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Preliminary Phytochemical Screening and *In vitro* Antioxidant Activity of Methanolic Extract of *Tropaeolum majus* L. Seed

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Authors' contributions

This work was carried out in collaboration between all authors. Author MUH designed the study and performed the statistical analysis. Author MW wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors MSI and AK managed the literature searches. Authors MU, BAC and SA supervised the research work. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the preliminary phytochemical screening and *in vitro* antioxidant activity of methanol extract of *Tropaeolum majus* L. (*T. majus*) seeds.
Methods: Phytochemical screening was performed by using standard methods. The antioxidant study was done by using *in vitro* method such as 2, 2-diphenyl-2-picrylhydrazyl (DPPH).
Results: Qualitative phytochemical analysis reveals presence of alkaloids, flavonoids and tannins. The extract showed moderate antioxidant activity against the tested method.

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Conclusion: There is an indication that seeds of *T. majus* contains important phytochemicals and an antioxidant capacity comparable with standard antioxidant compounds that may be linked to its beneficial effects on health.

Keywords: Tropaeolum majus L.; phytochemical; antioxidant; DPPH.

1. INTRODUCTION

Oxidative stress is a condition where the homeostasis between the oxidants and antioxidants are disturbed with inclination of more oxidative species being generated than the amount of counter-oxidative species being produced in defence [1]. Free radicals are the molecules with unpaired electrons and commonly called reactive oxygen species (ROS). Free radicals are generated during the process of cellular oxidation, some examples includes superoxide anion, hydrogen peroxide, hydroxyl and nitric oxide radical. These radicals are electrically charged, unstable and highly reactive in nature [2]. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases [3]. Natural antioxidants taken exogenously are helpful in scavenging the free radicals [4]. The user of natural antioxidants from fruits, vegetables and plants sources are increasing in recent years. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer [5,6]. The important natural antioxidants groups are polyphenols or flavonoids. These groups have beneficial effects on human health, mostly due to their ability to neutralize reactive oxygen species (ROS) and to have antioxidant activity [7].

Natural antioxidants are safer than the synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT). Because synthetic antioxidants causes liver damage and are carcinogenic [8]. Increased intake of food which is rich in antioxidants lowers the risk of degenerative diseases, particularly cardiovascular diseases and cancer [9]. Therefore, strict government rules regarding the safety of the food have necessitated the search for safer alternatives as food preservatives [10].

T. majus from family Tropaeolaceae commonly known as big-nasturtium [11]. In herbal medicine the plant is used as expectorant to relieve chest conditions, disinfectant, wound-healing and antibiotic [12,13].

Reviving the available literature nothing was traced regarding antioxidant activity of *T. majus* seed. This encourages the author to investigate such *in vitro* antioxidant activity in *T. majus* seed.

2. MATERIALS AND METHODS

2.1 Chemicals Used

Potassium mercuric iodide (BDH), Potassium iodide (BDH), Potassium bismuth iodide (Riedel de Haen), Picric Acid (Riedel de Haen), ammonia solution (Sigma Aldrich), sodium hydroxide (Riedel de Haen), lead acetate solution (Uni Chem), hydrochloric acid (Riedel de Haen), sodium chloride (Sigma), Acetic acid (Sigma Aldrich), Ferric chloride, Sulphuric acid (Sigma Aldrich), Methanol (Sigma Aldrich), DPPH (2,2-diphenyl-1- picryl-hydrazyl), Gallic acid (BDH), Dichloromethane (Sigma Aldrich). All the chemicals used including the solvents, were of analytical grade.

2.2 Plant Material

The seeds of *T. majus* were collected at the final stage of maturity from surroundings of Multan in the month of May 2013. The seeds were identified by Professor Dr. Altaf Ahmad Dasti, plant taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. The collecting seeds were cleaned to remove dust then dried under shade. The dried sample was powdered in electric grinder and used for solvent extraction.

2.3 Preparation of Seed Extract

The air dried seed sample of *T. majus* was first defatted by using *n*-hexane to remove lipids. For such a purpose, the dried seeds (250 g) were finely grounded and defatted in *n*-hexane (150 mL) over 12 hours. Then defatted sample was dried and soaked with methanol (200 mL) for 12 hours. Same process was repeated thrice. The

obtained extract (600 mL) was filtered by using muslin cloth and evaporated to dryness by using rotary evaporator (Rota vapor R-200, Buchi, Switzerland) [14].

2.4 Phytochemical Analysis

Phytochemical screening of seeds extract was conducted by standard qualitative analytical methods for identification of diverse phytocompounds such as alkaloids, saponins, Anthraquinones glycosides, flavonoids, tannins and cardiac glycosides.

2.4.1 Test for alkaloids

Methanol extract was dissolved in dilute HCl. After filtration through Whattman filter paper, filtrate was divided in five portions and test were performed according to specified methods [15].

2.4.1.1 Mayer's test

In 1 mL of filtrate 1 mL of potassium mercuric iodide solution (Mayer's reagent) was added. Formation of yellow colored precipitate indicates the presence of alkaloids.

2.4.1.2 Wagner's test

To the filtrate Wagner's reagent was added. Formation of brown precipitate indicates the presence of alkaloids.

2.4.1.3 Hager's test

Filtrate was treated with Hager's reagent. Yellow colored precipitate formation indicates the presence of alkaloids.

2.4.1.4 Dragendorff's test

To 2 mL of filtrate, 5 mL of distilled water was added. 2M hydrochloric acid was added until reaction occur. Then 1 mL of dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids [16].

2.4.2 Detection of saponins

2.4.2.1 Frothing test

Small amount of extract was diluted with 20 mL of distilled water and shakened for 15 minutes. Formation of foam layer indicates the presence of saponins [15].

2.4.2.2 Foam test

Small amount of extract was diluted with little amount of distilled water. Formation of persistent foam indicates the presence of saponins [15].

2.4.3 Detection of anthraquinones

2.4.3.1 Modified borntrager's test

The extract was treated with ferric chloride and heated in water bath for 5 minutes. The mixture was cooled and extracted with benzene. Benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides [17].

2.4.4 Detection of flavonoids

2.4.4.1 Alkaline reagent test

Few drops of sodium hydroxide was added in the extract. Formation of intense yellow color which become colorless on addition of dilute acid indicates the presence of flavonoids [17].

2.4.4.2 Lead acetate test

Few drops of lead acetate were added in 1 ml of extract. Yellow colour precipitate formation indicates the presence of flavonoids [17].

2.4.4.3 Shinoda test

A few fragments of magnesium ribbon were added in the extract and drop by drop hydrochloric acid. Occasionally green to blue colour appears after few minutes indicates the presence of flavonoids [18].

2.4.5 Test for tannins

2.4.5.1 Lead acetate test

To the extract few drops of 10% lead acetate were added. Formation of precipitate indicates the presence of tannins [19].

2.4.5.2 Ferric chloride test

A few drops of 1% neutral ferric chloride solution were added in the plant extract. Blackish- blue

color development indicates tannins presence [19].

2.4.6 Detection of cardiac glycosides

2.4.6.1 Keller-Killani test

In 5 ml extract, 2 ml of glacial acetic acid and one drop of ferric chloride solution was added. Concentrated sulphuric acid was added along the wall of the test tube. Appearance of blue color in the acetic acid layer indicates the presence of cardiac glycosides [19].

2.5 Antioxidant Activity: DPPH Based Free Radical Scavenging Assay

In 96-well plates, 10 µL sample of *T. majus* and 90µl of 100 µM methanol DPPH solutions were added. 100 µl resultant solution was mixed and incubated for 30 min at 37℃. For standard antioxidant drug gallic acid was used. The reduction in absorbance was measured at 517 nm using Synergy HT Bio Tek® USA microplate reader. The % age inhibition of DPPH was calculated from the following formula.

$$\text{Scavenging } \% = \frac{\text{Ac} - \text{Aa}}{\text{Ac}} \text{ X } 100$$

IC_{50} shows at which 50% of the radicals were scavenged by test samples of *T. majus.*

3. RESULTS

Preliminary phytochemical screening of *T. majus* seed contain alkaloids, flavonoids and tannins. DPPH scavenging based free radical scavenging assay shows that the seed extract have % RSA (radical scavenging activity) 82.48 % and the IC_{50} value was 156.13±5.08 µg/ mL. Gallic acid was used as a standard**.**

4. STATICALLY ANALYSIS

All the experiments were performed in triplicates and the data was reported as the ± SEM of the three measurements.

5. DISCUSSION

Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals [20]. The seeds of the plant *T. majus* contain alkaloids, flavonoids and tannins, which shows that *T. majus* have medicinal as well as biological potential. Phenolics compounds (flavonoids and tannins) the phytochemical confirmed to be present in seeds of *T. majus* have antioxidant activity. Flavonoids are becoming very popular because they have many health promoting effects. Apart from this tannic acid has very good antioxidant properties that is very beneficial. The presence of these phytochemicals in seeds of *T. majus* explains the antioxidant properties and its beneficial effect [21].

For the survival of living beings, oxygen is the most essential element on the earth. About 5% of the oxygen reduced to free radical for example hydrogen peroxide, nitric oxide, hydroxyl and superoxide during normal physiological and metabolic processes. [22]. Natural medicinal plants having activity of reducing free radical induced tissue injury, can be used therapeutically as a natural antioxidant [23].

Table 1. Results of preliminary phytochemical screening of methanol extract of *T. majus* seed

Sr. no.	Phytoconstituents	Test	Result
1.	Test for Alkaloids	Hager's test	Present
		Wagner's test	Present
		Mayer's test	Present
		Dragendorff's reagent	Present
2	Test for Saponins	Foam test	Absent
		Froth test	Absent
3.	Test for glycosides	Modified Borntrager's test	Absent
4.	Test for Flavonoids	Lead acetate test	Present
		Alkaline reagent test	Present
		Shinoda test	Present
5.	Test for Tannins	Gelatin test	Present
		Ferric chloride test	Present
6.	Cardiac Glycosides	Killer- Killani test	Absent

Table 2. Antioxidant activity of methanol extract of *T. majus* seed

Drug name	Concentration (mg/mL)	IC_{50}±SEM µg/mL	% RSA (Radical scavenging activity)
T. majus	0.5	156.13±5.08	82.48
Gallic acid	0.5	4.3±0.43	93.13

DPPH is a free radical after accepting a free electron become a stable diamagnetic molecule. DPPH radicals reduction capability is determined by at 517 nm induced by antioxidants. Higher the antioxidants in the extract the more DPPH reduction will be. High reduction is related to the high scavenging capability of the extract [24]. To evaluate the scavenging effect of the extract, DPPH reduction was investigated against positive control gallic acid. The scavenging effects of extract increased with their concentrations to similar extend.

The methanol extract of *T. majus* seeds have free radical scavenging activity 82.48% at concentration of 0.5 mg/mL which is near to the standard gallic acid. Gallic acid have free radical scavenging activity 93.13%. Hence the usage of *T. majus* seeds to be a natural antioxidant source and appears to be an alternative to synthetic antioxidants. Many plant extract exhibit efficient antioxidant properties due to their phytoconstituents, including phenolics. This method has been extensively used for screening antioxidants, such as polyphenols. The antioxidant effectiveness in natural sources has been reported to be mostly due to phenolic compound [25].

6. CONCLUSION

In conclusion, it could be suggested that the extract of *T. majus* seeds possess antioxidant activity. However, further extensive phyto-pharmacological studies are necessary to find out the active principles responsible for these activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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