Preliminary Phytochemical Screening and *In Vitro* Antioxidant Efficacy of Fruit Oil of *Martynia annua*

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Article Information

Received 08 December 2013

Received in revised form 05 Feb 2014

Accepted 10 Feb 2014

Keywords:

Martynia annua

DPPH

Superoxide

Polyphenol

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DOI: [10.20510/ukjpb/2/i1/91138](https://doi.org/10.20510/ukjpb/2/i1/91138)

Abstract

The free radicals play vital role in induction of various diseases. Natural antioxidants may help the body to protect itself from various types of oxidative damage which are linked to diseases such as cancer, diabetes, cardiovascular disorders and aging. *Martynia annua* belonging to Martyniaceae family are found to be a rich source of substances of phytochemical interest. The aim of the present study was to screen for phytoconstituent, and to determine the *in vitro* antioxidant activity of fruit oil of *M. annua*. The oil were extracted from fruit of *M. annua* by employing traditional methods. Preliminary phytochemical screening was performed to identify secondary metabolites in extracted oils. The *in vitro* antioxidant activity of oil was measured by means of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Super oxide free radical scavenging assay and total polyphenol content. The phytochemical investigation of oil of *M. annua* revealed the presence of Alkaloids, glycosides, flavonoids, fats, tannins and phenolic compound. The oil strongly scavenged DPPH radical and superoxide radical with the IC₅₀ being 87.56 µg/ml and 106.80 µg/ml respectively. The oil of *M. annua* exhibited 87.25±1.13 mg/100 gm of total polyphenol content. The outcomes justifies the oil of *M. annua* is a potential source of natural antioxidants.

1 Introduction

Pharmacology, medicinal plants and drugs are become popular words in these days. All are put together to work on a particular medicinal plant and go with their pharmacological, phytochemical studies etc. Some preliminary tests to be screened before preparation of drug¹. According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. The status of herbal medicine has been fast growing all over the world during the last few decades. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources².

Living cells may generate free radicals and other reactive oxygen species by products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases,

such as cancer, diabetes, aging, and other degenerative diseases in humans³. Medicinal herbs are an important source for the therapeutic remedies of various ailments⁴. Plant parts like fruits, tubers, flowers, leaves etc are consumed as principal or supplementary food and employed as medicines. *Martynia annua* belongs to family Martyniaceae (or Pedaliaceae), is a well-known small herbaceous annual plant, distributed throughout India. It is commonly known as the Cat's claw or Devil's claw refers to the inner woody capsule which splits open at one end into two curved horns or claws⁵. *Martynia annua* contains alkaloids, tannins, saponins, glycosides, flavonoids, anthocyanins, amino acid, steroids and phenols. From the available literature, it is known that the whole plant is used as medicine⁶. The leaves are used in epilepsy and applied locally to tuberculous glands of camel's neck; the juice of the leaves as a gargle for sore throat, fruit in inflammation, paste of the nut has beneficial effect when applied to the bites of venomous insects, and the leaf paste for wounds of domestic animals. The whole plant is also used by Santal tribals (India) for fever, hair loss, scabies, sores and carbuncles on the back⁷.

Antioxidants may play a role in helping to prevent diseases. Antioxidants are thought to help because they can neutralize free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective and that effectiveness declines with age. Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides ($\text{ROO}^\cdot$) and H_2O_2 and also by sequestering metal ions, scavenging active free radicals, repairing and/or clearing damage. Similarly, some antioxidants also induce the biosynthesis of other antioxidants or defence enzymes⁸. Looking its exclusively used in treatment of various disease by tribal people, we planned to evaluate the preliminary phytochemical and *in vitro* antioxidant activity of fruit oil of *M. annua*.

2 Materials and Methods

2.1 Plant collection and identification

The fresh fruit of *M. annua* were collected from the tribal area of Bilaigarh, Balodabazar District, Chhattisgarh State, India. The collected material was authenticated by Dr. A.P. Singh, Principal Scientist, Department of Agronomy College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Voucher specimen (AGRO/WC/13/254) was deposited in the herbarium of the department of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) for future reference.

2.2 Preparation of the crude extracts

The oil from fresh fruit was extracted by traditional method, which was developed by tribal people. For the extraction of oil two pots are arranged in vertical position and upper pot had holes on the center of bottom. The upper container was filled with dried fruits and closed with lid. The lower container was buried underground. The heat was applied on upper container for 1-2 hours. The oil in the fruit was melt and gets collected in the lower pot. The whole process is illustrated in figure 1. The collected oil was used for the further studies.

2.3 Preliminary Phytochemical analysis

Preliminary phytochemical screening was performed to identify secondary metabolites (phytoconstituent) in extracted oil^{9,10}.

2.4 In vitro antioxidant activity

2.4.1 Hydrogen-donating activity

This assay was used in many studies for testing antioxidant activity. 2,2-diphenyl-1-picryl-hydrazil stable radical (DPPH) evidently offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants. This assay was based on the reduction of a methanolic solution of the colored free radical DPPH by free radical scavenger. The degradation of DPPH was evaluated by comparison with a control sample without hydrogen-donating

compounds. The decrease in absorbance of DPPH at its absorbance maximum of 517 nm was proportional to the concentration of free radical scavenger added to DPPH reagent solution. Lower absorbance of reaction mixture indicated higher antioxidant activity¹¹⁻¹⁴.

In this study, methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of each extracts dissolved in methanol was added at different concentrations (50-250 $\mu\text{g/ml}$). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation^{15,16}.

$$\% \text{ AA} = 100 - \left\{ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{DPPH}}} \right\}$$

2.4.2 Superoxide scavenging activity

Superoxide scavenging was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8ml of an aqueous solution containing nitrobluetetrazolium (56 mM), EDTA (10 mM) and potassium phosphate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-250 $\mu\text{g/ml}$) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO¹⁷⁻¹⁹.

2.4.3 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 2.0 ml of the prepared extract was oxidized using Folin - Ciocalteu reagent (400 μl), and sodium carbonate solution (75 g/l) was then added to the reaction mixture to reach a 10.0 ml volume. After 2 h, the suspension was centrifuged for 10 min at 5000 rpm, and absorption was measured at a 760 nm wavelength. The amount was calculated using the gallic acid calibration curve²⁰⁻²³. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample.

2.5 Statistics Analysis

The data were reported as mean values $\pm$ standard deviation (SEM). Values representing the concentrations of investigated oil that cause 50% of neutralization/inhibition (IC_{50}) were determined by the linear regression analysis.

3 Results

3.1 Phytochemical study

The evaluation was done to determine the nature of phytoconstituent present in fruit extracts. The phytoconstituent may be useful indicator

of both efficacy and potential toxicity of plants. The phytochemical

screening of oil extracts of *M. annua* are exhibited in table 1.

Fig 1: Extraction of oil from fruit of *M. annua*

Table 1: Phytochemicals present in oil of *M. annua*

	Phytoconstituent	Oil
Alkaloids	Dragendorff's test	+
	Hager's test	+
	Legal's test	+
Glycosides	Keller killiani test	+
	Saponin glycoside	+
	Coumarin glycoside	-
	Molish test	-
Carbohydrates	Benedict's test	-
	Tannic acid test for starch	-
	5%FeCl ₃ solution	+
	Lead acetate solution	++
Tannins and Phenolic compound	Bromine water	+
	Acetic acid solution	-
	Dilute potassium permanganate solution	+
Flavonoids	Shinoda test	++
Steroid test	Liebermann burchard test	-
	Liebermann's reaction	-
Protein	Biuret test	-
	Ninhydrin test	-
Fat and oil test	Solubility Test	++
	Filter paper staining	+

+ = Detected, ++ = Strongly detected, - = Not detected

3.2 *In vitro* antioxidant activity

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichiometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in Table 2, oil of *M. annua* strongly scavenged DPPH radical with the IC₅₀ being 87.56 µg/ml (Fig. 2). The scavenging was found to dose dependent.

SOD is an important cellular antioxidant enzyme, which converts superoxide radical into H₂O₂ and O₂. We also looked for the protective effect of the both extract on antioxidant enzyme SOD in mitochondria exposed to H₂O₂. Table 3 gives the changes in the activity of SOD upon treatment with the both extracts. The oil of *M. annua* moderately scavenged superoxide radical with the IC₅₀ values of 106.80 µg/ml (Fig. 3).

The oil of *M. annua* was prepared for examination of the total phenolic content. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method. The total phenolic content in oil, expressed as gallic acid equivalents. The oil of *M. annua* exhibited 87.25±1.13 mg/100 gm of total polyphenol content.

4 Discussions

The phytochemical investigation of oil of *M. annua* revealed the presence of Alkaloids, glycosides, flavonoids, fats, tannins and phenolic compound. The flavonoids, tannins and phenolic compound are known to be useful in the treatment of various diseases such as cancer, hepatotoxicity, ulcerated tissue, cardiovascular diseases, diabetes etc. Hence, the presence of these bioactive components in oil of *M. annua*, may serve as a potential source of drug for traditional healers.

The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the oil. The rates of DPPH scavenging activity of oil are probably due to the presence phenolic compounds. Our study clearly indicated that the oil of *M. annua* exhibited high content of phenolic compounds which was significantly correlated with the DPPH radical scavenging activity.

From results, it was found that the oil showed strongly free radical scavenging activity. The oil donated their electrons to the superoxide and scavenge them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product^{24,25}. The findings of results revealed that the oil of *M. annua* displayed high content of flavonoids, which was significantly correlated with the superoxide radical scavenging activity.

Table 2: Free radical scavenging capacity of oil of *M. annua*

Concentration ($\mu\text{g/ml}$)	DPPH Scavenging %	
	Ethanol Extract	Ascorbic Acid
50	38.62 $\pm$ 0.56	96.43 $\pm$ 0.48
100	52.82 $\pm$ 0.73	-
150	69.73 $\pm$ 0.59	-
200	87.19 $\pm$ 1.02	-
250	98.21 $\pm$ 0.95	-
IC ₅₀	87.16	-

Values are mean $\pm$ SEM of six determinations

Fig. 2: IC₅₀ values of oil of *M. annua*

From the result of DPPH and superoxide radical scavenging activity it was observed that the oil of *M. annua* showed highest DPPH radical scavenging activity and maximum superoxide radical scavenging activity. It indicates the presence of different character of antioxidant components in crude oil of *M. annua*. From these results it can be concluded that antioxidant activity of oil depends on the presence of quality of active constituents, because each *in vitro* antioxidant model has different mechanism to reduce free radicals. Earlier many researchers have reported that the antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents.

It is well documented that plant flavonoids and phenols in general, are greatly effective free radical scavenging and antioxidants²⁶. Polyphenol and flavonoids are used for the prevention and cure of various diseases and skin disorders, which are mainly associated with free radicals. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions²⁷⁻²⁹. It has been reported that compounds such as the flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or

chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants³⁰. The findings of total polyphenol content of oil of *M. annua* support the study of DPPH and superoxide scavenging capacity of extracts.

Table 3: Superoxide scavenging capacity of oil of *M. annua*

Concentration ($\mu\text{g/ml}$)	Superoxide Scavenging %	
	Ethanol Extract	Ascorbic Acid
50	36.23 $\pm$ 0.84	89.12 $\pm$ 0.73
100	49.53 $\pm$ 0.57	-
150	59.47 $\pm$ 0.61	-
200	71.83 $\pm$ 0.49	-
250	86.28 $\pm$ 0.93	-
IC ₅₀	106.80	-

Values are mean $\pm$ SEM of six determinations

Fig. 3: IC₅₀ values of oil of *M. annua*

5 Conclusions

It is well known that free radicals play important role in leading of several diseases. The finding of *in vitro* antioxidant activity of fruit oil of *M. annua*, and this justified its uses in folkloric medicines. Further studies are in progress for the isolation of active constituents responsible for antioxidant activity.

6 References

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