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Study on *Cucumis melo* var. *utilissimus* Seeds for the Therapeutic Potential

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Abstract: The present study was designed to investigate *Cucumis melo* var. *utilissimus* seeds for their antioxidant and therapeutic potential. The antioxidant activity of methanolic extract was measured by DPPH method and hydrogen peroxide free radical scavenging activity. The methanolic extract of *Cucumis melo* var. *utilissimus* seeds (MECU) showed maximum antioxidant potential. Hence, it was further evaluated for anti-inflammatory activity by carrageenan induced rat paw edema and analgesic activity by tail immersion method and tail flick method at different concentrations, i.e., 100, 200 and 300 mg kg⁻¹. The significant results was found at maximum dose. The results suggested that methanolic seed extract possess great therapeutic potential.

Key words: Antioxidant activity, seeds, cucurbitaceae, DPPH, herbs, medicinal plant

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis to treat human diseases. The future of natural products drug discovery will be more holistic, personalized and involve use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients (Patwardhan and Hooper, 1992). There is emerging interest in the use of naturally occurring antioxidants for the management of a number of pathophysiological conditions, most of which involve free radical damage. The implication of oxidative stress in the etiology and progression of several acute and chronic clinical disorders has led to the suggestion that antioxidants can have health benefits as prophylactic agents (Soobrattee *et al.*, 2005).

The Cucurbitaceae family includes several species of cultivated plants that have great economical importance like Watermelon (*Citrullus lanatus* L.), Squash (*Cucurbita maxima* L.), Cucumber (*Cucumis sativus* L.) and Cantaloupe (*Cucumis melo* L.) (Ritschel *et al.*, 2004). Earlier studies on the cucurbitaceae family showed that cantaloupe pulp extract possesses high antioxidant and anti-inflammatory properties (Vouldoukis *et al.*, 2004). Antioxidant properties of seeds extract on streptozotocin induced diabetic rats has been reported by Sathishsekar and Subramanian (2005). The active principles in these vegetal extracts are principally water soluble or lipophilic antioxidant molecules. Most of these plant extracts contain various amounts of vitamin E, C, carotenes, triterpenoids and other flavonoids

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(Aruoma, 2003) and were used as potential antioxidant prophylactic agents for both health and disease management (Peng *et al.*, 2000; Clarkson and Thompson, 2000). *Cucumis melo* var. *utilissimus* is an annual creeping herb, cultivated in many parts of the country, especially in upper India and particularly in Uttar Pradesh and Punjab. Fruit has been traditionally used for the diuretic, antihelmentic and cooling effect (Nadkarni, 2002). But the seeds have not been explored yet. Increased attention has been paid in recent years on utilization of waste and by-products to regain their therapeutic potential. Thus components and properties of seeds are valuable and need to be examined (Brouns, 2002). The present study was undertaken to investigate the antioxidant and therapeutic potential of *Cucumis melo* var. *utilissimus* seeds.

MATERIALS AND METHODS

Plant Material

Cucumis melo var. *utilissimus* seeds were purchased from Local Grain Market Sector 26, Chandigarh (UT) in 2009. The seeds were authenticated and the voucher specimen No. 0387 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar, Punjab. 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.

Chemicals and Drugs

Diclofenac sodium obtained from Jackson Laboratories Pvt. Ltd. Amritsar, Morphine obtained from Government Medical College, Patiala, Carrageenan, ascorbic acid, methanol and ethyl acetate obtained from Central Drug House Pvt. Ltd. Mumbai, Silica Gel 60-120, potassium dihydrogen phosphate, sodium hydroxide, silica gel G and acetone obtained from E-Merk Pvt. Ltd. Mumbai, Hexane and chloroform obtained from Loba Chemie Pvt. Ltd. Mumbai.

Animals

The wistar albino rats of either sex were obtained from NIPER, Mohali. They were kept at standard laboratory diet, environmental temperature and humidity. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) 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 powdered seeds were extracted for 72 h with methanol and stirred every 18 h using a sterile glass rod. The filtrate obtained was concentrated on a rotary evaporator at 40°C. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on the water bath. The extract was stored at 4°C for further used for various investigations.

Phytochemical Screening

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

Quantitative Scavenging Activity on DPPH Radical

The antioxidant activity was measured by DPPH method (Gill *et al.*, 2009). About 1 mL (500-700 $\mu\text{g mL}^{-1}$) solution of methanolic seed extract was added to 1.5 mL of freshly prepared methanolic solution of DPPH (0.05 mM). The change in absorbance at 517 nm was measured after 30 min by a spectrophotometer against a blank solution. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as reference drugs. Each reading was performed in triplicate. Percentage inhibition was calculated by using the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

Where:

A_0 = Absorbance of the negative control

A_s = Absorbance of the sample

Hydrogen Peroxide Radical Scavenging Activity

The methanolic seed extract 1 mL (50-250 $\mu\text{g mL}^{-1}$) solution was mixed with 2.4 mL, phosphate buffer (0.1 M, pH 7.4) and 0.6 mL of H_2O_2 solution (43 mM). After 10 min the absorbance was measured at 230 nm using spectrophotometer against a blank solution. The percentage inhibition was calculated. Each reading was performed in triplicate (Buyukbalci and Sedef Nehir, 2008).

Anti-Inflammatory Study

The carrageenin-induced rat paw edema assay was carried out according to Winter *et al.* (1962). Extract as well as standard drug were prepared with 1% Carboxy Methyl Cellulose (CMC) to administration in experimental animals. The animals were divided into 5 groups of 6 animals each.

Experimental Design for Anti-Inflammatory Action:

Group I (Disease Control): Carrageenan (1%) was administered in the plantar surface of rat

Group II (Standard): Diclofenac sodium (12.5 mg kg^{-1} , p.o.)

Group III (MECU 100): Methanolic extract (100 mg kg^{-1} , p.o.)

Group IV (MECU 200): Methanolic extract (200 mg kg^{-1} , p.o.)

Group V (MECU 300): Methanolic extract (300 mg kg^{-1} , p.o.)

Paw edema was induced by injecting 0.1 mL carrageenan (1%) prepared in physiological saline into the sub plantar tissues of the left hind paw of each rat. The methanolic seed 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 plethysmometer. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group. Diclofenac sodium was used as reference drug.

$$\text{Inhibition of edema (\%)} = \frac{V_e - V_t}{V_e} \times 100 \quad (2)$$

Where:

V_c = The average increase in paw volume of control

V_t = The average increase in paw volume after the administration of test and standard drug

Analgesic Screening

Extract as well as standard drug were prepared with 1% Carboxy Methyl Cellulose (CMC). The animals were divided into 5 groups of 6 animals each.

Experimental Design for Analgesic Action:

Group I (Control Group): Vehicle (1% CMC, p.o.)

Group II (Standard Group): Morphine (10 mg kg⁻¹ p.o.)

Group III (MECU 100 Group): Methanolic extract (100 mg kg⁻¹ p.o.)

Group IV (MECU 200 Group): Methanolic extract (200 mg kg⁻¹ p.o.)

Group V (MECU 300 Group): Methanolic extract (300 mg kg⁻¹ p.o.)

Tail Flick Test

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 recorded using tail flick analgesimeter at 0, 30, 60, 90 and 120 min time interval after the drug administration. A cut off time was maintained 15 sec to avoid injury of tail in mice. The temperature was maintained at 52±0.5°C.

Tail Immersion Test

The tail of mice (1 cm from the terminal part of tail) was immersed in warm water kept constant at 52.5±0.5°C. The reaction time of the tail-flick response was determined at 0, 30, 60, 90 and 120 min after the administration of drugs. A cut off time was maintained 15 sec to avoid injury of tail in mice (Palanichamy and Nagarajan, 1999).

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

Preliminary phytochemical screening of methanolic seed extract showed the maximum presence of chemical constituents such as protein and amino acid, triterpenes, carbohydrates, sterols and tannins (Table 1). The maximum scavenging effect of MECU extract on the DPPH radical was 73.7% at a concentration of 300 µg mL⁻¹ was comparable to the scavenging effects of ascorbic acid (Table 2). The scavenging effect of MECU extract on the H₂O₂ radical was 57.52% at a concentration of 200 µg mL⁻¹ was comparable to the scavenging effects of ascorbic acid (Table 3). Carrageenan administration was shown to significantly rise in the paw volume as compared to normal control group. The methanolic seed extract (100, 200 and 300 mg kg⁻¹) exhibited statistically significant reduction of

Table 1: Phytochemical screening of *Cucumis melo* var. *utilissimus* seeds extract

Chemical tests	Methanol
Alkaloids	-
Flavonoids	-
Protein and amino acid	++
Triterpenoids	+++
Saponin	-
Carbohydrates	++
Phytosterols	++
Tannins	++

+: The presence of compounds, -: The absence of compounds

Table 2: DPPH antioxidant activity of the *Cucumis melo* var. *utilissimus* seeds extract

Concentration of extract ($\mu\text{g mL}^{-1}$)	Scavenging of DPPH radical (%)	
	Methanol extract	Ascorbic acid
100	49.3 \pm 0.64	60.4 \pm 0.47
200	61.5 \pm 0.37	71.7 \pm 0.42
300	73.7 \pm 0.71	84.8 \pm 0.26

Table 3: H_2O_2 radical scavenging activity of the *Cucumis melo* var. *utilissimus* seeds extract

Concentration of extract ($\mu\text{g mL}^{-1}$)	Inhibition of H_2O_2 (%)	
	Methanol extract	Ascorbic acid
25	33.60 \pm 0.02	40.75 \pm 0.01
50	44.85 \pm 0.04	55.37 \pm 0.04
100	51.40 \pm 0.01	62.45 \pm 0.01
200	57.52 \pm 0.01	70.65 \pm 0.01

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

Groups	Dose (mg kg^{-1}) orally	Mean paw volume (mL)			Inhibition at 3rd h relative to control
		1 h	2 h	3 h	
Control	1 % CMC	0.49 \pm 0.02	0.58 \pm 0.003	0.68 \pm 0.008	--
Diclofenac	12.5	0.47 \pm 0.01 ^a	0.36 \pm 0.008 ^a	0.22 \pm 0.05 ^a	67.6
MECU	100	0.48 \pm 0.04	0.54 \pm 0.03	0.60 \pm 0.04	11.7
MECU	200	0.46 \pm 0.2 ^{ab}	0.44 \pm 0.005 ^{ab}	0.41 \pm 0.02 ^{ab}	39.7
MECU	300	0.43 \pm 0.02 ^{ab}	0.37 \pm 0.008 ^{ab}	0.31 \pm 0.005 ^{ab}	54.4

Values are Mean \pm SEM of 6 animals in each group. ^a p <0.05 vs. Control, ^b p <0.05 vs. Diclofenac sodium

Table 5: Analgesic effect of the methanolic extract of *Cucumis melo* var. *utilissimus* seeds by tail flick

Group (mg kg^{-1})	Tail withdrawal latency				
	0 min	30 min	60 min	90 min	120 min
Control	2.9 \pm 0.1	3.3 \pm 0.01	2.7 \pm 0.10	3.2 \pm 0.04	3.1 \pm 0.04
Morphine (10)	3.3 \pm 0.03	5.4 \pm 0.02 ^a	8.6 \pm 0.02 ^a	10.3 \pm 0.02 ^a	9.7 \pm 0.05 ^a
MECU (100)	3.1 \pm 0.02	3.8 \pm 0.03	4.5 \pm 0.01	4.8 \pm 0.10	4.1 \pm 0.02
MECU (200)	2.8 \pm 0.05	4.3 \pm 0.04 ^{ab}	5.4 \pm 0.01 ^{ab}	6.8 \pm 0.04 ^{ab}	6.4 \pm 0.03 ^{ab}
MECU (300)	2.9 \pm 0.05	4.8 \pm 0.01 ^{ab}	7.8 \pm 0.01 ^{ab}	8.7 \pm 0.04 ^{ab}	8.2 \pm 0.01 ^{ab}

Values are Mean \pm SEM of 6 animals in each group. ^a p <0.05 vs. Control, ^b p <0.05 vs. Morphine

percentage inhibition of paw volume at dose dependent manner as compared to carrageenan control group (Table 4). The methanolic seed extract were exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the normal control group. The MECU extract (300 mg kg^{-1}) were shown significant central analgesic activity by tail flick method (Table 5). The MECU extract (200 and 300 mg kg^{-1}) were shown significant results as compared to the 100 mg kg^{-1} pre-treated group by tail immersion method (Table 6).

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

Group (mg kg ⁻¹)	Tail withdrawal latency				
	0 min	30 min	60 min	90 min	120 min
Control	3.3±0.003	2.40±0.03	2.2±0.01	2.48±0.06	2.41±0.2
Morphine (10)	3.5±0.03	6.20±0.1 ^a	11.2±0.01 ^a	15.80±0.01 ^a	15.10±0.01 ^a
MECU (100)	3.4±0.3	3.20±0.01	4.2±0.04	5.90±0.1	5.30±0.01
MECU (200)	3.3±0.02	4.56±0.05 ^{ab}	7.6±0.01 ^{ab}	10.20±0.3 ^{ab}	9.50±0.05 ^{ab}
MECU (300)	3.1±0.04	5.86±0.05 ^{ab}	9.3±0.01 ^{ab}	12.90±0.05 ^{ab}	12.20±0.01 ^{ab}

Values are Mean±SEM of 6 animals in each group. ^ap<0.05 vs. Control, ^bp<0.05 vs. Morphine

DISCUSSION

Intensive research was conducted over the last 10 years to promote the antioxidant nutritional medicine in the nutraceutical field (Lee *et al.*, 2003; Dugas *et al.*, 1999). The antioxidant and anti-inflammatory effects of melon extracts have been already described (Murcia *et al.*, 2001; Campanella *et al.*, 2003) but little was known on the potent pharmacological effect of the MECU extract. In the present work we demonstrated that the MECU extract possesses significant antioxidant properties as revealed by DPPH and H₂O₂ method. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Lee *et al.*, 2003). Scavenging of H₂O₂ by antioxidants may be due to donation of electrons to H₂O₂, thus neutralizing it to water (Bhalodi *et al.*, 2008). Carageenan induced inflammation is a useful model to detect oral action of anti-inflammatory agents (Di Rosa *et al.*, 1971). The development of oedema in the paw of the rat after the injection of carageenan is due to release of histamine, serotonin and prostaglandin like substances (Vinegar *et al.*, 1969). The MECU extract showed significant anti-inflammatory effect. Triterpenoids isolated from the various species of Cucurbitaceae family has been reported to have anti-inflammatory activity (Mohammad, 2009). Tail immersion and tail methods were carried out to evaluate the analgesic potential of seed extract. The duration as well as the intensity of analgesia of MECU extract was dose dependent. The analgesic effect was clearly greater at concentration of 300 mg kg⁻¹ than the lower concentration of 100 mg kg⁻¹. Thermal stimuli induced fall in tail withdrawal latency is most sensitive to central acting analgesics like narcotic drugs (Furst *et al.*, 1988). In the present study, we concluded that the significant antioxidant activity in the MECU extract is essential for its therapeutic potential.

CONCLUSION

Thus from the above study it may be concluded that the methanolic extract of *Cucumis melo* var. *utilissimus* seeds has significant antioxidant activity which may be responsible for its great therapeutic potential.

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REFERENCES

- Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Res.*, 523: 9-20.
- Bhalodi, M., S. Shukla and A.K. Saluja, 2008. *In-vitro* Antioxidant activity of the flowers of *Ipomoea aquatica* Forsk. *Pharmacogn Mag.*, 4: 226-226.
- Brouns, F., 2002. Soya is flavones: A new and promising ingredient for the health foods sector. *Food Res. Int.*, 35: 187-193.
- Buyukbalci, A. and E. Sedef Nehir, 2008. Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. *Plant Foods Hum. Nutr.*, 63: 27-33.
- Campanella, L., A. Bonanni, G. Favero and M. Tomassetti, 2003. Determination of antioxidant properties of aromatic herbs, olives and fresh fruit using an enzymatic sensor. *Anal. Bioanal. Chem.*, 375: 1011-1016.
- Clarkson, P.M. and H.S. Thompson, 2000. Antioxidants: What role do they play in physical activity and health?. *Am. J. Clin. Nutr.*, 72: 637-646.
- Di Rosa, M., J.P. Giroud and D.A. Willough, 1971. Studies of the acute inflammatory response induced in rats in different sites by Carrageenan and Turpentine. *Pathology*, 104: 15-29.
- Dugas, B., A. Mercenier, I. Lenoir-Wijnkop, C. Arnaud, N. Dugas and E. Postaire, 1999. Immunity and probiotics. *Immunol. Today*, 20: 387-390.
- Furst, S., K. Gyires and J. Knoll, 1988. Analgesic profile of rimazolium as compared to different classes of painkillers. *Drug Res.*, 4: 552-557.
- Gill, N.S., M. Garg, R. Bansal, S. Sood, A. Muthuraman, M. Bali and P.D. Sharma, 2009. Evaluation of antioxidant and antiulcer potential of *Cucumis sativum* L. seed extract in rats. *Asian J. Clin. Nutr.*, 1: 131-138.
- Harborne, J.B., 1973. *Phytochemical Methods*. Chapman and Hall, London, pp: 1-32.
- Lee, S.E., H.J. Hwang, J.S. Ha, H.S. Jeong and J.H. Kim, 2003. Screening of medicinal plant extracts for antioxidant activity. *Life Sci.*, 73: 167-179.
- Mohammad, S.A., 2009. Lupeol, novel anti-inflammatory and anti-cancer dietary triterpene. *Can. Lett.*, 285: 109-115.
- Murcia, M.A., A.M. Jimenez and M. Martinez-Tome, 2001. Evaluation of the antioxidant properties of Mediterranean and tropical fruits compared with common food additives. *J. Food Prot.*, 64: 2037-2046.
- Nadkarni, K.M., 2002. *The Indian Materia Medica*. 4th Edn., Popular Prakashan Pvt. Ltd., Bombay.
- Palanichamy, S. and S. Nagarajan, 1999. Analgesic activity of *Cassia Alata* leaf extract and kaempferol 3-O- sophoroside. *J. Ethnopharmacol.*, 29: 73-78.
- Patwardhan, B. and M. Hooper, 1992. Ayurveda and future drug development. *Int. J. Altern. Complement. Med.*, 10: 9-11.
- Peng, J., G.L. Jones and K. Watson, 2000. Stress proteins as biomarkers of oxidative stress: Effects of antioxidant supplements. *Free Radical Biol. Med.*, 28: 1598-1606.
- Ritschel, P.S., T.C. Lins, R.L. Rodrigo Lourenço Tristan, G.S. Buso, J.S. Buso and M.E. Ferreira, 2004. Development of microsatell markers from an enriched genomic library for genetic analysis of melon (*Cucumis melo* L.). *BMC. Plant Biol.*, 4: 9-9.
- Sathishsekar, D. and S. Subramanian, 2005. Original article antioxidant properties of *Momordica Charantia* seeds on Streptozotocin induced diabetic rats. *Asia Pac. J. Clin. Nutr.*, 14: 153-158.

- Soobrattee, M.A., V.S. Neergheen, A. Luximon-Ramma, O.I. Aruoma and T. Bahorun, 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Res.*, 579: 200-213.
- Vinegar, R., W. Schreiber and R. Hugo, 1969. Biphasic development of carrageenan oedema in rats. *J. Pharmacol. Exp. Ther.*, 166: 95-103.
- Vouldoukis, I., D. Lacan, C. Kamate, P. Coste,, A. Calenda and D. Mazier, 2004. Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. extract rich in superoxide dismutase activity. *J. Ethnopharmacol.*, 94: 67-75.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced oedema in hind paw of the rats as an assay of anti-inflammatory drug. *Proc. Soc. Exp. Biol. Med.*, 111: 544-547.