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## Evaluation of Therapeutic Potential of Traditionally Consumed *Cucumis melo* Seeds

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**Abstract:** The objective of present study was to evaluate the therapeutic potential of *Cucumis melo* seeds traditionally consumed and it was studied for free radical scavenging potential by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and DPPH method. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema in rats. Analgesic activity was evaluated by tail immersion and tail flick methods in mice. The percentage scavenging of DPPH radical and H<sub>2</sub>O<sub>2</sub> radical was 74.9% at 300 µg mL<sup>-1</sup> and 58.91% at 200 µg mL<sup>-1</sup> respectively concentration of. The H<sub>2</sub>O<sub>2</sub> scavenging effect of MECM was 58.91%. The percentage reduction of paw edema was 56.6% at the dose of 300 mg kg<sup>-1</sup>. The high dose caused significant reduction in pain. Thus it can be concluded from the above results that *Cucumis melo* seeds have potent anti-inflammatory, antioxidant and analgesic activity.

**Key words:** *Cucumis melo*, antioxidant, analgesic, anti-inflammatory

### INTRODUCTION

Plant extracts have been used as medicines, nutrition and other industrial purposes. The Natural products today symbolize safety in contrast to the synthetic drugs. Numerous evidences have shown that increased consumption of fruits and vegetables reduce the risk of various pathological events such as cancer, cardiovascular diseases and cerebrovascular diseases (Goodwin and Brodwick, 1995; Rimm *et al.*, 1996). This is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent damages caused by free radicals (Stahelin *et al.*, 1991).

Free radicals are continuously produced in the human body. Overproduction of free radicals results in oxidative stress which leads to the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. The human body counteracts oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through foods and supplements. Plants and herbs have been reported to exhibit antioxidant activity which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Valko *et al.*, 2000).

Many medicinal plants, such as *Salvia sclarea*, *Rosmarinus officinales*, *Chrysanthemum multiflorum* and *Albizia amara* have been studied for their antioxidant activity (Chen *et al.*, 2005; Miliuskas *et al.*, 2004; Kumar *et al.*, 2008a; Yesil-Celiktas *et al.*, 2007). Many researchers have paid attention towards the Cucurbitaceae family. Plants such as *Sechium edule* and *Lagenaria siceraria* have been studied for their medicinal properties (Dire *et al.*, 2007; Fard *et al.*, 2008). The seeds and fruits of various plants of the family, such as *Momordica chirantia*, *Citrullus colocynthis*, *Benincasa hispida*, have been evaluated for their antioxidant, anti-inflammatory and analgesic activities (Gill *et al.*, 2010a,b; Semiz and Sen, 2007; Kumar *et al.*, 2008b; Marzouka *et al.*, 2010). The seeds of *Cucumis sativum* and *Cucumis melo* var. *utilissimus* are reported to possess antioxidant activity (Gill *et al.*, 2009, 2010a). *Cucumis melo* is main plant of this family. This plant is used to treat cardiovascular diseases, diabetes, urinary diseases. Previous study showed that pulp extract of *Cucumis melo* possesses high antioxidant and anti-inflammatory properties. However, antioxidant assessment on the seeds of *Cucumis melo* has not been carried out yet. So the present study was carried out to evaluate the antioxidant, anti-inflammatory and analgesic potential of *Cucumis melo* seeds (Mariod and Matthaus, 2008).

## MATERIALS AND METHODS

**Plant material:** The seeds were purchased from the local market of Ropar (India) in August 2010. The healthy looking seeds were selected for authentication and voucher specimen number 0388 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (India). The seeds were cleaned, washed, dried at room temperature and powdered at low temperature.

**Drugs and chemicals:** All chemical reagents used were of analytical grade which were procured from different companies (Loba Chem, Mumbai and Merck Limited, Mumbai). Sample of morphine and diclofenac sodium was procured from Government Medical College and Hospital, Patiala. Ascorbic acid, Hydrogen peroxide were obtained from Sigma Chemical Co., USA.

**Animals:** The wistar albino rats and swiss albino mice of either sex were obtained from Sanjay biological, Amritsar. Institutional Animal Ethics Committee duly approved the experimental protocol and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg No. 874/ac/05/CPCSEA).

**Extraction:** The seeds of *Cucumis melo* (500 g) were cleaned, shade dried, coarsely powdered and extracted with various solvents (methanol, ethyl acetate, chloroform etc.) for 72 h at room temperature. The extract was evaporated and concentrated under reduced pressure in rotary evaporated. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on the water bath. These crude extracts were further used for various investigations such as antioxidant, anti-inflammatory, analgesic activity (Uchikoba *et al.*, 1998; Banerjee *et al.*, 2008).

**Phytochemical screening:** The crude extracts were analyzed for alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and phenolic acids using standard procedures of analysis (Harborne, 1973).

### Free radical scavenging activity

**Quantitative evaluation of the DPPH free radical scavenging activity:** DPPH scavenging activity of cantaloupe extract was determined according to the method described with slight modifications. In brief, 1 mL of methanolic extract of *Cucumis melo* seeds (MECM) at various concentrations were respectively added to 1 mL 0.05 mM DPPH in methanol and was made up to 5 mL with methanol. Then, the mixtures were vortexed vigorously

and allowed to stand in the dark for 60 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan) at 517 nm using methanol as blank. Ascorbic acid was used as standard. Control was prepared by diluting 1 mL of 0.05 mM DPPH with 4 mL of methanol. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \{(A_c - A_t)/A_c\} \times 100$$

where,  $A_c$  = Absorbance of the control reaction,  $A_t$  = Absorbance in presence of the sample of the extracts (Singh *et al.*, 2002).

**Hydrogen peroxide radical scavenging activity:** In this 1 mL of extract (25-200  $\mu\text{g mL}^{-1}$ ) was added to 2.4 mL of 0.1 M phosphate buffer (pH 7.4), and then 0.6 mL of a 43 mM solution of  $\text{H}_2\text{O}_2$  in the same buffer were added. The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was taken as control. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows:

$$\text{Percentage scavenged } [\text{H}_2\text{O}_2] = [(A_c - A_t)/A_c] \times 100$$

$A_c$  = Absorbance of the control

$A_t$  = Absorbance in the presence of extract and standard (Wettasinghe and Shahidi, 2000).

### Anti-inflammatory activity

**Carrageenan-induced rat paw edema:** Wistar rats were divided into 5 groups each consisting of 6 animals.

- Group I (Disease control): Carrageenan (1%) was administered in the plantar surface of rat.
- Group II (Standard): Diclofenac sodium (12.5 mg  $\text{kg}^{-1}$ , p.o.)
- Group III (MECM 100): Methanolic extract (100 mg  $\text{kg}^{-1}$ , p.o.)
- Group IV (MECM 200): Methanolic extract (200 mg  $\text{kg}^{-1}$ , p.o.)
- Group V (MECM 300): Methanolic extract (300 mg  $\text{kg}^{-1}$ , p.o.)

Five groups each consisting of six wister rats were used. Edema was induced by subcutaneous injection (0.1 mL) of 1% freshly prepared suspension of carrageenin in normal saline, into the plantar side of the right hind paw of each rat. Methanolic extract of *Cucumis melo* (100, 200, 300 mg  $\text{kg}^{-1}$  p.o) and Diclofenac sodium (12.5 mg  $\text{kg}^{-1}$  p.o) as a standard drug were given 30 min before the

carrageenin injection. Control animals received the vehicle only. The volume of the paw was measured at intervals of 60, 120, 180 min by the mercury displacement method using a plethysmograph. The percentage of inflammation decrease was calculated in relation to carrageenan control group and calculated according to the following formula:

$$\text{Percentage inhibition} = [(V_t - V_c) / V_c] \times 100$$

$V_c$  = The inflammatory increase in paw volume in control group of animals.

$V_t$  = The inflammatory increase in paw volume in drug-treated animals (Winter *et al.*, 1962).

**Analgesic activity:** Swiss albino mice of either sex were divided into 5 groups each consisting of 6 animals.

- Group I(Control): Vehicle (1% CMC, p.o.)
- Group II(Standard): Morphine (10 mg kg<sup>-1</sup> p.o.)
- Group III(MECM 100): Methanolic extract (100 mg kg<sup>-1</sup> p.o.)
- Group IV(MECM 200): Methanolic extract (200 mg kg<sup>-1</sup> p.o.)
- Group V (MECM 300): Methanolic extract (300 mg kg<sup>-1</sup> p.o.)

**Tail immersion method:** Male Swiss albino mice weighing 20-25 g were divided into six groups each containing five animals. The tail of the mouse was immersed to a constant level (3 cm) in a water bath maintained at 55±0.5°C. The control group was fed with the vehicle (1% CMC) orally. The Standard group was treated with morphine (10 mg kg<sup>-1</sup> p.o.). The methanolic extract was administered to other groups in doses 100, 200 and 300 mg kg<sup>-1</sup> orally. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animals. The reaction time was the time taken by the mice to deflect their tails. The reaction time of the tail-flick response was determined at 0, 30, 60, 90 and 120 min after the administration of drugs. A significant increase in reaction time compared with control animals was considered a positive analgesic response (Patwardhan and Hooper, 1992).

**Tail flick method:** Swiss albino mice of either sex were divided into six groups each consisting of five animals. The tail of mice was placed on the radiant heat source (1 cm distance from the nichrome wire) of an analgesimeter and time taken by the animals to withdraw its tail from the radiant heat source was taken as the reaction time. The temperature was maintained at 55° to 56°C. Reaction time was recorded at 0, 30, 60, 90 and 120 min after the drug administration. A cut off time of 15 sec was maintained to prevent tissue damage (Davies *et al.*, 1946).

**Statistical analysis:** All the results were expressed as Mean±standard error of means (SEM). The data was statistically analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple range tests by using Sigmastat Version-2.0 Software. The p<0.05 was considered to be statistically significant.

## RESULTS

**Phytochemical screening:** The results in Table 1 reveals that methanolic extract contain maximum amount of tannins, alkaloids, steroids, triterpenoids, carbohydrates, proteins and amino acids using standard procedures. Further studies were carried out by using the methanolic extract.

**DPPH radical scavenging activity:** DPPH reacts with antioxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The highest antioxidant activity of MECM was found to be 74.9% at concentration of 300 µg mL<sup>-1</sup> as shown in Table 2.

**Hydrogen peroxide radical scavenging activity:** The free radical scavenging activity of the MECM was evaluated by H<sub>2</sub>O<sub>2</sub> scavenging method. The H<sub>2</sub>O<sub>2</sub> scavenging effect of MECM was 58.91% at a concentration of 200 µg mL<sup>-1</sup> which was comparable to the scavenging effect of ascorbic acid (Table 3).

Table 1: Phytochemical screening of MECM

Chemical constituents	Ethyl acetate	Chloroform	Methanol
Flavonoids	-	-	-
Amino acid	-	+	++
Triterpenoids	+	+	++
Saponin	-	-	-
Carbohydrates	+	+	++
Tannins	+	+	++
Alkaloids	+	-	+

+: Presence of chemical constituent, -: Absence of chemical constituent, ++: Maximum presence of chemical constituents

Table 2: Percentage scavenging of DPPH radical

Conc. of extract (µg mL <sup>-1</sup> )	Percentage scavenging of DPPH radical	
	Methanol extract	Ascorbic acid
100	52.8±0.28	64.7±0.87
200	63.3±0.54	73.6±0.62
300	74.9±0.76	82.5±0.58

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

Table 3: Percentage scavenging of H<sub>2</sub>O<sub>2</sub>

Conc. of extract (µg mL <sup>-1</sup> )	Percentage scavenging of H <sub>2</sub> O <sub>2</sub>	
	Methanol extract	Ascorbic acid
25	35.2±0.02	42.75±0.01
50	48.9±0.04	55.89±0.04
100	52.50±0.01	61.45±0.01
200	58.91±0.01	71.65±0.01

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

Table 4: Effect of MECM on carrageenin induced paw edema in rats

Treatment	Dose (mg kg <sup>-1</sup> ) orally	Mean paw volume (mL)			Percentage inhibition of edema
		60 min	120 min	180 min	
Disease control	1% CMC	0.47±0.02	0.55±0.06	0.69±0.008	-
Diclofenac	12.5	0.42±0.008	0.36±0.01	0.23±0.005	66.6
MECM	100	0.45±0.004	0.51±0.04	0.59±0.05	14.4
MECM	200	0.44±0.01	0.42±0.009	0.39±0.02	43.4
MECM	300	0.41±0.01	0.37±0.1	0.30±0.005	56.6

The values are Mean±SEM of 6 animals

Table 5: Analgesic effect of the MECM by tail immersion test

Groups	Dose (mg kg <sup>-1</sup> ) orally	Tail withdrawal latency (sec)				
		0 min	30 min	60 min	90 min	120 min
Control	1% CMC	2.58±0.05	2.64±0.02	2.52±0.03	3.43±0.05	2.77±0.04
Morphine	10	2.46±0.23	5.86±0.12 <sup>a</sup>	9.93±0.18 <sup>a</sup>	11.65±0.03 <sup>a</sup>	10.49±0.41 <sup>a</sup>
MECM	100	2.52±0.13	2.82±0.08	3.75±0.05	5.32±0.02	4.91±0.26
MECM	200	2.79±0.06	3.78±0.06 <sup>ab</sup>	5.63±0.14 <sup>ab</sup>	7.47±0.07 <sup>ab</sup>	6.63±0.04 <sup>ab</sup>
MECM	300	2.45±0.03	4.93±0.03 <sup>ab</sup>	7.24±0.05 <sup>ab</sup>	9.17±0.01 <sup>ab</sup>	8.87.41±0.25 <sup>ab</sup>

Values are Mean±SEM of 6 animals in each group. <sup>a</sup>p<0.05 compared with control group, <sup>b</sup>p<0.05 compared with morphine treated group

Table 6: Analgesic effect of the MECM by tail flick test

Group	Dose (mg kg <sup>-1</sup> ) orally	Tail flick latency (sec)				
		0 min	30 min	60 min	90 min	120 min
Control	1% CMC	2.21±0.01	2.16±0.27	2.38±0.07	2.32±0.03	2.26±0.03
Morphine	10	2.12±0.14	4.53±0.06 <sup>a</sup>	6.48±0.14 <sup>a</sup>	8.79±0.03 <sup>a</sup>	7.64±0.01 <sup>a</sup>
MECM	100	2.18±0.13	2.86±0.07	3.86±0.05	4.23±0.14	3.82±0.03
MECM	200	2.26±0.20	3.71±0.23 <sup>ab</sup>	4.78±0.03 <sup>ab</sup>	5.59±0.34 <sup>ab</sup>	4.94±0.02 <sup>ab</sup>
MECM	300	2.25±0.10	4.65±0.25 <sup>ab</sup>	6.57±0.01 <sup>ab</sup>	7.21±0.47 <sup>ab</sup>	6.42±0.04 <sup>ab</sup>

Values are Mean±SEM of 6 animals in each group. <sup>a</sup>p<0.05 compared with control group, <sup>b</sup>p<0.05 compared with morphine treated group

**Anti-inflammatory activity:** Table 4 shows the results of the anti-edematous effect of orally administered MECM on carrageenin induced paw edema in rats. At dose of 100 mg kg<sup>-1</sup>, MECM caused a reduction in paw edema (18.3%) 3 h after the subplantar injection of carrageenin. Doses of 200 and 300 mg kg<sup>-1</sup> caused a significant reduction of paw edema (43.4 and 56.6%, respectively).

**Analgesic activity:** Table 5 and 6 depicts the analgesic activity shown by MECM by tail immersion and tail flick method. Maximum analgesic effect was observed at 90 min interval.

The results of the current study demonstrate that *Cucumis melo* seeds displays potent anti-inflammatory, antioxidant, analgesic activity. Results have revealed that MECM seeds possess *in vitro* free radical scavenging activity. Hence, the extract was further evaluated for its *in vivo* anti-inflammatory and analgesic potential (Peters *et al.*, 1997).

## DISCUSSION

Tail flick and tail immersion methods were carried out evaluate the analgesic potential of *Cucumis melo* seeds. Free radicals are involved during pain stimulation and antioxidants show reduction in such pain. Thus methanolic extract of seeds possess analgesic property by

inhibiting the free radical formation. Anti-inflammatory effect of natural products has frequently been studied by Carrageenan induced rat paw edema. Carrageenan-induced accumulation of leukocytes in the pleural cavity, as well as the enhancement of LTB<sub>4</sub> levels in pleural exudate occur after inflammatory stimulus. Migration of neutrophils to the affected area constitutes an important pro-inflammatory factor, as they liberate toxic oxygen radicals in the extracellular medium. *Cucumis melo* inhibited the leukocyte influx and raised LTB<sub>4</sub> levels. It also possesses significant free radical scavenging activity (Borgeat *et al.*, 1976; Peters *et al.*, 1999). The literature also reveals the antioxidant capacity of the pumpkin seed extract against DPPH free radical formation (Xanthopoulou *et al.*, 2009). The seeds of *Citrullus lanatus* are found to possess antioxidant, anti-inflammatory and analgesic activities (Gill *et al.*, 2009). The seed extract of *Momordica Charantia* normalizes the impaired antioxidant status in streptozotocin induced-diabetes by scavenging of free radicals (Sathishsekar and Subramanian, 2005).

Thus the seeds of *Cucumis melo* can be used as source of antioxidants in the treatment of pain and inflammation. The triterpenoids isolated from various species of cucurbita family have been reported to possess anti-inflammatory activities (Miro, 1995). The cucurbitacins from *Cucurbita andreana* were evaluated

for their anti inflammatory and inhibitory effects on the growth of cancer cell lines (Jayaprakasam *et al.*, 2003). Thus the triterpenoids might be responsible for the free radical scavenging, analgesic and anti-inflammatory activity.

### CONCLUSION

Free radical scavenging activity of traditionally consumed *Cucumis melo* seeds were proved as it causes reduction in carrageenan-induced rat paw edema along with decrease in algesia in mice models.

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