h. The difference between 1 h and subsequent hours reading was taken as actual edema volume.

The four groups are as follows:

Group I: served as control and treated with carrageenan;
Group II: standard group, ibuprofen 50 mg/kg
Group III: served with *Martynia annua* extract at dose of 100 mg/kg
Group IV: served with *Martynia annua* extract at dose of 200 mg/kg

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 1: Percentage Yield of crude extracts of *Martynia annua* extract

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Martynia Annua*	Pet ether	290	1.64	0.56%
2		Methanol	299	6.22	2.08%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Present
2.2	Legal's test	Present	Present

2.3	Killer-Killiani test	Present	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

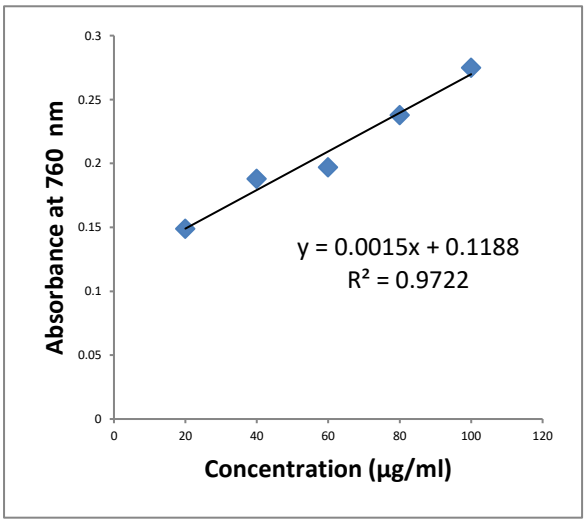
Table 3 Standard table for Gallic acid and Rutin

S. No.	Concentration (µg/ml)	Absorbance (TPC)	Absorbance (TFC)
1.	20	0.149	0.180
2.	40	0.188	0.209
3.	60	0.197	0.279
4.	80	0.238	0.325
5.	100	0.275	0.338

Table 4 Total Phenolic Content and Total Flavonoids content of extract *Martynia Annua*

Extracts	Methanol
Total Phenolic content (mg/gm equivalent of Gallic acid)	54 mg/gm
Total Flavonoid content (mg/gm equivalent of rutin)	17.66 mg/gm

Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 9, No.3, September : 2024



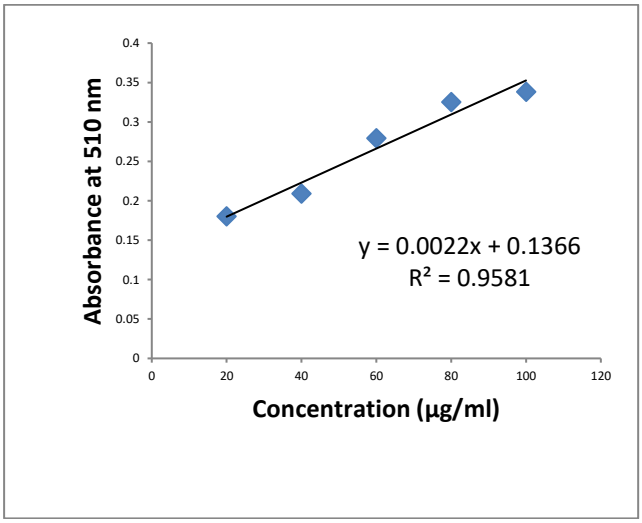


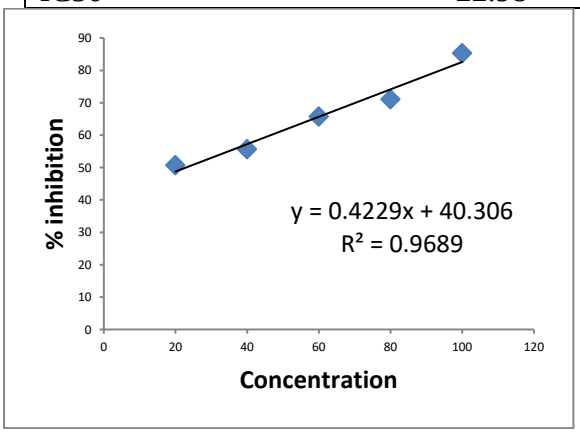
Figure 1: Represent standard curve of Gallic acid and Rutin

3.4 *In vitro* Antioxidant Assays

3.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid and methanol extract of *Martynia Annua*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.489	50.70
40	0.440	55.64
60	0.340	65.72
80	0.287	71.06
100	0.146	85.28
Control	0.992	
IC50		22.98



Concentration (µg/ml)	Absorbance	% Inhibition
20	0.522	43.75
40	0.470	49.35
60	0.453	51.18
80	0.418	54.95
100	0.370	60.12
Control	0.928	
IC50	50.47	

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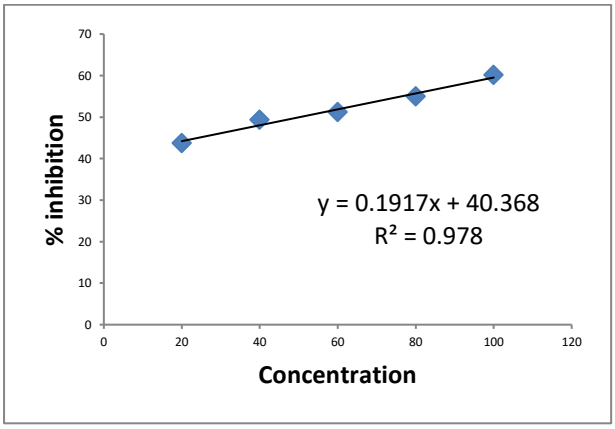

Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid and the Percentage Inhibition *Vs* Concentration of extract of *Martynia Annua*

3.5 Evaluation of *in vitro* anti-inflammatory activity of *Martynia annua* extract: -

Table 6: Effect of Diclofenac sodium (Standard) and Effect of *Martynia annua* extract on Protein Denaturation

S. No	Con. of extract (µg/mL)	Absorbance at 660nm		% Inhibition		IC_{50} Value	
		Diclofenac sodium	*Martynia annua* extract	Diclofenac sodium	*Martynia annua* extract	Diclofenac sodium	*Martynia annua* extract
1.	Control	1.104	1.056	-	-		
2.	20	0.734	0.804	33.51	23.86		
3.	40	0.617	0.737	44.11	30.20		
4.	60	0.537	0.580	51.35	45.07	54.65 µg/ml	66.74 µg/ml
5.	80	0.426	0.428	61.41	59.46		
6.	100	0.302	0.307	72.64	70.92		

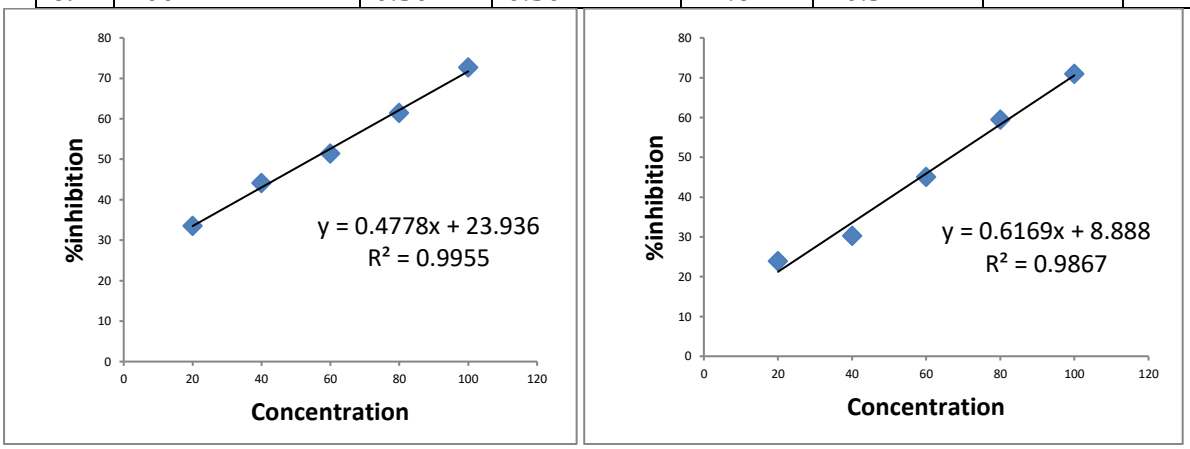

Figure 3: Graph represents the standard curve of Percentage inhibition *Vs* Concentration of Diclofenac sodium (Standard) and *Martynia annua* extract

3.6 Carrageenan-Induced Edema in Rats

The anti-inflammatory activity of *Martynia annua* against carrageenan induced paw edema has been shown in and the results were comparable to that of ibuprofen a protype of non-steroidal anti-inflammatory agent.

Table 7: Effect of extract *Martynia annua* against carrageenan induced paw edema in rats (n=6)

Treatment	1 h	2 h	3 h	Average reading	% of inhibition

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ISSN: 0970-2555
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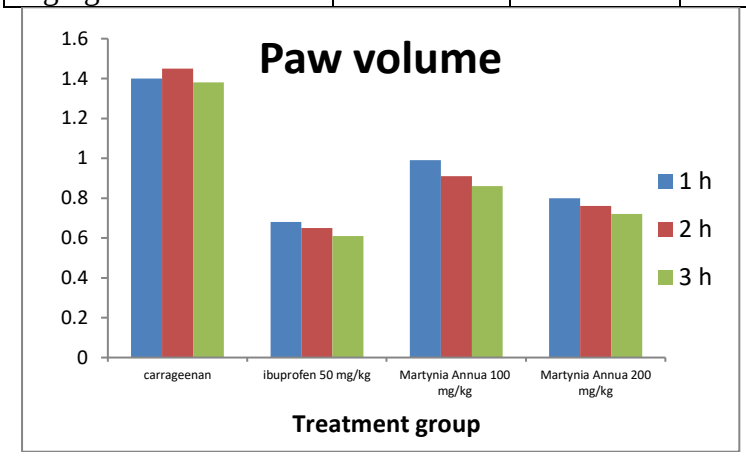
carrageenan	1.40±0.07	1.45±0.06	1.38±0.04	1.41	0
Ibuprofen 50 mg/kg	0.68±0.05	0.65±0.07	0.61±0.03	0.64	59
Martynia annua 100 mg/kg	0.99±0.10	0.91±0.12	0.86±0.09	0.92	38
Martynia annua 200 mg/kg	0.80±0.08	0.76±0.03	0.72±0.11	0.76	42



Graph 1: **Carrageenan -induced rat paw edema**

3.7 Images

4. CONCULSION

In this study, the phytochemical examination of *Martynia annua's* methanolic extract revealed the existence of several bioactive components, such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenols, proteins, and amino acids. Quantitative assays revealed considerable total phenolic content (TPC) and total flavonoid content (TFC), as evaluated with gallic acid and rutin as standards, respectively. The extract had significant antioxidant activity, with a DPPH radical scavenging percentage of 60.12% and an IC50 value of 50.47 µg/ml. However, it was less powerful than

ascorbic acid, which had an IC50 value of 22.98 µg/ml. Acute toxicity experiments showed that the extract is safe up to a dose of 2000 mg/kg body weight, which supports the use of 100 mg/kg and 200 mg/kg doses in future studies. In a protein denaturation experiment, the extract demonstrated anti-inflammatory activity with an IC50 value of 66.74 µg/ml. Furthermore, the extract considerably reduced paw thickness in a carrageenan-induced paw edema model in rats, with the highest inhibition reported at a dose of 200 mg/kg, indicating that *Martynia annua* has considerable anti-inflammatory characteristics comparable to ibuprofen. These findings emphasize *Martynia annua's* therapeutic potential and call for additional research into its medical applications.

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