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Antioxidant Activity and Pharmacological Evaluation of *Cucumis melo* var. agrestis Methanolic Seed Extract

R. Arora, M. Kaur and N.S. Gill
Rayat Institute of Pharmacy, S.B.S. Nagar, Punjab Technical University, Ropar, Punjab-144533, India

Correspondence Author: Rashmi Arora, Rayat Institute of Pharmacy, S.B.S. Nagar, Punjab Technical University, Ropar-144533, India Tel: +91-8146991679

ABSTRACT

Traditional medicinal plants in India had a glorious past and have a promising future. People throughout the world use medicinal plants and have great faith on them for their effectiveness due to their inherent medicine properties. The present study was to evaluate antioxidant, antiinflammatory and analgesic activities of *Cucumis melo* var. agrestis methanolic seed extract on traditional background. Free radical scavenging potential was evaluated by hydrogen peroxide and 1,1-diphenyl-2-picrylhydrazyl method. Anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema. Analgesic activity was evaluated by acetic acid-induced writhing response in albino mice and tail immersion method in albino rats. The seed extract was found to have significant scavenging activity 75.59% at 300 μg mL^{-1} by 1,1-dipheny1-2-picrylhydrazy1. picrylhydrazyl method and 69.86% at 400 μg mL^{-1} by Hydrogen peroxide method as compared to standard (ascorbic acid). In case of antiinflammatory the maximum percentage inhibition by rat paw edema was 61.6% at 300 mg kg^{-1}. Further the extract showed maximum analgesic activity i.e. 70.6% at 300 mg kg^{-1} by acetic acid induced writhing method and increased the pain threshold significantly after 60 min at 300 mg kg^{-1} by tail immersion method. The present study indicates that the seeds of *Cucumis melo* var. agrestis has significant antioxidant, anti-inflammatory and analgesic properties.

Key words: Antioxidant activity, *Cucumis melo* var. agrestis, anti inflammatory, analgesic activity

INTRODUCTION

Nature always stands as a golden mark and it has provided the natural products from plant, animal and minerals to cure all aliments of mankind (Verma and Singh, 2008). India is a home of traditional medicine systems which possesses large extent of higher plants approximately 400,000 species in the world as compared to animal's species that are about 5-10 millions (El Saed and Al Barak, 2011). About 80% of world population utilize plant as their primary source of medicinal agents (Shafaei *et al*., 2011). Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals (Dubey *et al*., 2004). The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore, be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Jothimanivannan *et al*., 2010). Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. Some free radicals are good because they

enable your body to fight inflammation, kill bacteria and control the tone of smooth muscles which regulate the working of internal organs and blood vessels (Pala and Gurkan, 2008).

On the other hand the overproduction of Reactive oxygen species like hydroxyl radical, superoxide anion radical, hydrogen peroxide radical can contribute to oxidative stress (Braca *et al.*, 2002). 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 (Koneru *et al.*, 2011). Endogenous defence systems are capable of stabilising or deactivating most of the free radicals before they attack cells. But these systems may not be completely efficient requiring them to depend on exogeneous anti-oxidants from natural sources. Antioxidant activities mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quencher (Anokwuru *et al.*, 2011). Presently, there is amplified interest worldwide to identify antioxidant components which are pharmacologically effective or have low or no side effects and can be used in food industry and preventive medicine (Ramya *et al.*, 2011). Many plants, citrus fruits and leafy vegetables as source of ascorbic acid, Vitamins E and phenolic compounds, possess the ability to reduce the oxidative damage (Ashawat *et al.*, 2007). Generally, antioxidant is identified as major health beneficial compounds reported from varieties of medicinal plants and are source of alternative medicine (Pham Huy *et al.*, 2008).

The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many plants which has been unexplored in the field of medicine or Science (Reddy *et al.*, 2010). One of such plant is *Cucumis melo* var. agrestis. The fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as wild melon, small gourd and wild musk melon. The fruit is usually consumed as a vegetable due to its good nutritional value (Dahot *et al.*, 1999). Seeds oil of Cucumis melo var agrestis was evaluated for the antifungal activity in Nigeria. This fruit contained carbohydrate, protein, lipids, Water soluble vitamins were ascorbic acid, phenylalanine, glutamine and asparagine were found to be present in the aqueous extract of premature chibber fruit (Adekunle and Oluwo, 2008). These results suggested that *Cucumis melo* var. agrestis fruit contains sufficient amount of all essential nutrients which could become a good source of human food.

From the literature review, Cucurbitaceae family includes several species of cultivated plants that have great economic importance like *Momordica charantia* (Semiz and Sen, 2007) *Citrullus Colocynthsis* (Kumar *et al.*, 2008) *Cucumis sativum, Benincasa hispida, Citrullus lanatus* L., *Cucumis melo* (Sood *et al.*, 2009a,b; Gill *et al.*, 2011) *Cucurbita maxima* L., has been evaluated for their antioxidant, anti-inflammatory and analgesic activities. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. So, the present investigation was carried out to evaluate the antioxidant, anti-inflammatory and analgesic activities of *Cucumis melo* var. agrestis in wistar albino rats and swiss albino mice.

MATERIALS AND METHODS

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

Chemical and drugs: 1,1-Diphenyl-2-picrylhydrazyl and Hydrogen peroxide were obtained from Sigma chemical Co. Acetylsalicylic acid was supplied by Aspro Nicholas Pakistan Ltd. Diclofenac sodium was obtained from Jackson Laboratories Pvt. Ltd. Pentazocine was obtained from Ranbaxy-Paonta Sahib. Carrageenan, ascorbic acid, methanol and ethyl acetate obtained from Central Drug House Pvt. Ltd. Mumbai. Sodium chloride, acetic acid, silica gel G, obtained from E-Merk Pvt. Ltd. Mumbai.

Experimental animals: The animal study was carried out during the month of Feb-May (2011). 30 Wistar rats (animals were divided in five groups each containing six rats having average weight of 160-170 g) and 60 Swiss albino mice (Animals were divided in 10 groups having average weight of 20-25 g) of either sex were purchased from Guru Angad Dev Veterinary and Animal Sciences University. The animals were housed in standard cages at room temperature (25±4°C) with food and water ad libitum. Twenty-four hours before the experiments they were transferred to the laboratory and were maintained only with water ad libitum. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animal was carried out as per the guidelines of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration no. 874/ac/05CPCSEA.

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°C. The concentrated extract was suspended in distilled water and partitioned successively with hexane and used for further investigation (Gill *et al.*, 2011).

PHYTOCHEMICAL SCREENING

The crude extract was subjected to preliminary phytochemical screening for evaluation of major phytochemical constituents such as alkaloids, tannins, carbohydrates, proteins, flavonoids, steroids, anthraquinones, coumarin, glycosides, terpenoids and phenolic acids using standard procedure of analysis (Aiyegoro and Okoh, 2010).

FREE RADICAL SCAVENGING ACTIVITY

Qualitative method by 1,1-Diphenyl-2-picrylhydrazy1: 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 reagent (methanol 0.2%) depicting antioxidant activity (Motlhanka, 2008).

Quantitative method by 1,1-Diphenyl-2-picrylhydrazyl: The free radical scavenging activity of test samples were measures by 1,1-diphenyl-2-picrylhydrazyl (Kaur *et al.*, 2008). The percentage scavenging of 1, 1-diphenyl-2-picrylhydrazyl was calculated as:

$$\% \text{ scavenging activity} = \frac{A_0 - A_s}{A_1} \times 100$$

where, A_0 is the absorbance of negative control A_s is the absorbance of sample.

Hydrogen peroxide method: The ability of *Cucumis melo* var. agrestis seed extract to scavenge hydrogen peroxide was determined (Ebrahimzadeh *et al.*, 2010). The percentage scavenging of hydrogen peroxide was calculated as:

$$\% \text{ scavenging activity} = \frac{A_0 - A_1}{A_1} \times 100$$

where, A_0 is the absorbance of negative control A_1 is the absorbance of sample.

Pharmacological evaluation

Antiinflammatory activity

Carrageenan-induced paw edema test in rats: The study was performed in five groups of animal each containing six rats.

Group 1 (Disease control)	:	Carrageenan (1%) was administrated in the plantar surface of rat
Group 2 (Standard)	:	Diclofenac sodium at the dose of 12.5 mg kg^{-1}, p.o
Group 3 (MECMVA 100 group)	:	Methanolic extract at the dose of 100 mg kg^{-1}, p.o+carrageenan treated group
Group 4 (MECMVA 200 group)	:	Methanolic extract at the dose of 200 mg kg^{-1}, p.o+carrageenan treated group
Group 5 (MECMVA 300 group)	:	Methanolic extract at the dose of 300 mg kg^{-1}, p.o+carrageenan treated group

Paw edema was induced by injecting 0.1 mL carrageenan (%) prepared in physiological saline into the sub plantar tissues of the left hind paw of each rat. The methanolic extract was administered orally 30 min before carrageenan administration. The paw volume was measured at intervals of 1, 2 and 3 h by the mercury displacement method using a plethysometer. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group. Diclofenac sodium was used as reference drug (Ratheesh and Helon, 2007; Sood *et al.*, 2009b).

$$\text{Percentage inhiibition} = \frac{V_c - V_t}{V_C} \times 100$$

where, V_c and V_t represent mean increase in paw volume in control and treated group, respectively.

Analgesic activity

Acetic acid induced writhing test: Animals were divided in five groups each containing six mice. Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test) a chemical visceral pain model. Mice were injected intraperitoneal (i.p) with 10 mL kg^{-1} of 0.6% acetic acid solution. Immediately after administration of acetic acid the animals were placed in glass cages and the number of 'stretching' per animal was recorded during the following 15 min. Methanolic extract of *Cucumis melo* var. agrestis was administrated orally at doses of (100, 200 and 300 mg kg^{-1}) and acetylsalicylic acid (10 mg kg^{-1}) was administrated 30 min before the acetic acid injection (Rasika and Ravindra, 2009; Vijay and Vijayvergia, 2010).

$$\text{Inhibiton}(\%) = \frac{\text{mean number of writhings (control)} - \text{mean no of writhings (test)}}{\text{Mean number of writhings (control)}} \times 100$$

Tail immersion method: Male Swiss albino mice weighing 20-25 g were divided into 5 groups each containing 6 animals. A significance increase in reaction time compared with control animal was considered as positive analgesic response (Mondal *et al*., 2009).

Statistical analysis: All the results were expressed as Mean±Standard Error of Means (S.E.M). The data was statistically analysed by one way Analysis of Variance (ANOVA) followed by Tukey's multiple range test (Keselman *et al*., 1976) by using sigma stat version-2.0 software. The $p<0.05$ was considered to be statistically significant.

RESULTS

Phytochemical screening: Preliminary phytochemical screening of methanolic extract of *Cucumis melo* var. agrestis seeds showed the presence of alkaloids, triterpenoids, carbohydrate, protein, flavonoids, Phytosterols (Table 1).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity: The reduction capability of 1,1-diphenyl-2-picrylhydrazyl was determined by the decrease in its absorbance at 517 nm induced by antioxidants. As 1-diphenyl-2-picrylhydrazyl reacts with antioxidant gets converted into 1, 1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The results are shown in Table 2. The methanolic extract of *Cucumis melo* var agrestis showed concentration dependent scavenging activity. The highest radical scavenging activity of methanolic extract was found to be 75.59% at concentration 300 μg mL^{-1} in case of quantitative analysis and in case of qualitative analysis, purple colour of 1,1-diphenyl-2-picrylhydrazyl changed to yellow colour.

Hydrogen peroxide radical scavenging activity: 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. Result of hydrogen peroxide scavenging activity was shown in Table 3. The maximum hydrogen peroxide scavenging activity of *Cucumis melo* var agrestis methanolic extract was 69.86% at a concentration of 400 μg mL^{-1}. which are comparable to scavenging activity of ascorbic acid.

Antiinflammatory activity: The effect of methanolic extract of *Cucumis melo* var. agrestis (100, 200 and 300 mg kg^{-1}) in carrageenan induced rat paw edema as shown in Table 4. One-way

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, +: Minor presence of chemical constituents, -: Absence of chemical constituents

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

	Percentage scavenging of DPPH radical	
Concentration of extract (μg mL^{-1})	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	85.40±5.10

Values are the average of triplicate experiments and represented as Mean±SEM

Table 3: Percentage scavenging of Hydrogen peroxide

	Percentage scavenging of DPPH radical	
Concentration of extract (μg mL^{-1})	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±SEM

Table 4: Effect of methanolic extract of *Cucumis melo* var. agrestis seeds on carrageenan induced rat paw edema

	Mean paw volume (mL)			
Group (mg kg^{-1})	1 h	2 h	3 h	% Inhibition at 3 h
Disease control	0.67±0.004^{a}	0.78±0.059^{a}	0.86±0.157^{a}	0
Diclofenac (12.5)	0.63±0.0008^{b}	0.46±0.006^{b}	0.29±0.101^{b}	66.2
MECMVA (100)	0.60±0.009^{a}	0.53±0.074^{a}	0.44±0.063^{a}	48.8
MECMVA (200)	0.59±0.021^{a}	0.50±0.023^{a}	0.38±0.105^{a}	55.8
MECMVA (300)	0.61±0.001^{b}	0.48±0.0011^{b}	0.33±0.022^{b}	61.6

Data are represented as Mean±SEM statistical analysis was done by one way ANOVA followed by Tukey's test. ^{a}: $p<0.05$, vs. control, ^{b}: $p<0.05$ vs. diclofenac sodium

ANOVA test was applied to evaluate the significant p values and $p<0.05$ was considered as significant. The extract caused maximum reduction in paw edema i.e. 61.6% at 300 mg kg^{-1} in case of carrageenan induced model.

Analgesic activity

Acetic acid induced writhing test: The Methanolic extract of *Cucumis melo* var agrestis (100, 200 and 300 mg kg^{-1}, s.c.) suppressed the acetic acid-induced writhing response significantly ($p<0.05$) at dose of 300 mg kg^{-1}. The standard drug, acetylsalicylic acid in increasing doses produced increased inhibition of writhing movements. The results were found to be highly significant in comparison to the control as shown in Table 5.

Tail immersion method: Methanolic extract of *Cucumis melo* var. agrestis showed dose dependent activity against conduction of heat induced analgesia in mice. Medium 200 mg kg^{-1} and High 300 mg kg^{-1} doses showed significant ($p<0.05$) difference in analgesic activity when compared with control group and standard group as shown in Table 6.

Table 5: Evaluation of analgesic activity of methanol extract of the seeds of *Cucumis melo* var agrestis by acetic acid induced writhing in mice

Group	Treatment	Dose (mL kg^{-1})	Avg No. of writhing	Percentage inhibition
I	Control	3	51.26±0.041	0.0
II	Acetylsalicylic acid	10	10.05±0.95[b]	80.3
III	Methanol extract	100	45.13±0.917[a]	11.9
IV	Methanol extract	200	23.42±0.675[a]	54.3
V	Methanol extract	300	15.04±0.145[b]	70.6

Data are represented as Mean±SEM Statistical analysis was done by one way ANOVA followed by Tukey's test.[a]: $p<0.05$, [b]: $p<0.05$ as compared to control group

Table 6: Analgesic effect of methanolic extract of *Cucumis melo* var. agrestis seeds by tail immersion test

	Mean time latency (time in sec)			
Group (mg kg^{-1})	15 min	30 min	45 min	60 min
Control	3.60±1.044[a]	3.74±1.088[a]	3.79±0.0236[a]	3.76±0.575[a]
Pentazocine (30)	6.12±0.0007b	8.03±0.0123[b]	8.36±0.036[b]	8.56±0.483[b]
MECMVA (100)	4.01±0.396[a]	4.53±0.854[a]	4.62±0.148[a]	4.19±0.457[a]
MECMVA (200)	5.12±0.057[b]	6.28±0.106[b]	7.24±0.058[b]	6.03±0.0964[b]
MECMVA (300)	6.31±0.588[a]	7.05±0.638[a]	8.14±1.0987[a]	7.40±0.794[a]

Data are represented as Mean±SEM. Statistical analysis was done by one way ANOVA followed by Tukey's test. [a]: $p<0.05$ vs. Control group, [b]: $p<0.05$ vs. Pentazocine

DISCUSSION

Cucurbitaceae plants are highly useful as they have good potential against many health ailments. In the present study the phytochemical study of *Cucumi melo* var. agrestis methanolic seed extract showed the presence of flavonoids, terpenoids, alkaloids and phenolic compounds. These phytoconstituents may be responsible for various activities. The antioxidant activity of seed extract showed potent radical scavenging activity. It may be due to donation of electrons to hydrogen peroxide and thus neutralise it to water (Bhalodi *et al.*, 2008). The plant *Cucumis melo* var. agrestis has explored only for its antifungal activity (seed oil) (Adekunle *et al.*, 2008). But the other plants of this family (cantaloupe) has explored for antioxidant activity by (Ismail *et al.*, 2010). Antioxidant activity of Cucurbitacin glycosides from cucurbitaceae plants has been reported by Tannin-Spitz *et al.* (2007).

As free radicals are responsible for the pain stimulation and the antioxidants are highly useful in reducing the pain (Gill *et al.*, 2011). The analgesic activity was evaluated by writhing test and tail immersion test. The writhing test is generally used for screening of antinociceptive effects (Reanmongkol *et al.*, 2007). The extract showed significant analgesic activity as compared to standard. No scientific report exists on the analgesic activity of *Cucumis melo* var. agrestis plant. However, the plant of this family wilbrandia ebracteata has been reported for analgesic activity by Gonzalez and Di Stasi (2002). The extract showed similar results as compare to previously studied plants of Cucurbitaceae family.

Free radicals also cause inflammation by increasing the activity of genes involved in making proinflammatory cytokines such as interluki-6 (IL-6) TNFα, interferons etc. (Parke and Sapota, 1996). The experimental model exhibits a high degree of reproducibility by carrageenan-induced rat paw edema. The extract inhibited the edema in the early and in late phase of an acute inflammation with maximum inhibitory effect showed at dose 300 mg kg^{-1}.

Hence, these results seems to support the view that *Cucumis melo* var. agrestis has influence on proinflammatory cytokines because cytokines believed to be regulator of inflammation (Parke and Sapota, 1996). No scientific report has been available on antiinflammatory activity of *cucumis melo* var. agrestis plant but a similar report on antiinflammatory activity has been available on this family plants. Triterpenoids isolated from the various species of cucurbitaceae family has been reported to have anti-inflammatory activity by Saleem (2009). The seeds of *Citrullus lanatus* and Citrullus colocynthsis aqueous extract are found to possess antioxidant, anti inflammatory and analgesic activities (Gill *et al.*, 2010; Marzouk *et al.*, 2010). All the results justifies that the present work done is in support with the previous research done on the other plants of the same family. Thus, the *Cucumis melo* var. agrestis methanolic seed extract have potent antioxidant, anti-inflammatory and analgesic activity and it can be useful for therapeutic potential.

CONCLUSION

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

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