

Research Article

Phytochemical Screening and Estimation of Flavonoids in Leaves, Roots and Stem of *Martynia annua* Linn.

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Abstract

In present investigation, preliminary phytochemical screening of leaves, roots and stem extracts of *Martynia annua* was carried out. The leaves, root and stem powder was subjected to methanolic, ethanolic and aqueous extraction followed by qualitative screening for major phytochemicals which confirmed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids and tannins. Terpenoids were detected in aqueous extract of stem only. Anthocyanides were not detected in any extract. The percentage of total flavonoid content in leaves, roots and stem extracts was estimated as 0.122 ± 0.002 , 0.0677 ± 0.001 and 0.0724 ± 0.002 respectively.

Keywords: Phytochemical screening, *Martynia annua*, flavonoid content

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Introduction

India is one of the ancient traditional herbal ayurvedic knowledge system which employs a large number of plant species such as Ayurveda (2000 species), Siddha (1121 species), Unani (751 species) and Tibetan (337 species) [1]. Use of different plants as a source of various medicines has been inherited from the onset of human civilization and is an important component of the healthcare system [2]. Medicinal plants are used for nutritional as well as medicinal needs. Important herbal products include herbal teas, functional foods, medicinal raw materials, essential oils, flavoring and dietary supplements [3]. The fact of using medicinal plants as a medicine lies in the reason that they contain chemical compounds of therapeutic values [4]. These are the naturally occurring compounds found in the medicinal plants, vegetables, fruits, flowers, leaves, roots, stem bark and work together with nutrients and fibers to act as a defense system and protect humans against diseases. These compounds are called secondary metabolites that often play an important role in plant defense against herbivory [5] and other interspecies defenses. These chemical compounds form the backbone of the modern medicines or drugs [6].

Martynia annua L. belongs to family Martyniaceae (or pedaliaceae), is a well-known small herbaceous annual plant, distributed throughout India in waste places, rubbish heaps and road sides. The plant is commonly known as Devil's claw (English), Bichu (Hindi), Kakanasika (Sanskrit) and Vichchida (Gujarati) [7]. The fruit splits open at one end into two curved horns or claw [8]. It is an endemic plant of Mexico but also found commonly in India and subcontinent [7]. In Ayurveda, the plant is known as Kakanasika, which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis [9]. The leaves and fruits are the biologically active part of this plant [10, 11]. The leaves of *M. annua* are edible and used as antiepileptic and antiseptic, applied locally to tuberculous glands of neck, the juice of leaves as a gargle for sore throat and the leaf paste for wounds of domestic animals [12, 13]. The fruit is considered alexiteric and useful in inflammations while fruit ash mixed with coconut oil is applied on burns [9]. Seed oil is applied on abscesses and for treating itching and skin affections [7]. The Ayurvedic pharmacopoeia of India recommended the seeds of *M. annua* for arresting the greying of hair [9]. The fruits of *M. annua* are used as local sedative and also used as antidote to scorpion stings to venomous bites and stings [14]. The root extract of *M. annua* showed anthelmintic activity, antifertility activity, antinociceptive and CNS depressant activity [15]. The stem extract has also been reported to contain the antioxidant activity. In literature, it is well documented that the whole plant is used as medicine [16]. The present study was undertaken to screen the leaves, roots and stem of *M. annua* for their phytochemicals.

Material And Methods

Collection and processing of plant samples

The plant samples of *M. annua* were collected from Kundam block of Jabalpur district in Madhya Pradesh. The plant parts (leaves, roots and stem) were separated and washed in tap water to remove foreign and dust particles. The cleaned materials were cut into pieces and dried in shade. The dried materials were powdered using grinder and stored in polythene bags for further chemical analysis.

Extraction of plant materials

100 mg of powdered plant materials was kept overnight in 25 ml of different solvents viz. aqueous, ethanol and methanol. The extracts were filtered using whatman filter paper no.1 and filtrates were used for phytochemical screening.

Phytochemical screening of extracts

The extracts were subjected to preliminary phytochemical testing following the standard methods [17, 18].

Test for Alkaloids

2 ml of filtrate was taken in a test tube followed by addition of 1% HCL. Creamish colour indicated the presence of alkaloids.

Test for Anthocyanides

1 ml of filtrate with 5 ml of dilute 10-12% HCL. Presence of pale pink colour indicated the presence of Anthocyanides.

Test for Cardiac glycosides

2 ml of filtrate was taken followed by addition of 1ml of glacial acetic acid, FeCl_3 and conc. H_2SO_4 . Green blue precipitate or brown ring indicated the presence of cardiac glycosides.

Test for Flavanoids

5 ml of diluted ammonia solution was added to a portion of filtrate of plant extract followed by addition of conc. HCL. Yellow colour indicated the presence of flavanoids.

Test for Phenols

2 ml filtrate was taken in a test tube and added to it 1 ml of 1% FeCl_3 . Brown precipitate or haziness indicated the presence of phenols.

Test for Saponins

1 ml of filtrate was mixed with 5ml of distilled water, shake vigorously for a stable froth. Persistence of froth indicated the presence of saponins.

Test for Steroids

2 ml of filtrate was taken followed by addition of 2 ml of acetic anhydride and conc. H_2SO_4 . Blue green ring indicated the presence of steroids.

Test for Tannins

2 ml of filtrate was taken followed by an addition of 2ml of FeCl_3 . Brownish colour or precipitate indicated the presence of tannins.

Test for Terpenoids

5 ml of extract was mixed in 2 ml of chloroform and 3 ml of conc. H_2SO_4 was carefully added. Appearance of reddish brown colouration at interface was showed the presence of terpenoids.

Estimation of flavonoids

Total flavonoids were determined by the aluminum chloride calorimetric method [19]. 0.5 g sample was weighed and kept in 95% ethanol for 24 hours. It was then filtered and volume was made up to 25 ml with 80% ethanol. 0.5ml of filtrate was then mixed with 1.5 ml of 95% ethanol, 0.1ml of 10% AlCl₃, 0.1 ml of potassium acetate and 2.8 ml distilled water. The tubes were then incubated at room temperature for 30 minutes. Observation was measured at 415 nm. Flavonoid content of the samples was calculated from the standard graph of quercetin (**Figure 1**). Percentage of flavonoids was calculated by using the following formula:

$$\text{Flavonoid (\%)} = \frac{\text{Conc. of standard}}{\text{O.D. of Standard}} \times \frac{\text{O.D. of Sample}}{\text{Weight of sample}} \times \frac{\text{Total volume makeup}}{\text{Volume taken}} \times 100$$

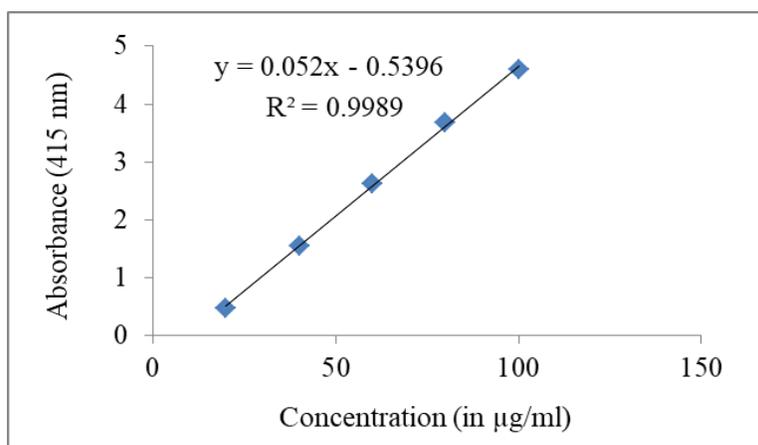


Figure 1 Standard graph of quercetin

Results and discussion

The results of phytochemical screening of aqueous, ethanol and methanol extracts of leaves, roots and stem of *M. annua* are presented in **Table 1** which revealed the presence of saponins in extracts of root, stem and leaves each. Alkaloids and tannins were found present in all the extracts except the methanolic extract of leaves. Phenols were detected in all extracts of roots and stem but in leaves, phenols were found in ethanolic extract only. Cardiac glycosides were found in ethanolic and methanolic extracts of leaves and stem while these were detected in ethanolic extract of roots only. Cardiac glycosides were not detected in aqueous extract. All three i.e. aqueous, ethanolic and methanolic extracts of roots were found to contain steroids while only ethanolic extracts of leaves and stem contained steroids. All extracts except aqueous and methanolic extracts of roots and leaves respectively showed the presence of flavonoids. Terpenoids were detected in aqueous extract of stem only.

Total flavonoid content in ethanolic extracts of leaves, roots and stem of *M. annua* is given in **Table 2** which indicated that maximum flavonoid content was found in leaves (0.122±0.002%) followed by roots (0.0677±0.001%) and stem (0.0724±0.002%). Lodhi *et al.*, (2016) [20] reported that ethanolic extract of *M. annua* leaves exhibited total flavonoid content of 12.62 ± 4.69% which is more as reported in our study.

Table 1 Qualitative phytochemical screening of leaves, roots and stem of *M. annua*

S. No.	Phytochemical constituents	Aqueous extract			Ethanol extract			Methanol extract		
		Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem
1	Alkaloids	++	++	+	+	+	++	-	++	++
2	Tannins	+	+	+	+	++	++	-	++	++
3	Phenols	-	+	+	+	++	+	-	++	+
4	Cardiac glycosides	-	-	-	+	+	+	+	-	+
5	Steroids	-	+	-	+	+	+	-	+	-
6	Saponins	+	+	++	+	+	++	++	+	+
7	Flavonoids	+	-	+	+	++	+	-	+	++
8	Terpenoids	-	-	+	-	-	-	-	-	-
9	Anthocyanides	-	-	-	-	-	-	-	-	-

(+) = detected, (++) = detected in higher amount and (-) = not detected

Table 2 Flavonoid content in leaves, roots and stem of *M. annua*

S. No.	Plant samples	Total flavonoid content (%)
1	Leaves	0.122±0.002
2	Root	0.0677±0.001
3	Stem	0.0724±0.002

Preliminary phytochemical screening actually helps in isolating and characterizing the chemical constituents present in the plant extracts and the knowledge of the chemical constituents is desirable to understand herbal drugs and their preparations and finally in discovering the actual value of folkloric remedies [21]. Phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides, phenols, saponins and tannins present in different extracts exhibit a number of biological activities and protect from most of the chronic diseases [22, 23].

Kalaichelvi and Dhivya (2016) [24] reported the presence of alkaloids, carbohydrates, proteins, glycosides, phenolic compounds, saponins, tannin and terpenoids in aqueous leaf extract and alkaloids, carbohydrates, flavonoids, proteins, glycosides, phenolic compounds, phytosterols, saponins and tannin and in ethanolic leaf extract. Padmapriya *et al.*, (2016) [25] carried out phytochemical screening of methanolic, chloroform and ethanolic extracts of leaves, flowers and stem of *M. annua*. The study showed positive results for carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, terpenoids, phenols, proteins, steroids and phylobatannins for methanolic leaf extract. Methanolic flower extract showed positive results for eight phytochemicals, carbohydrates, tannins, saponins, flavonoids, quinones, phenols, coumarins and proteins. Methanolic stem extract registered positive results for carbohydrates, flavonoids, quinones, cardiac glycosides and coumarins. The chloroform extracts of leaf, stem and flowers did not show positive results in both leaf and stem extract for the above said chemicals. Flowers extract registered significant results for carbohydrates and quinones only. Ethanolic leaf extract showed positive results for alkaloids, quinones, terpenoids and phenols while flower and stem extracts registered negative results.

Conclusion

In present investigation, phytochemical screening of targeted species revealed that all extracts i.e. aqueous, methanolic and ethanolic of leaves, roots and stem were found to contain a certain categories of chemical compounds which authenticate the use of leaves, roots and stem of *M. annua* as the potential source of useful drugs in traditional system of medicine to cure diseases and improve human health. Moreover, further investigation can be planned for purification, identification and characterization of active lead compounds of pharmaceutical importance from this plant.

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