Research Article

Available online through
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IJRAP
ISSN 2229-3566

ANTIOXIDANT ACTIVITY OF *CUCUMIS MELO* VAR. *AGRESTIS* SEEDS FOR THEIR THERAPEUTIC POTENTIAL

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Received on: 19/06/2011 Revised on: 23/07/2011 Accepted on: 12/08/2011

ABSTRACT
The present study was an endeavor to evaluate antioxidant activity of methanolic extract of *Cucumis melo* var. *agrestis* seeds for their therapeutic potential. *In- vitro* antioxidant activity was performed by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) and Hydrogen peroxide (H_2O_2). The methanolic seed extract was found to have significant scavenging activity 75.59% at 300 µg/ml by 1,1- diphenyl-2-picryl-hydrazyl method and 69.86% at 400 µg/ml by Hydrogen peroxide method as compared to standard (ascorbic acid). Presence of phytochemicals like triterpenoids, alkaloids, tannins, flavonoids, coumarin glycosides, carbohydrates might contribute to observed antioxidant activity.
Keywords*:* *Cucumis melo* var. agrestis seeds, Cucurbitaceae, Antioxidant activity, DPPH, H_2O_2, medicinal plant.

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INTRODUCTION

Free radicals are produced by the body to aid in the metabolic processes, such as digestion and the conversion of food into energy. They are actually quite helpful in many of the body's natural functions. When excessive free radicals are produced in our cells can attack the cell membranes (the outer coat of the cell) causes cell and tissue damage.[1] Radicals can also break strands of DNA (the genetic material in the cell). This oxidative damage caused by the free radicals is considered to play a causative role in ageing and several stress related diseases including cataracts, cognitive dysfunction, cancer, myocardial infarction, diabetes and several heart disease.[2]

ROS/RNS are both playing a dual role as deleterious and beneficial species, since they can be either harmful or beneficial to living systems.[3] In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress.[4] Our bodies try to protect us from free radical damage by producing enzymes that neutralize them. However, they are not capable of handling this function without antioxidants provided by our diets. Antioxidants are protective molecules also referred to as free radical scavengers and hence prevent and repair damage done by these free radicals.[5] Fruits and vegetables are the main source of antioxidants in the diet, are associated with lower risk of degenerative disease.[6] Health problems such as heart disease, macular degeneration, diabetes, cancer are all contributed by oxidative damage. Antioxidants may also enhance immune defense and therefore lower the risk of cancer and infection.

Many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans[7]. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future [8]. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor.

Cucumis melo var. agrestis is commonly known as wild melon, small gourd, wild musk melon is an annual climber belongs to family cucurbitaceae. The fruits can be used as a cooling light cleanser or moisturizer for the skin and has stomachic properties. They are also used as

a first aid treatment for burns and abrasions. Seeds are antitussive, digestive, febrifuge and vermifuge. The extract of seed oil was reported for Antifungal activity.[9] So our present study was carried out to evaluate the antioxidant activity of *Cucumis melo* var. agrestis seeds for their therapeutic potential.

MATERIALS AND METHODS

Cucumis melo var. agrestis seeds were purchased from local grain market of Bathinda in 2010. The seeds were authenticated with the voucher speicemen No.0396 & 1522/120 has been deposited in the Botanical and Environment Science Department, Guru Nanak Dev University, Amritsar, Punjab and NISCAIR Delhi. The seeds were cleaned, washed, dried at room temperature for 2 days and coarsely powdered. The sample was kept in light- protected air tightened containers.

Drugs and Chemicals

DPPH (1,1- diphenyl-2-picrylhydrazyl) was obtained from Sigma Chemical Co .Pvt Ltd. Methanol, ethyl acetate, hexane and sodium hydroxide were of analytical grade and purchased from SD fine chemicals, Merk, Qualigen and Loba chemicals.

Extraction

The powdered seeds were extracted for 72 h for methanol at room temperature. The solvent was filtered off and residue macerated again with the fresh solvent. Both solvents were combined and concentrated under reduced pressure on a rotary evaporator (Hedolph) at 40^0C.The concentrated extract was suspended in distilled water and partitioned successively with hexane and used for further investigation.[10]

Phytochemical Screening

The crude extract was subjected to preliminary phytochemical screening for evaluation of major phytochemical constituents such as alkaloids, tannins, saponins, flavonoids, steroids, anthroquinones, coumarin glycosides, terpenoids and phenolic acids using standard procedure of analysis.[11]

Antioxidant Assay

In vitro models based on reaction between unstable radicals such as DPPH and hydrogen peroxide with plant based antioxidants was used to evaluate antioxidant potential of *Cucumis melo* var. agrestis methanolic seeds extract. Ascorbic acid was used as standard for both methods.

Free radical scavenging activity using DPPH method

Qualitative method

Methanolic seed extract was spotted onto TLC plate and dried. It was developed with mobile phase (Hexane: Ethyl acetate 8: 2). The plate showed yellow coloration when sprayed with 1,1- Diphenyl-2- picrylhydrazyl (DPPH) reagent (methanol 0.2 %) depicting antioxidant activity.[12,13]

Quantitative method

The free radical scavenging activity of test samples were measures by 1,1-diphenyl-2-picrylhydrazyl (DPPH).[14] Briefly, a 0.1 mM solution of DPPH in methanol and 1.5 ml of this solution was added to 0.5 ml of extract solution in methanol at different concentration (50-300µl/ml). The mixture was shaken vigorously and allow to stand at room temperature for 30 min. then the absorbance was measured at 517nm using a spectrophotometer.(Shimadzu UV- 1700 Pharma spec). A blank without DPPH was used to remove the influence of the color of samples. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as a reference drug. All measures were carried out in triplicate. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_O - A_S}{A_O} \times 100$$

Where, A_0 is the absorbance of negative control A_S is the absorbance of sample respectively.

Free radical scavenging activity using hydrogen peroxide

The ability of *Cucumis melo* var. agrestis seed extract to scavenge hydrogen peroxide was determined.[15] An aliquot of 0.6 ml of hydrogen peroxide (43Mm) and 1.0 ml of various concentration of extracts prepared using phosphate buffer (200 – 400µg/ml) were mixed, followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4). The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm. All measures were repeated triplicate. For each concentration, mixture without sample was taken as a control and mixture without hydrogen peroxide was taken as a blank. Ascorbic acid was used as a standard compound. The percentage scavenging of hydrogen peroxide was calculated as:

$$\text{Scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

RESULTS

Preliminary phytochemical screening of methanolic extract of *Cucumis melo* var. agrestis seeds showed the presence of alkaloids, triterpenoids, carbohydrate, protein, amino acid, coumarin glycosides (Table 1). The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm induced by antioxidants. As DPPH reacts with antioxidant gets converted into 1, 1-diphenyl-2- picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The methanolic extract of CMVA showed concentration dependent DPPH radical scavenging

activity. The highest radical scavenging activity of methanolic extract of CMVA was found to be 75.59% at concentration 300µg/ml in case of quantitative analysis and showed yellow coloration in case of qualitative analysis. The results are shown in (Table 2, Graph 1). Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of increase in the hydroxyl radicals in the cells. The hydrogen peroxide scavenging activity of methanolic extract of CMVA is shown in (Table 3, Graph 2).The maximum hydrogen peroxide scavenging activity of CMVA was 69.86 % at a concentration of 400µg/ml. which are comparable to scavenging activity of ascorbic acid.

DISCUSSION

Free radicals contribute to more than one hundred disorders in human. Due to negative effects of synthetic antioxidants nowadays, much attention has been placed on phytoconstituents. Many of the phytoconstituents are beneficial and many of them are acting as natural antioxidants.[16]The results of the present investigation were suggestive of the potential of solvent extracts in scavenging free radical. According to our study, the presence of phytoconstituents such as flavonoids and phenolic compounds, triterpenoids, carbohydrates, proteins and coumarin glycosides in different extracts but methanolic extract showed the maximum presence of phytoconstituents.

Further, *in vitro* antioxidant activity of the extract was evaluated by DPPH and H_2O_2 method. However, Qualitative method was showed that DPPH is decolourised due to the presence of antioxidant compound in methanolic extract while Quantitative method was measured from the decrease in absorbance at 517 nm owing to rapid hydrogen donating ability of DPPH, it react with antioxidants and gets converted into 1,1- diphenyl- 2- picryl hydrazine. Scavenging activity by H_2O_2 may be due to donation of electrons to hydrogen peroxide and thus neutralise it to water. The methanolic seed extract was found to have significant scavenging activity 75.59% at 300 µg/ml by DPPH method and 69.86% at 400 µg/ml by H_2O_2 method as compared to standard (ascorbic acid**).**

CONCLUSION

From the above study it may be concluded that methanolic extract of *Cucumis melo* var. agrestis seeds has significant antioxidant activity which may be responsible for its therapeutic potential. Thus, seed extract can be used to treat diseases caused by free radicals.

ACKNOWLEDGMENT

Thanks to Professor A. C. Rana and all faculty members of Rayat institute of pharmacy for their encourgment and support. We are also grateful to Rayat Bahra education and Research Trust for their unconditional help to carry out this project. Manpreet Kaur conducted the phytochemical screening of the extract and also evaluated the free radical scavenging activity by DPPH and hydrogen peroxide method with the assistance of Rashmi arora.

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Table 1: Phytochemical screening of *Cucumis melo* var agrestis

Chemical constituents	Results
Alkaloids	+ +
Flavonoids	+
Triterpenoids	++
Anthraquinone	-
Carbohydrates	+
Proteins	+
Phytosterols	+
Tannins	-

++ : Maximum Presence of chemical constituents + : Minimum presence of chemical constituents. -: Absence of chemical constituents

Table: 2 Percentage scavenging of 1,1- Diphenyl – 2 Picrylhydrazyl radical

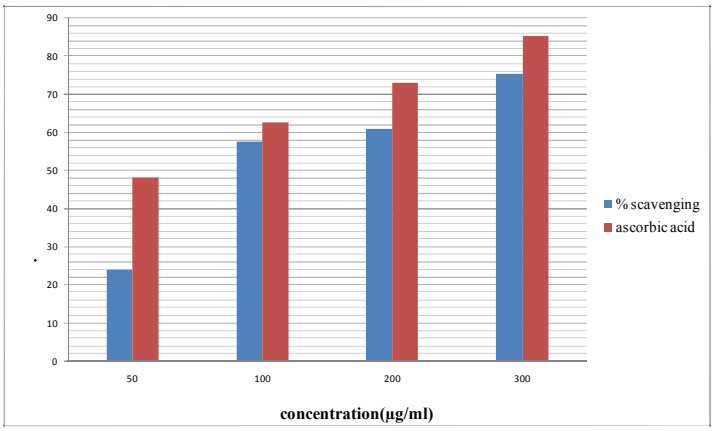
Concentration of extract (µg/ml)	(%) percentage scavenging of DPPH radical	
	Methanolic extract	Ascorbic acid
50	24.01 ± 7.098	48.03 ± 5.078
100	57.59 ± 8.934	62.74 ± 9.34
200	61.02 ± 9.023	73.03 ± 4.26
300	75.59 ± 6.716	85.4 ± 5.10

Values are the average of triplicate experiments and represented as mean ± S.E.M.

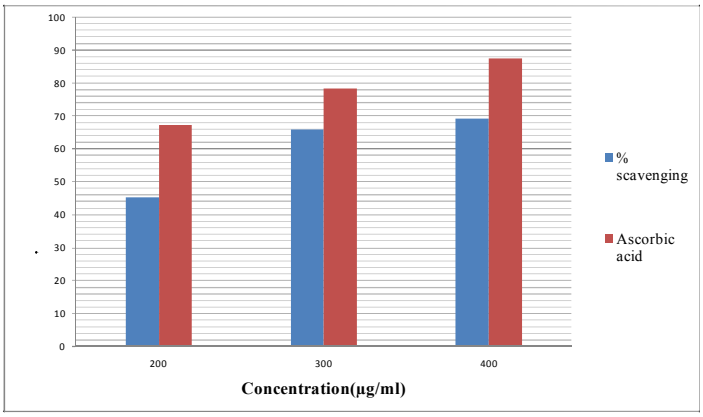
Table: 3 Percentage scavenging of Hydrogen peroxide

Concentration of extract (µg/ml)	(%) Percentage scavenging of hydrogen Peroxide	
	Methanolic extract	Ascorbic acid
200	45.23 ± 5.35	67.4 ± 3.67
300	65.86 ± 4.12	78.5 ± 2.72
400	69.86 ± 3.98	87.5 ± 4.20

Values are the average of triplicate experiments and represented as mean ± S.E.M.



Graph 1: DPPH Scavenging activity



Graph 2: Hydrogen peroxide scavenging activity

Source of support: Nil, Conflict of interest: None Declared