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## Phytochemical Screening and Analysis of Antioxidant Activity from The Botanical Extract of The Plant *Martynia Annua*

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 23 Nov 2023	Medicinal plants, enriched by nature with potent compounds, hold great promise in developing natural drugs with minimal side effects. This study focuses on Martynia annua, a plant species renowned for its medicinal potential. We aimed to identify and quantify phytochemicals in its leaf extracts and evaluate their in vitro antioxidant activity. Methanol, hexane, and water were used as solvents for extraction. Qualitative analysis revealed a range of pharmacologically active phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenols, fixed oils, and terpenoids. Methanolic extract exhibited the highest diversity, with eight distinct compounds. Quantitative analysis showed that methanolic extract contained the most phenols (0.5 mg/g) and carbohydrates (12 mg/g), while hexane had the highest flavonoid content (319 mg/g), and aqueous extract displayed the most protein (53.57 mg/g). Antioxidant assays demonstrated the superior performance of methanolic extract, with maximum inhibitory percentages of 92.99% (DPPH), 94.28% (FRAP), 76.11% (ion chelating), and an IC50 value of 52.17 µg/ml, indicating its remarkable antioxidant potential. These findings underscore Martynia annua as a valuable source for phytochemicals with significant antioxidant properties, particularly in the methanolic leaf extract, holding promise for future therapeutic developments."
CC-BY-NC-SA 4.0	Keywords: Martynia annua, drug, DPPH, FRAP, antioxidant.

## 1. Introduction

The mother nature has gifted our country with an enormous wealth of medicinal plants, and therefore India is said to be the medicinal garden of the world. In the 21st century, the pharmacological properties of the medicinal plants will definitely lead to the promising future drugs for healing human ailments (Shakya and Arvind Kumar, 2016). For the purpose of health care, over three- quarters of the global population depends on plant and plant-based extracts. Though 20,000 medicinal plants have been reported in India, only 7,000 plants areas being used by the traditional communities to cure diseases. As the modern drugs are prepared from synthetic compounds it may cause side effects while herbal drugs are safe to use and rarely cause side effects. This in turn makes people to choose products synthesized from plants (Gupta et al., 2020; Shakila et al., 2017). Biologically active molecules and lead structures can be provided by the plants used in Ayurveda for the development of modified derivatives with good activity and/or reduced toxicity. Some of the useful plant drugs include taxol, vincristine, vinblastine, tubocurarine, podophyllotoxin, oligoxigenin, digitoxigenin, gitoxigenin, camptothecin, artemisinin, curamin, allicin, capscicine, pilocarpine, atropine, aspirin, codeines, morphine and ephedrine. Phytochemicals possess antioxidant, anti-microbial, antidiabetic and other properties as per recent studies (Sheng et al., 2022). For the purpose of medicine in some cases, crude extracts of medicinal plants may be used. On the other hand, paramount importance will be given to the isolation, identification, and the elucidation of the mechanism of action of a drug. Hence, the work of both traditional medicine and single- active compounds is important.

In India, about 2500 plant species serve as a source of medicine. Over a decade, there has been a tremendous increase in the study of medicinal plants in various parts of the world. To enhance the development of herbal treatment, therapeutic effects, chemical structure of the plant and other properties

may vary (Narayanan et al., 2022). Among different medicinal plants, *Martynia annua* belongs to the plant group Martyniaceae and is commonly known as devils' claw or unicorn plants. This versatile plant previously came under the family Pedaliaceae, commonly known as the sesame family. It is indigenous to the new world and is found primarily in subtropical areas. Leaves and fruits are the most biologically active parts. It is characterized by having an unilocular and bicarpellate ovary with parietal placentation. This plant possess various pharmacological effectson human body as the stem, leaves, bark, flowers, fruits and seeds are rich in secondary metabolites (Zarina Arshad et al., 2017). The seeds of the plant have the potential of healing itchiness and skin infections (Gupta et al., 2015; Jadhav et al., 2015). The extracts of the plant possess good antidiabetic (Jitender Malik, 2019), gastroprotective (Saurabh soni, 2019) and immune modulatory activities (Jitender Malik, 2017).

The plant is a native of Mexico and is commonly known as Devils claw in English, Kakanasika in Sanskrit, Vichchida in gujarati and Bichu in Hindi. This plant belonging to the family Martyniaceae possesses glandular hairs composed of one to several celled uniseriate stalks and a several - celled head, giving the plant a sticky texture by covering its surface. The characteristic feature of the family is the occurrence of woody, beaked fruits. When the fruits tend to mature, the fleshy exocarp senesces and sloughs off, thereby revealing the woody endocarp at the bottom. It has also been observed that the fruits of this plant dehisce longitudinally along abiaxal and adiaxal sutures and tend to produce two sharps, pointed hooked horns as the beak splits. At times, the sharp seed pods get attached to the feet of the animals and include some of the largest hitchhiker fruits in the world. Fruits are also used as local sedatives. This plant in traditional medicine is used to cure tuberculosis, inflammation, wounds and sore throats. The plant extract possesses various phytoconstituents which could be used to synthesize the nanoparticle with valuable therapeutic effect (Narayanan et al., 2022; Prema et al., 2022). The objective of this review is to give information on the therapeutic efficacy of *Martynia annua* on cancer and also on the various research studies done on this plant.



Figure 1 Martynia annua

Scientific classification of this plant is given below (Senthil Kumar Raju et al., 2023): -

Kingdom Plantae Division Magnoliophyta Class Angiosperms Order Lamiales Family Martyniaceae Genus Martynia Species *Martynia annua* 

It usually grows in wastelands and seeds are brown to black, 2 to each pod. Leaves simple, opposite to alternate, long-petioled, exstipulate, entire to sinuate, inflorescence racemose, cordate at base. Flowers perfect, pedicelled, hypogynous, zygomorphic, each axillary to bract. Calyx of 5 sepals, bibracteolate at base, more or less unequally 5- lobed, zygomorphic, either synsepalous and spathaceous, split abaxially to base. Corolla sympetatous, the throat campanulate, aestivation imbricate, somewhat 2-lipped. Androecium of 4 didynamous, alternate stamens, anthers dorsiixed, becoming more or less connate, with placentoids, bilocular at maturity, locules dehiscing longitudinally and introrsely. Pollen

spheroidal, reticulate, apparently non aperturate. Gynoecium syncarpous, sensitive to touch, stigma 2lipped, lips thin, style elongate, included in or slightly exserted from the throat. Somatic and gametic chromosome number reported for *Martynia annua* indicate diploid numbers  $2n=32^{[15]}$ . Nomenclature of the fruit is bit problemtic as it is called by different names. Capsule with deciduous pericarp, "a woody many –seeded capsule" "a dorupaceous capsule", "a ligneous drupe", "a drupaceous follicle", "subdrupaceous capsule", "a pod" and for simpler classification, authors call it as a fruit. In gardens, *Martynia annua* are grown as ornamental plants. It is actually a widely distributed weed and are noxious. This plant is native to subtropical and tropical regions and can be found throughout India, Central America, Burma and Mexico (Bansode et al., 2017; Bansode et al., 2021; Gupta et al., 2021; Jamshaid et al., 2018). The aim of the study is to carry out the phytochemical screening and analyse the antioxidant activity from the botanical extracts of *Martynia annua*.

## 2. Materials And Methods

#### **Collection and Preparation of plant material**

The whole plant material of *Martynia annua* was collected from the Padappai of kancheepuram district, Tamilnadu during the rainy season and washed thoroughly. The plant material was shade-dried for a period of three weeks and it was finely powdered using an electrical blender. The finely powdered plant material (25g) was subjected to Soxhlet extraction for a period of 4 days in the increasing order of polarity i.e hexane, methanol and water. Further the extract was concentrated using a rotary evaporator and was stored at 4°C for further use (Zhang et al., 2019)

#### **Qualitative Phytochemicals Analysis**

To determine the presence or absence of various phytoconstituents like saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, carbohydrates and phenols the botanical extracts obtained from Soxhlet extraction were subjected to undergo preliminary phytochemical screening. Preliminary phytochemical screening was carried out for the extract as per standard methods (Raipuria et al., 2018).

#### **Test for Saponins**

To aqueous plant extract 5.0 ml of distilled water was mixed in a test tube and mixed vigorously. Presence of saponin was identified by froth formation.

#### Test for Flavonoid

Alkaline reagent test: Concentrated yellow colour was produced when aqueous plant extract was mixed with 2ml of 2.0% NaOH mixture. The result indicated the presence of flavonoids.

#### **Test for Terpenoids**

5ml of aqueous plant extract was added with 2.0 ml of chloroform and evaporated in waterbath and then it was subjected to boiling with 3ml of concentrated sulphuric acid. Formation of grey colour indicated the presence of terpenoids.

#### Test for glycosides.

To 10ml of aqueous plant extract and 1 ml of concentrated sulphuric acid, 4ml of glacial acetic acid with 1 drop of 2.0% FeCl<sub>3</sub> was mixed. Cardiac steroidal glycoside was confirmed by the formation of brown ring between the layers.

#### **Test for Alkaloids**

Dragendorff test- Presence of alkaloid was confirmed by the presence of orange-red colour precipitate when one drop of dragendorff reagent was added to 5mg extract taken in tube.

#### **Test for Proteins**

Biuret test- 5mg of plant extract was mixed with few drops of biurets reagent and was allowed to boil for 1-5 minutes. Presence of protein was confirmed by the formation of red or violet colour.

#### **Test for Aminoacids**

Ninhydrin test- To 5mg of plant extract 2ml of 0.2% ninhydrin solution was added and it was then boiled in water bath for 2 minutes. Presence of aminoacid was confirmed by the occurrence of violet colour.

## **Test for Carbohydrates**

Fehling's test- To 5mg of botanical extract few drops of benedicts reagent was added and allowed to boil. Reddish brown precipitate indicated the presence of carbohydrates.

## **Test for Phenols**

Powdered samples of leaves were boiled along with 20ml of distilled water and filtered. Occurrence of brownish green or blue colour was observed when 3-4 drops of 0.1% ferric chloride was added to the filtrate. It indicated the presence of phenols.

## QUANTIITATIVE PHYTOCHEMICAL ANALYSIS

Hexane, methanol and aqueous extracts of *Martynia annua* were subjected to quantitative analysis inorder to determine the amount of metabolites present in them.

#### Determination of total phenolic content

2g of botanical powder was soaked in different solvents such as methanol, water and hexane. It was placed in an orbital shaker for the time period of 24 hours. After filtering the residues, the filtrate was subjected to evaporation. The extracts were centrifuged at 10,000 rpm for 15 minutes. From the supernatant, 20  $\mu$ l of extract was prepared and it was made up to 3ml with distilled water. The tubes were then placed in the incubator for 3 minutes at 45°C after adding 0.5 ml of Folin Ciocalteu's phenol reagent to all the tubes. 2ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added to all the tubes after 3 minutes and kept for incubation. Absorbance was measured at 650nm (Guyo Jilo Molole et al., 2015). The total phenol content was calculated using the formula,

C(GAE)=C×V/M

Where C=Concentration of sample from the curve obtained (mg/ml)

V= Volume used during the assay (ml) and

M= mass of the sample used during the assay (g)

#### **Determination of total flavonoid**

By slightly modifying the spectrophotometry method of Elin Novia Sembiring, flavonoid contents were determined. In a mortar and pestle, one gram of dry powder was weighed, and it was ground using 200 ml of 80% aqueous methanol. Clear filtrate was obtained after filtering the ground sample. 3 ml of distilled water and 0.3 ml of 5% sodium nitrite were added to the test tube containing an aliquot of the sample (0.5 ml). For 5 minutes, the solution was allowed to stand at room temperature after vertexing and 0.6 ml of 10% aluminium chloride was added. In the test tube, 2ml of 1M sodium hydroxide was added after 6 minutes\ with distilled water, the solution was made up to 10 ml. The absorbance was finally read at 510 nm (Elin Novia Sembiring., 2022). The total flavonoid content was calculated as quercetin equivalent (mg QE/g) using the formula,

 $X= (A.M_0/ A.M_0)$ Where, A= absorption of sample, A\_0= absorption of standard (quercetin), M= weight of sample (mg/ml) and M\_0 = weight of the quercetin in solution (mg/ml)

#### **Protein estimation assay**

The assay is based on the observation that when the acidic solution of coomassie brilliant blue G-250 binds to protein, the absorbance maximum shifts from 465nm to 595nm. There occurs a visible colour change when the anionic form of the dye is stabilized by hydrophobic and ionic interactions. Over a 10-fold concentration range, the extinction coefficient of a dye-albumin complex solution is constant, thereby making the assay useful (Zanini et al., 2022).

To atleast one assay tube containing 100 $\mu$ l sample, unknowns can be diluted to obtain between 5 and 100 $\mu$ g protein. Equal volume of 1M NaOH can be added to each sample followed by vortexing. NaOH can be added to standards as well. In 100 $\mu$ l volume standard protein (albumin or gamma globulin) can be prepared in a range of 5 to 100  $\mu$ g. Finally, absorbance can be measured at 595 nm after adding 5 ml of dye reagent.

#### **Total Carbohydrates**

1 ml of phenol and 5ml of concentrated sulphuric acid was added to 1ml of the sample and the mixture was mixed thoroughly. The OD readings can be taken at 490nm after allowing the solution to stand in boiling water bath for 15 minutes. Using the standard graph prepared by D-glucose, the amount of total carbohydrates was calculated and the values are expressed as  $\mu g/ml$ (Albalasmeh et al., 2013).

## **Antioxidant Activity**

## **DPPH Assay**:

By assessing the DPPH free radical assay, the percentage of antioxidant activity of the each substancecan be calculated. As per the methodology described by Thasneem (2022), the DPPH radical scavenging activitywas measured. the sample were made to react with methanol solution containing stable DPPH radical. The constituents of reaction mixture included 0.5 ml of sample, 1 ml of methanol and 1 ml of DPPH radical solution (0.5mm in methanol). DPPH gets reduced, when it is reacted with an antioxidant compound which can donate hydrogen.Using UV - VIS Spectrophotometer the change in colour from deep violet to light yellow were readat 517 mm . 1 ml of methanol, serve as blank and the control solution was prepared by mixing DPPH radical solution (1.0 ml) and methanol (1.0 ml). The scavenging activity percentage was determined according to, Percentage of inhibition = control OD - sample O.D / control O.D x 100

#### Ferric reducing antioxidant power assay

Estimation was done spectrometrically for the antioxidant capacity of the medicinal plants following the procedure of Anoob Kumar (2021).

The method is based on the fact that, due to the action of electron donating antioxidants at low PH, there occurs reduction of Fe 3+ TPTZ complex (coloured complex) to Fe2+ - tripyridyltriazine (blue colouredcomplex) - By measuring the change in absorbance at 593 mm, the monitoring can be done for reactions.

By mixing 300 mm acetate buffer, 10 mL TPTZ in 40 mm HCL and 20 mm FeCl3-6H2O in proportion of 10:1:1, the ferric reducing antioxidants power (FRAP) reagent was prepared at 37-degree celcius using 1-5 ml variable micropipette (3.995 ml). FRAP reagent was pipetted and mixed with diluted plant sample of 5 microlitre.

After 20 minutes of incubation at 37-degree celcius, the ferric tripyridyltriazine (Fe3+ TPTZ) complex was reduced to (fe2+) form followed by the formation of intense blue color complex and the absorbance was measured at 593 nm against a reagent blank (3.995 ml) FRAP reagent + 5 microlitre distilled water.

By plotting the absorbance at 593 nm different concentration of FeSo4, the calibration curve was prepared. against the concentration of standard antioxidant trolon. the concentration of FeSo4 were in turn plotted.

The FRAP values were expressed as mg of trolon equivalent per gram of sample and it was obtained. when the comparison was done between the absorbance change in the test mixture with those obtained from increasing concentrations of Fe3+.

## ABTS radical scavenging assay

By ABTS radical cation decolorization assay, free radical scavenging activity of plant samples were determined (Mansoor et al 2022).

By storing in dash at room temperature for 12-16 h, there occurs reaction between 7mm ABTS in water and 2.45 mm potassium persulfate (1:1) leading to the formation of + cation radical.

To obtain an absorbance of 0-70 degree at 734 nm ABTS. + solution was diluted methanol. Absorbance can be measured at 30 minutes after mixing 3.995 ml of diluted ABT. + solution with 5 microlitre of plant extract. each assay, as appropriate amount of solvent blank was run.

Percentage inhibition of absorbance at 734 nm was calculated using the formula, ABTS + scavenging effect (%) =  $(AB-AA)/AB \times 100$ ; Where AB is absorbance of ABTS radical + methanol; AA is the absorbance of ABTS radical + sample extract /standard. Trolox was used as the standard.

#### Ion chelating assay

By the method of Ilhami Gulcin (2022) the extracts chelatin of ferrous ion can be estimated. To 1ml of varying concentration of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml),50 microlitre of 20 mm FeCl2 was added .By adding 0.2 ml of 5mm feroxine solution , the reactions can be initiated . after shaking

vigrorously the mixture can be allowed to stand for 10 minutes at room temperature. the absorbance was thereafter measured at 562 nm. The percentage inhibition of ferrozine -Fe2+ complex formation was calculated as  $[(A_0-A_s)/A_s] \times 100$ 

Where  $A_o$  was the absorbance of the control as was the absorbance of the extract/standard. As positive control, Na2EDTA was used.

## 3. Results and Discussion

#### **Phytochemical screening**

The phytochemical analysis of the various extracts of Martynia annua is shown in Table 1.

		Martynia annua L.			
S.No.	Phytocomponents	Methanol Extract	Hexane Extract	Aqueous Extract	
1.	Alkaloids	+	+	+	
2.	Carbohydrates	+		+	
3.	Glycosides	+		+	
4.	Saponins	+	+	+	
5.	Proteins	+			
6.	Aminoacids		+		
7.	Phenols	+			
8.	Fixed Oils	+		+	
9.	Terpenoids	+	+	+	
(+) - Presence () - Absence					

Table 1. Qualitative Phytochemical analysis

From the qualitative findings presented in Table 1, it has been observed that the extracts of *Martynia annua* contained alkaloids, carbohydrates, glycosides, saponins, proteins, phenols, amionoacids, fixed oils, and terpenoids. However, most of the secondary metabolites were present in the methanolic extract. Next to methanolic extract aqueous extract showed higher variety of phytoconstituents compared to hexane extract. Aminoacid was found to be present only in the hexane extract.

#### Quantitative analysis

**Table 2.** Quantitative phytochemical analysis

Quantitative phytochemical analysis results for total phenols, flavonoids, proteins and carbohydrates were obtained using three botanical extracts and the results of quantitative phytochemical analysis is presented in Table 2.

S.No.	Parameters	Absorbance					
<b>5.</b> 110.		MAM	MAH	MAW	LNM	LNH	LNW
1.	Total Phenols	0.50 mg/g	0.42 mg/g	0.48 mg/g	0.38 mg/g	0.43 mg/g	0.52 mg/g
2.	Total Flavonoids	212 mg/g	319 mg/g	287 mg/g	202 mg/g	329 mg/g	106 mg/g
3.	Total Proteins	48.28 mg/g	44.14 mg/g	53.57 mg/g	43057 mg/g	43 mg/g	56.8 mg/g
4.	Total Carbohydrates	12 mg/g	7 mg/g	9 mg/g	4 mg/g	6 mg/g	11 mg/g

## **Total phenols**

The standard used for the determination of phenolic content was gallic acid. The total phenolic content of the methanolic extract were 0.50mg/g and 0.42mg/g for hexane and 0.48 mg/g for aqueous extract. The result indicated that the methanolic extract of *Martynia annua* possess high amount of phenol when compared to hexane and aqueous extract.

## Total flavonoid

The standard used for the determination of flavonoid content was quercetin. The total flavanoid content of the methanolic extract were 212 mg/g and 319 mg/g for hexane and 287mg/g for aqueous extract. The result indicated that the hexane extract of *Martynia annua* possess high amount of flavanoid when compared to methanolic and aqueous extract.

#### **Estimation of protein**

The standard used for the determination of protein content was bovine serum albumin. The total protein content of the methanolic extract were 48.28mg/g and 44.14 mg/g for hexane and 53.57mg/g for aqueous extract. The result indicated that the aqueous extract of *Martynia annua* possess high amount of protein when compared to methanolic and hexane extract.

## **Estimation of carbohydrate**

The standard used for the determination of carbohydrate content was glucose. The total carbohydrate content of the methanolic extract were 12mg/g and 7 mg/g for hexane and 9mg/g for aqueous extract. The result indicated that the methanolic extract of *Martynia annua* possess high amount of carbohydrate when compared to hexane and aqueous extract.

#### **Antioxidant Activity**

In the present study extracts of *Martynia annua* were tested using three different solvents (methanol,hexane and aqueous) for their free radical scavenging activity using DPPH assay, ABTS assay,Ion chelating assay and FRAP assay. It was observed that the plant extracts showed good potency for scavenging free radicals. The extracts were tested on a concentration range (20-100 $\mu$ g/ml) and it was also observed that as the concentration of botanical extract increases, the activity also increases. In all the cases the methanolic extracts proved to be a better antioxidant than the hexane and aqueous extracts.

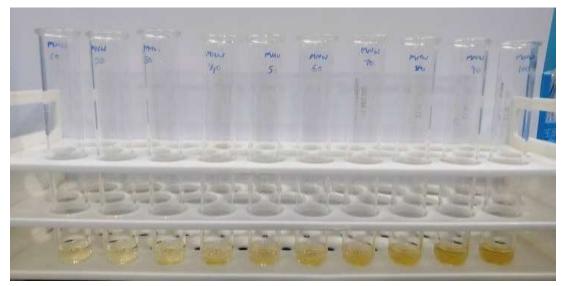


Figure 2. DPPH scavenging activity (%) of plant extract at different concentration

Concentration (µg/ml)	Methanol extract	Hexane extract	Aqueous extract	Quercetin
20	14.44±0.00	3.93±0.00	7.87±0.00	13.52±0.01
40	34.79±0.00	23.19±0.00	14.87±0.00	23.53±0.01
60	78.55±0.00	35.44±0.00	40.7±0.00	41.96±0.01
80	82.93±0.00	47.92±0.00	55.79±0.00	48.82±0.02
100	92.99±0.00	56.45±0.00	70.89±0.00	72.94±0.03
IC <sub>50</sub> (µg/ml)	52.17	83.47	65.55	81.93

Table 3. DPPH scavenging activity (%) of plant extract at different concentration

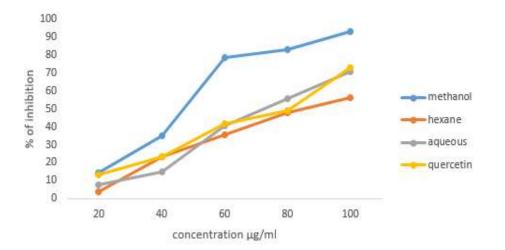
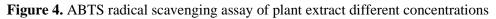


Figure 3. DPPH scavenging activity (%) of plant extract at different concentration

The IC<sub>50</sub> values were calculated for all the three extracts. In DPPH assay the methanolic extract showed maximum inhibitory percentage of 92.99% at concentration of 100  $\mu$ g/ml giving IC<sub>50</sub> value of 52.17 $\mu$ g/ml.With increasing polarity the pattern of increasing antioxidant activity has been observed.

## ABTS ASSAY



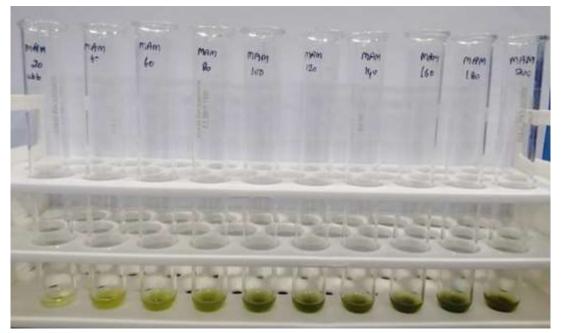


Table 4. ABTS radical scavenging assay of plant extract at different concentrations.

Concentration (µg/ml)	Methanol extract	Hexane extract	Aqueous extract	Ascorbic acid
20	6.53±0.00	$7.67 \pm 0.00$	6.81±0.00	33.10±1.33
40	19.88±0.04	15.62±0.00	34.37±0.00	45.94±1.48
60	43.18±0.00	25.85±0.00	61.93±0.00	48.64±1.86
80	54.26±0.00	35.79±0.00	64.48±0.00	56.08±1.29
100	64.77±0.00	41.47±0.00	67.32±0.00	71.62±1.86
IC <sub>50</sub> (µg/ml)	75.12	120.56	51.16	61.67

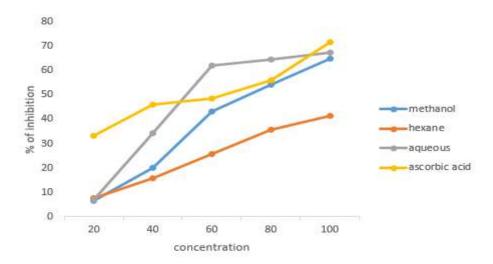


Figure 5. ABTS radical scavenging assay of plant extract at different concentrations.

Then DPPH, ABTS was considered to be more reactive and it involves the transfer of electrons. In ABTS assay the aqueous extract showed maximum inhibitory percentage of 67.32% at concentration of 100ug/ml giving IC<sub>50</sub> value of 51.16  $\mu$ g/ml. In this study the assay of ABTS was performed at varying concentrations and it showed good results than that of ascorbic acid whose IC<sub>50</sub> value was 61.67  $\mu$ g/ml.

## Frap Assay

Figure 6. Ferric reducing antioxidant power assay of plant extract different concentrations



Table 5. Ferric reducing antioxidant power assay of plant extract different concentrations

Concentration (µg/ml)	Methanol extract	Hexane extract	Aqueous extract	Quercetin
20	24.28±0.00	20.11±0.00	12.85±0.00	18.52±1.28
40	40.98±0.00	35.71±0.00	24.28±0.00	25.23±0.98
60	57.14±0.00	48.57±0.00	40.87±0.00	36.70±1.06
80	75.71±0.00	62.85±0.00	57.14±0.00	48.41±0.99
100	94.28±0.00	82.85±0.00	67.14±0.00	68.05±0.67
IC <sub>50</sub> (µg/ml)	51.47	61.76	72.06	82.62

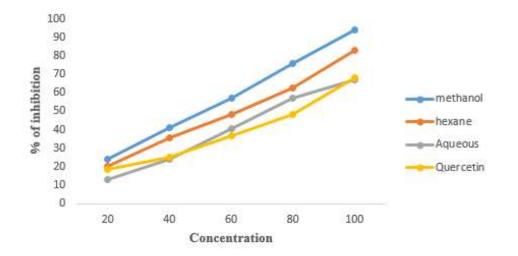


Figure 7. Ferric reducing antioxidant power assay of plant extract different concentrations

This assay is considered to be a good reflector to determine the antioxidant properties of the plant. It has also been reported that the plants possessing high reducing power is said to carry high antioxidant activity too<sup>30</sup>. The reducing power potential of all the three plant extracts had increased with the dose, however it has been observed that the three extracts showcased low reducing power than that of Ascorbic acid. In FRAP assay the methanolic extract showed maximum inhibitory percentage of 94.28 % at concentration of  $100\mu g/ml$  giving IC<sub>50</sub> value of 51.47  $\mu g/ml$ .

#### Ion Chelating Assay

Figure 8 Ion Chelating Assay of plant extracts at different concentrations.

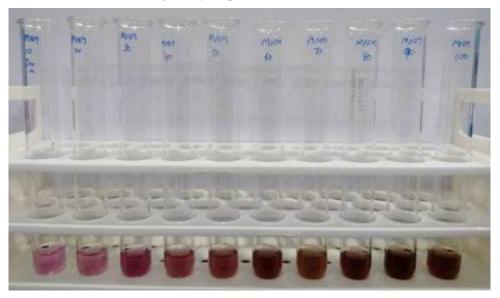


Table 6. Ion Chelating Assay of plant extracts at different concentrations.

Concentration (µg/ml)	Methanol extract	Hexane extract	Aqueous extract	Di-Sodium EDTA
20	13.43±0.00	14.92±0.00	13.43±0.00	32.83±0.98
40	28.35±0.00	23.88±0.00	28.35±0.00	41.79±1.04
60	47.76±0.00	38.8±0.00	41.79±0.00	46.26±1.35
80	58.2±0.00	46.26±0.00	55.22±0.00	52.23±0.98
100	76.11±0.00	55.22±0.00	68.65±0.00	59.70±0.80
IC <sub>50</sub> (µg/ml)	63.21	86.16	71.06	64.85

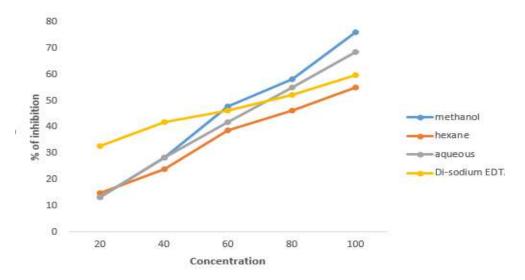


Figure 9. Ion Chelating Assay of plant extracts at different concentrations.

From the above table 4 and figure 5 it has been observed that the methanolic extract possessed more ability than the hexane and aqueous extracts. the methanolic extract showed maximum inhibitory percentage of 76.11% at concentration of 100ug/ml giving IC<sub>50</sub> value of 63.21  $\mu$ g/ml.

#### 4. Conclusion

Martynia annua, although considered a weed, possesses significant therapeutic efficacies and various bioactive compounds that represent the medicinal characteristics of the plant. This plant contains various phytochemicals such as saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, carbohydrates, and phenols. The study revealed that the methanolic extract contained a higher number of compounds. Quantitative analysis also illustrated a significant amount of phenols, flavonoids, carbohydrates, and proteins. The methanolic extract exhibited a higher level of antioxidant activity compared to other extracts, and this study paves the way for future research to isolate the bioactive compounds responsible for this activity. The curative potential of the plant inspires further research activities on this remarkable plant and the development of a novel drug for the future treatment of various diseases.

#### **Conflicts Of Interest**

The authors declare no conflicts of interest.

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