

Antioxidant Activities and Phytochemical Screening of *Martynia annua* Fruit Extract

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Martynia annua (cat's claw, bichu) is part of *Martyniaceae* family. For centuries, extracts of leaves, roots, stems, fruits and seeds of *M. annua* have been used to cure epilepsy, inflammation, tuberculosis, skin infections etc. Present investigations revealed qualitative phytochemical screening and bioactivities of fruit extracts of *M.annua* in solvents of different polarities. The qualitative phytochemical analysis exhibited the presence of alkaloids, flavonoids, glycosides, saponins, tannins, anthocyanins, steroids, amino acids and phenols. DPPH (1,1-diphenyl-2-picryl hydrazyl free radical scavenging, reducing power assay and lipid peroxidation inhibition assay) likability in different solvents were explored which revealed that with increase in concentration of extracts resulted increase in degree of reduction. The outcome of the present studies revealed that the fruit extracts of *M. annua* have eminent antioxidant activity.

Keywords: *Martynia annua*, Phytochemical screening, Antioxidant, DPPH, Lipid peroxidation inhibition, reducing power assay.

For thousands of years, plants have been good source of medicine to treat illness and maintain health. Mostly fruits, flowers, leaves, stem, barks and seeds of plants are rich in secondary metabolites that produce definite pharmacological effects on human body. *M. annua* is an upright short-lived herbaceous plant. The young fruits are oblong and green with long beak(claw), but when dry, becomes woody with two sharp hairy curved hooks.¹ *M. annua* belongs to family *Martyniaceae*. It is native to Mexico, Central America, mostly naturalized in northern Australia and South Eastern Asia. It is commonly known as cat's claw, devil's claw and bichu. The fruits and leaves are biologically active parts of this plant.² It is used for the treatment of epilepsy,

inflammation and tuberculosis.³ The leaves of the *M. annua* are edible and used as antiepileptic, antiseptic and applied locally to tuberculosis glands of the neck.⁴ The juice of the leaves is used as a gargle for sore throat and the leaf paste for wounds of domestic animals.⁵ The unripe fruits of *M.annua* found to have antioxidant activity³ and the ash of fruits mixed with coconut oil are used to cure burns.⁴ The fruits are also used as local sedative and antidote to scorpion stings.⁶ Seed oil is used for abscesses and treating itching and skin infections. The seeds of *M.annua* are used for prevention of graying of hair.⁷ The whole plant is used for fever, hair loss, scabies and abscess on the back.⁸

An antioxidant is a substance that prevents or delays oxidation of other molecules.

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Free radicals are produced during oxidation which can be trapped by antioxidants. In plants, natural exogenic antioxidant substances are present i.e. vitamins phenolic acids, flavonoids, phenolic diterpenes, oils and plant pigments like anthocyanins scavenge free radicals such as peroxide, hydroperoxide or lipid peroxidation. Free radical and reactive oxygen species (ROS) are basically the main causes of several disorders in humans like cancer, heart disease, ageing, diabetes, Alzheimer's, Parkinson's diseases⁹ by inhibiting a reaction cycle. Different methods are used to assess the antioxidant and free radical scavenging activity. *In vitro* antioxidant activity is mostly measured by DPPH method developed by Biols (1958), hydrogen peroxide scavenging assay,¹⁰ nitric acid scavenging activity,¹¹ ferric reducing antioxidant power assay,¹² and reducing power method.¹³ Present investigation reports DPPH, lipid peroxidation and ferric reducing power assay activities of the fruit extracts of *M. annua*.

Study protocol

DPPH assay

By using stable free radical, 1,1-diphenyl-2-picrylhydrazyl, the odd electron of nitrogen in DPPH is reduced by receiving hydrogen from antioxidants to corresponding hydrazine.¹⁴

Reducing power assay

In this method potassium ferrocyanide, ferric chloride and trichloroacetic acid form a colored complex with antioxidant compound increase in absorbance of reaction mixture indicates the increase in antioxidant activity.

The present study revealed the qualitative phytochemical analysis and *in vitro* antioxidant activities of ethanolic fruit extract partitioned in different solvents (ethanol, methanol, *n*-hexane, ethyl acetate, water) by scavenging effect on 1,1-diphenyl-2-picrylhydrazyl, ferric reducing power and lipid peroxide inhibition assay to protect the oxidative damage.

MATERIAL AND METHODS

The fruits of *M. annua* were collected from local market in december 2012 and identified by Dr. Rubina Dawar, Plant Taxonomist, Department of Botany, University of Karachi. The fruits of *M. annua* were dried (20kg) crushed and soaked in 30L of ethanol for 3 weeks. The extraction with

ethanol was repeated thrice at room temperature. The extract was evaporated in rotary evaporator under pressure to yield a gum (1789 g). This gum was suspended in water (1L) and extracted with different solvents yielding *n*-hexane soluble fraction (910g), ethyl acetate soluble fraction (364g) and *n*-butanol (386 g). All the chemicals used were of analytical grade (Merck) and BDH.

Phytochemical analysis

Qualitative phytochemical analysis of fruit extracts of *M. annua* in (Ethanol, ethyl acetate, *n*-Hexane, *n*-Butanol, water) for the identification of alkaloids, flavonoids, terpenoids, saponins, tannins, anthocyanins, steroids and cardiac glycosides were done by prescribed methods.¹⁵⁻¹⁷

Test for Alkaloids

- Dragendorff's Test: 5 mg of each extract was mixed with 1% HCl (5 mL) and heated on water bath for 5 minutes. After filtration, 2mL filtrate was treated with solution of potassium bismuth iodide (Dragendorff's reagent). Formation of red ppt indicated the presence of alkaloids.

- Mayer's Test: 2 mL of filtrate was treated with 1 mL potassium mercuric iodide solution (Mayer's reagent). Formation of cream coloured ppt indicated the presence of alkaloids.

Test for Flavonoids

- Alkali Test: 5mg of each extract was dissolved in water. Filtrate was treated with 2mL of 10% NaOH To yield yellow coloured solution formed. On addition of HCl it became colorless showing the presence of flavonoids.

- Filtrate was treated with 2 mL of dilute ammonia solution. Yellow color disappear after addition of 1 mL sulphuric acid indicated the presence of flavonoids. c. Lead acetate test: 2 mg of each extract (ethanol, ethyl acetate, *n*-hexane, *n*-butanol, water) was treated with few drops of lead acetate solution. Formation of yellow solution showed the presence of flavonoids.

Test for Terpenoids and Steroids

Salkowski's Test: 2mg of each extract was mixed with 2 mL of chloroform then 3 mL of sulphuric acid was added through the walls of test tube to form a layer. A reddish brown colour of interface showed the existence of terpenoids or steroids.

Test for Saponins

Foam Test: 5 mg of each extract was shaken with 2 mL of water. Foam produced

persisted for ten minutes proving the presence of saponins.

Test of Tannins

Ferric chloride test: 5 mg of each extract was boiled with 10 mL of water then filtered. Few drops of 0.1% ferric chloride were added in the filtrate. Bluish green ppt indicated the presence of tannins.

Potassium dichromate test: Filtrate(above) was treated with few drops of potassium dichromate, dark colour is developed showed presence of tannins.

Test for Phenols

2 mg of each extract were mixed with few drops of ferric chloride. Bluish black coloured appearance showed the presence of phenols.

Test for carbohydrate

Molish Test: 2 mg of each of extract was dissolved in water then ethanol was added followed by few drops of α -naphthol. Sulphuric acid was added through the walls of test tube. Violet ring appeared at the junction of two layers indicating the presence of carbohydrates in the fruits.

Test for glycosides

Keller-Killiant Test: 1 mg of each of extract was mixed 5 mL of water. 2 mL of glacial acetic acid with few drop of ferric chloride were added. The sulphuric acid was then poured through the wall of test tube. Formation of brown ring at the interface proved the existence of glycosides.

Test for Anthocyanins

2 mg of each extract was treated 2 mL (2N) HCl. Reddish pink colour appeared which was changed to purple after addition of ammonia. proving the existence of anthocyanins.

Antioxidant activity

DPPH Free radical scavenging activity

Determination of DPPH radicals scavenging activity was estimated by method given by Ishikawa,¹⁸ and with slight modification by Ayoola GA, Sofidiya T (2006).¹⁹

The radical scavenging activities of different concentration of fruit extracts of *Martynia annua* in water, ethanol, *n*-hexane, *n*-butanol and ethyl acetate against DPPH were determined by spectrophotometer at 517 nm. The stock solution (1M) of DPPH was prepared in methanol. The 0.01 mM solution of DPPH was prepared by adding 90 mL of methanol to 10 mL of stock solution. Vitamin C (Ascorbic acid) was used as an antioxidant standard. The concentrations of standard and fruit extracts prepared were 10, 20, 30, 40, 50 and 60 mg/mL. The solutions of fruit extracts were treated with stable DPPH radical. The reaction mixture was prepared by adding 3 mL of 0.1 mM solution of DPPH in 1 mL each of the different concentration of fruit extracts of *M.annua* and allowed to stand for 30 minutes in the dark. The inhibition of DPPH radical by the reaction of antioxidant was observed by change in colour from deep violet to light yellow. A blank solution was prepared containing same amount of DPPH and methanol. Tests were carried out in triplicate and the absorbance was recorded at 517 nm by using double beam UV/Visible spectrophotometer. The percent inhibition was calculated by following formula.

$$\% \text{ Inhibition} = 100 \times (A_b - A_a / A_b).$$

where A_b is absorbance of blank and A_a is absorbance of extract.

Table 1. Phytochemical Analysis of Fruit Extract of *Martynia Annua*

Compounds	Aqueous	Ethanol	<i>n</i> -Hexane	Ethyl Acetat	<i>n</i> -Butanol
Alkaloids	-	+	+	-	-
Flavonoids	+	+	+	+	+
Terpenoids	+	+	-	+	-
Saponins	+	-	+	+	+
Steroids	-	+	+	+	-
Tannins	+	+	+	+	+
Phenols	+	+	-	+	+
Glycosides	+	-	-	+	+
Anthocyanins	+	-	-	+	-

% inhibition of both samples and blanks was calculated for each concentration. Graphs were plotted against % inhibition and concentration.

Ferric reducing antioxidant power assay (frap)

The reductive power capacity of water, *n*-butanol, ethanol, ethyl acetate and *n*-hexane extracts of *M. annua* fruit (method used was carried out by Oyaizu *et al* 1986). The 1 mL of each extract of increasing concentration (60, 40, 30, 2, 10 mg/mL) was mixed with phosphate buffer (0.2 M, 6.6 pH) and 2.5 mL potassium ferrocyanate (1w/v). The reaction mixtures were incubated at 50°C for 20 mins. 10% trichloroacetic acid (2.5mL) was

added to each solution and centrifuged at 3000 rpm for 10 minutes. Supernatant of each solution was mixed with water (2.5 mL) and 0.1% of FeCl_3 (0.5 mL). The absorbance was recorded at 700 nm using double beam UV/Visible spectrophotometer. The antioxidant activity of extracts was measured by the intensity of absorbance.

Lipid peroxidation inhibition assay

Lipid peroxidation is a chemical process in which unsaturated fatty acids of lipids are damaged by free radicals and oxygen under lipoperoxide formations. Lipoperoxides are unstable and decompose to form reactive carbonyl

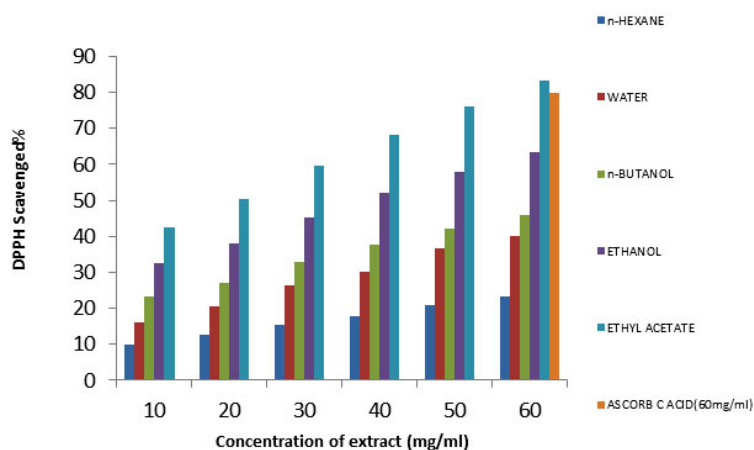


Fig. 1. DPPH free radical scavenging activity of fruit extracts of *Martynia annua* in water, *n*-hexane, ethyl acetate, ethanol and *n*-butanol fractions.

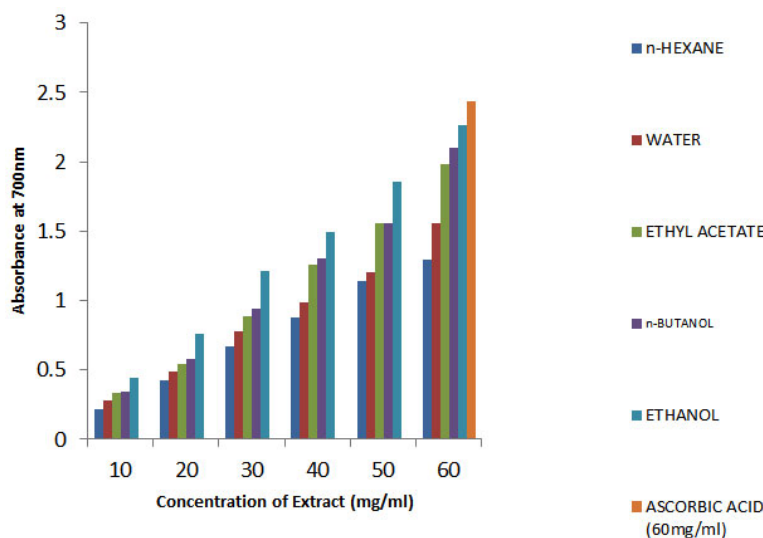


Fig. 2. Reducing power assay of *n*-hexane, water, ethyl acetate, *n*-butanol, and ethanol fractions of leaves extract of *Martynia annua*.

compounds, that damage cells by binding with free amino groups of proteins. One of oldest method for determination of lipid peroxides is iodometric method.²⁰⁻²¹ The principle of this method is based upon ability of lipid peroxidases to oxidize iodide (I⁻) to iodine (I₂), which is further titrated using sodium thiosulphate solution in the presence starch solution as indicator. The procedure used to measure the peroxide value was prescribed by Hortwitz(2002). The 60 mg/mL of each mustard oil treated with fruit extract (ethanol, ethyl acetate, *n*-hexane, *n*-butanol and water). were incubated at 60± 5°C for 4, 8, 12, 16, 20 and 24 days.²²

After incubation, 0.5mL of freshly prepared solution of potassium iodide was added followed by the addition of 30 mL distilled water. The reaction mixture was titrated with 0.1 N sodium thiosulphate in the presence of starch solution. Peroxide value was measured as milliequivalent peroxide per kg oil.

Milliequivalent of peroxide/Kg of oil = $S \times N \times 1000 / \text{Wt of sample}$

S = Volume of sodium thio sulphate used.

N = Normality of sodium thiosulphate.

RESULT AND DISCUSSION

Phytochemical analysis

The qualitative phytochemical screening of fruit extracts of *Martynia annua* (Table 1)

revealed the pressence of secondary metabolites such as flavonoids, terpenoids, saponins, tannins, anthocyanins, steroids and cardiac glycosides. Flavonoids and Tannins are major group of compounds that act as a primary antioxedants or free radical scavengers.

DPPH radical scavenging activity

Figure 1: shows the DPPH scavenging activity of different concentration of fruit extract of *Martynia annua* in water, ethanol, *n*-hexane, ethyl acetate and *n*-butanol. DPPH is nitrogen centered free radical having an odd electron that reacts with a compound that has a H-donating ability. It is reduced to diphenyl picryl hydrazine. After the reaction, the solution changes the colour from dark-purple to yellow which gives a strong absorbance at 517nm. This analysis showed an increase in DPPH scavenging activity with increase in concentration of extracts. The percentage of inhibition was maximum in a extract having 60 mg/mL concentration. *n*-Hexane extract exhibited 28.06% inhibition, water extract 40.69%, ethyl acetate extract 60.53%, *n*-butanol extract 63.49% and ethanol showed 83.43% inhibition with reference of ascorbic acid (60mg/mL) which revealed 95.8%. Among all, ethanolic fractions possessed highest antioxidant activity.

Reducing power assay

Figure 2 showed: The reducing power capacity of water, *n*-butanol, ethanol, ethyl

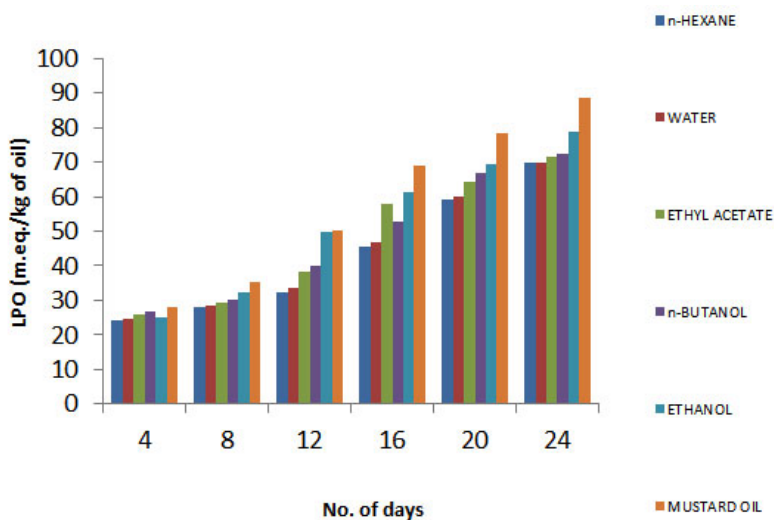


Fig. 3. Lipid peroxide value (meq.Peroxide/kg of oil) of *n*-hexane, water, ethyl acetate *n*-butanol and ethanol fractions of fruit extract of *Martynia annua*

acetate and *n*-hexane extracts of *M.annua* fruits. Antioxidant components present in the extracts converted Fe^{+3} into Fe^{+2} (Benzie, Strain 1996). The results revealed that increase in reducing power activity with an increase in antioxidant i.e. increase in concentration of fruit extracts. Among all, ethanolic fractions were found to possess highest antioxidant capability.

Lipid peroxidation inhibition assay

Fig 3 showed the lipid peroxidation inhibition assay of fruit extracts of *Martynia annua* in water, ethanol, *n*-hexane, ethyl acetate and *n*-butanol. The results exhibited significant protective efficiency of fruit extracts to protect the tissue from oxidative damage. Ethanol extract showed the highest LPOI activity.

CONCLUSION

The present investigation revealed phytochemical screening of the fruit extracts of *Martynia annua*. The qualitative analysis exhibited the presence of secondary metabolites such as flavonoids, alkaloids, steroids, tannins, saponins and glycosides. The fruit extracts also exhibited antioxidant potential which indicated that it can help to improve immune system. Antioxident activities measured by different methods like DPPH free radical scavenging, ferric reducing power and oxidative stress mechanism by lipid peroxidative assays. The ethanolic fraction showed maximum extent but in water these activities were also significant. The phenolic compounds and flavonoids are responsible of antioxidant activities.

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