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# PRELIMINARY PHYTOCHEMICAL SCREENING AND *In vitro* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF DIFFERENT PARTS OF *Martynia annua* L.

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**Abstract**

Free radicals act as an chemicals they are highly unstable and it cause damage to other molecules by extracting electrons from them in order to attain the stability. Antioxidant plays a vital role in protecting the biological systems against the development of more chronic diseases and disorders. The aim of the present study was to screen the phytoconstituents, and to assess the *in vitro* antioxidant activity of various parts of *Martynia annua* plant belonging to the family Martyniaceae. The *in vitro* antioxidant activity of MaF, MaL and MaS was measured by means of the 1, 1-diphenyl-2-picrylhydrazyl DPPH assay. The phytochemical investigation of oil of *M. annua* revealed the presence of glycosides, steroids, and terpenoids compounds. Among this MaF shows the antioxidant activity than the MaL and MaS. The outcomes justifies the flower *M. annua* is a potential source of natural antioxidants.

**Key words**: Antioxidants, Free radicals, Phytochemicals and *Martynia annua.*

## 1. Introduction

Medicinal plants offer alternative remedies with enormous opportunities to produce income, employment and foreign exchange for under developing countries. Many traditional healing herbs and their parts have been shows its medicinal value and it can be used to prevent, alleviate or cure several human ailments. India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to herbal medicine and it employs a large number of plant species which includes Ayurveda (2000 species), Siddha (1121 species), Unani (751 species) and Tibetan (337 species) (Vijay Wagh *et al*., 2013).

Phytochemistry is the term that deals with chemicals which was derived from plants. There are large numbers of secondary metabolic compounds found in plants. The chemical substances of the medicinal plants it has own capacity of exerting a physiological action on the human body were the primary features. The bioactive compounds of plant such as alkaloids, flavanoids, tannins and phenolic compounds were considered to be more significant. The phytochemical research usually has been done based on the ethno-pharmacological information forms the effective approach in the discovery of new anti-infective agents from higher plants (Gajalakshmi *et al*., 2012).

In ancient Indian system of medicine is mainly based on making the use of most of our native plants. The oxidation induced by the means of reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate the development of various disorders, such as cancer, liver injury and cardiovascular diseases.

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Therefore, the consumption of fruits and vegetables has proven to substantially reduce the risk of cardiovascular diseases, cancers and neurodegenerative diseases including Parkinson's and Alzheimer's diseases. Although, the our own body possesses defense mechanisms, enzymes and antioxidant nutrients which arrest the damaging properties of ROS, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and it causes irreversible oxidative damage. Antioxidant agents are helpful in lowering the risk of various oxidative damages occurring in cell. Several antioxidant agents including chemical and biological materials are available for usage nowadays. Synthetic antioxidant compounds which are used commercially in food processing industries known which is actually leads to side-effects. Recently restriction in the use of some synthetic antioxidants is being imposed because of their carcinogenicity. This has attracted a great deal of research interest in the identification and exploitation of natural antioxidants (Pazhanisamy and Ebenezer, 2013).

*Martynia annua* belongs to the family Martyniaceae and it is a herbaceous erect, branched, glandular hairy annual herb. Fruits are hard, woody with 2-sharp re-curved hooks and seeds are oblong. It is commonly known as Bichchhu, used in epilepsy and applied locally to tuberculosis glands of camel's neck. The juice of leaves is used as a gargle for sore throat, fruits used for inflammation, leaf paste has beneficial effect when applied to the bites of venomous insects and wounds of domestic animal (Santram Lodhi, 2011). The present investigation was carried out to evaluate the phytoconstituents present in the different parts of *Martynia annua* plant and its antioxidant activity.

## 2. Materials and Methods

### Collection of plant material

*Martynia annua* flower, leaves and stem were collected from Tirupattur, Vellore district, Tamilnadu, India. The collected plant parts were washed thoroughly in tap water, shade dried, finely powdered and stored for further research.

### Preparation of organic extracts of various parts of *Martynia annua*

Twenty gram of flower, leaves and stem powder of *Martynia annua* was extracted with 100 ml of methanol for 24 hours. The extract was then distilled and evaporated to dryness. The concentrated extract were then accurately weighed and stored in small vials at 20 $^{\circ}$C for further studies.

### Qualitative screening of phytochemical constituents

Phytochemical screening was performed using standard procedures. The presence or absence of alkaloids, flavonoids, saponins, phenols, glycosides (Raaman, 2006), tannins, reducing sugar (Iyengar, 1995), phytosterol, terpenoids (Siddiqui and Ali, 1997) and Anthraquinones (Ayoola *et al*., 2008) were detected in flower, leaves and stem of *Martynia annua*.

#### *Test for Alkaloids*

Small amount of solvent free extract was dissolved in Diluted HCl. A volume of 1.2 ml of this extract was mixed with 0.1 ml of Mayer's reagent. Formation of white precipitate shows the presence of alkaloids (Evans, 1997).

#### *Test for Flavonoids*

A pinch of the extract was dissolved in 5 ml of distilled water. Ten % of Sodium hydroxide was prepared and mixed with the extract. Formation of yellow colour which disappears by the addition of Diluted HCl shows the presence of flavonoids (Trease and Evans, 2002).

#### *Test for Glycosides*

Two ml of the sample was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus that is a glycone portion of glycoside (Trease and Evans, 1985).

***Total Phenolic content***

The mixture was prepared dissolving 0.1 mg of sample in 1 ml of methanol. From the mixture, 20 µl was taken and 180 µl of water was added. Then, 0.5 ml of Folin's phenol reagent, 0.5 ml of water 1 ml of 7.5 % sodium carbonate was added to the mixture. Then, it was kept 2 hours for incubation. Absorbance was read at 726 nm by spectrophotometer. Gallic acid was used as phenol standard and expressed as Gallic acid equivalent (Wolfe and Liu, 2003).

***Test for Saponins***

A pinch of the extract was dissolved in 1 ml of distilled water. It was warmed in the heating mantle for 2 min at 60 °C. Then, 0.5 ml of distilled water was added to it and shaken well. Appearance of froth on the top layer shows the presence of saponins (Sofowara, 1993).

***Test for Steroids***

A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroid, development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids (Gibbs, 1974).

**Figure – 1: *Martynia annua* Plant**

***Test for Tannins***

About 2 ml of the sample was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. Formation of green precipitate was indication of presence of tannins (Odebiyi and Sofowora, 1978).

***Test for Terpenoids***

A small amount of extract was dissolved in 1 ml of chloroform and 1 ml of conc. sulphuric acid formation of reddish brown coloration confirms the presence of Terpenoids (Sofowara, 1993).

**Determination of DPPH Radical Scavenging activity (Mensor *et al*., 2001)**

DPPH (1,1'- diphenyl-2-picryl hydrazyl hydrate) radical reacts with an antioxidant compound, which can donate hydrogen and gets reduced. The change in colour from deep violet to yellow can be measured at 518 nm. A methanolic solution of 0.1 mM DPPH (0.5 ml) was added to equal volume of the sample homogenate (20 % homogenate was prepared in Tris EDTA buffer, pH 7.2) and allowed to react at room temperature. DPPH in methanol without plant extract served as positive control. After 30 minutes, the mixture was centrifuged and the absorbance of the supernatant was measured at 518 nm and converted into percentage radical scavenging activity as follows.

Scavenging activity (%) = A518 (Sample) – A518 (Blank)/ A518 (Blank) × 100

**Figure – 2: *Martynia annua* flowers**



**Figure – 3: *Martynia annua* stem**

**Figure – 4: *Martynia annua* leaves**

## 3. Results and Discussion

Phytochemicals are the biologically active substances which are present in the parts of plants. It was also reported that the pharmacological activity of the medicinal plants was mainly due to the presence of the phytochemicals. In the present research, preliminary phytochemical analysis of various parts of *Martynia annua* plant was tested and the results were furnished in Table – 1. Phytochemicals like Tannins, Terpenoids, Steriods and Phenols are present in methanolic extract of *Martynia annua* flower (MaF). Phenols, Glycosides and Steriods are presnt in the methanolic extract of *Martynia annua* leaves. In the methanolic extract of *Martynia annua* stem, Phenols Steriods and Terpenoids are present. Similar results were reported by Aris *et al*. (2009) who showed that the total phenolic content of three different extracts of fruits of *Ficus deltoidea* var *angustifolia*. It was also found that the hexane extract showed the highest phenolic concentration of 259 mg/g of GAEs followed by methanol extract (245 mg/g of GAE s) and chloroform extract (159-2 mg/g of GAE s). Sengul *et al*. (2009) reported that the total phenolic content varied of the crude extracts of *Viscum album* and *Crocus sativus* had the highest total phenolic contents 42 -29 mg GAE/g DW respectively. Mayur *et al*. (2010) reported that the methanolic extract of *Carpesium abrotanoides* was found to possess considerable amount of phenols. Santhi *et al*. (2012) concluded that the aqueous extract of *Nerium olender* and *Momordica charantia* leaves revealed the presence of carbohydrate, cholesterol, proteins, amino acids, alkaloids, flavaonoids, tannins, saponins, cardiac glycosides, terpenoids and phlobaotinins followed by ethanol, ethyl acetate, diethyl ether and chloroform.

The DPPH (1,1'- diphenyl-2-picryl hydrazyl hydrate) radical reacts with an antioxidant compound, which can donate hydrogen and gets reduced. The change in colour from deep violet to yellow can be measured at 518 nm. DPPH Radicals Scavenging activity of various parts of *Martynia annua* (Flower, Leaf and Stem) was investigated in the present research and the results were furnished in Table – 2. The antioxidant activity of *Martynia annua* plant parts was estimated at 0 minutes and 16 minutes and the Scavenging activity percentage of DPPH radicals was maximum at 16 minutes when compared to 0 minutes. Among the three plant parts of *Martynia annua* tested, highest Scavenging activity was noticed in the methanolic flower extract of *Martynia annua* (37.64 % at 0 minutes and 73.54 % at 16 minutes) followed by the methanolic leaf extract of *Martynia annua* (21.03 % at 0 minutes and 56.45 % at 16 minutes) and the lowest scavenging was recorded in the methanolic stem



extract of *Martynia annua* (37.64 % at 0 minutes and 73.54 % at 16 minutes).

**Table – 1: Preliminary phytochemical analysis of various parts of *Martynia annua* plant**

| Phytoconstituents | MaF | MaL | MaS |
|---|---|---|---|
| Alkaloids | - | - | - |
| Flavanoids | - | - | - |
| Total phenolic content | + | + | + |
| Glycosides | - | + | - |
| Steriods | + | + | + |
| Saponins | - | - | - |
| Tannins | + | - | - |
| Terpenoids | + | - | + |

"+" = Present and "-" = Absent

**Table – 2: DPPH Radicals Scavenging activity by various parts of *Martynia annua***

| Plant extract | 0 Min. | Percentage (%) | 16 Min. | Percentage (%) |
|---|---|---|---|---|
| MaF | 0.151 | 37.64 | 0.164 | 73.54 |
| MaL | 0.254 | 21.03 | 0.270 | 56.45 |
| MaS | 0.347 | 6.03 | 0.368 | 40.64 |

## 4. Conclusion

The results of the present study clearly indicated that the presence of significant level of phytochemical compounds enhances the free radical scavenging activity in various parts of *Martynia annua* plant that could protect against oxidant and free radical injuries. Thus, the flower of *Martynia annua* was effective source and it could be employed in all medicinal preparations to combat diseases associated with oxidative stress including cancer, diabetes and related disorders.

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