

Anti-inflammatory and Antioxidant Activities of *Cucumis sativus* Leaves

Fatema Nasrin¹, Israt Jahan Bulbul¹, Fahima Aktar² and Mohammad A. Rashid²

¹Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh

²Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

This study was carried out to evaluate the anti-inflammatory and antioxidant activities of *Cucumis sativus* Linn. (Family: Cucurbitaceae) leaves. The methanolic extract of *C. sativus* leaves (MCS) was investigated for anti-inflammatory activities in Long Evans rat model at two different doses of 150 and 250 mg/kg body weight and the effects were compared with the standard, indomethacin (10 mg/kg body weight). It exhibited highest anti-inflammatory activity at the dose 250 mg/kg. In the formalin test, the extract at both doses (150 and 250 mg/kg body weight) significantly prevented the increase in volume of paw edema ($P < 0.05$ and $P < 0.001$). In carrageenan-induced paw edema test the MCS significantly ($P \leq 0.001$) reduced inflammation by 57.35 % (150 mg/kg body weight) and 72.06% (250 mg/kg body weight) in comparison to the standard drug, indomethacin (79.41%) at the end of 5h. MCS was also screened for DPPH scavenging activity, total antioxidant capacity, reducing ability as well as total phenolics content to assess its antioxidant potential. Total phenolic content and total antioxidant capacity of MCS were found to be 262.31 mg/g equivalent of gallic acid and 267.2 mg/g equivalent of ascorbic acid, respectively. The IC_{50} of free radical scavenging of DPPH was 13.06 $\mu\text{g/ml}$ while that of standard ascorbic acid was 13.17 $\mu\text{g/ml}$. The reducing power of MCS was found to be concentration dependent.

Key words: Anti-inflammatory, carrageenan, paw edema, antioxidant.

Introduction

Although inflammation is a defense mechanism, an uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses (Kumar *et al.*, 2004). Worldwide, there is an increasing concern in finding new anti-inflammatory remedies, which are not only effective, but also nontoxic; in this respect plant extracts are in focus. Recently special scientific interest has been focused on natural foods, medicinal plants and phytoconstituents to utilize their well-known antioxidant power (Kukic *et al.*, 2006). The free radicals in human body develops a great number of pathological states like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc. (Ozgen *et al.*, 2006).

Cucumis sativus Linn. (Family: Cucurbitaceae) is an annual, rather coarse, fleshy, prostrate or climbing vine. Different parts of this plant is traditionally used in headache, as cooling and diuretic, nutritive and demulcent, and emetic in acute indigestion in children (Hisahiro *et al.*, 2008). Several studies have shown multiple biological

activities of different parts of *C. sativus*. These include antidiabetic (Karthiyayini *et al.*, 2009), antiulcer (Swapnil *et al.*, 2012), moisturizing (Prashant *et al.*, 2005), antioxidant and analgesic properties (Kumar *et al.*, 2006) of the fruit extracts. The seed extracts were found effective on controlling the loss of body weight in diabetic rats (Minaiyan *et al.*, 2006) and against tapeworms (Elisha *et al.*, 2006). Cytotoxic, antifungal (Joysree *et al.*, 2012) and antibacterial (Tang *et al.*, 2010) activities have been reported from leaves and stems extracts. Chemical studies have demonstrated the presence of cucurbitasides B, C and ferredoxin in leaves (Joshi, 2003; Nadkarni and Nadkarni, 2006) and α - and β -amyrins, sitosterol and cucurbitasides (Abou-Zaid *et al.*, 2001) in seeds. No report on anti-inflammatory activity was found for the *C. sativus* leaves.

So, an attempt was made to assess scientifically the *in vivo* anti-inflammatory and *in vitro* antioxidant activities of methanolic extract of *C. sativus* leaves.

Materials and Methods

Collection of sample and preparation of extract: The leaves of *C. sativus* were collected from Gazipur in the

month of March, 2012. The plant was taxonomically identified in Bangladesh National Herbarium, Dhaka, Bangladesh, where a voucher specimen (DACB no-34479) has been deposited. The fresh leaves were first washed with water to remove adhering dirt and then dried at 45 °C for 36 hrs in an electric oven, then powdered with a mechanical grinder, passed through sieve # 40 and stored in a tight container. The dried powdered material (1kg) was taken in a clean, flat bottom glass container and soaked in methanol for seven days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The total filtrate was concentrated to dryness, *in vacuo* at 40 °C to render the methanol extract (390 g) of brownish red color.

Animals: Long Evans rats of either sex weighing about 135-150 g were used for the experiments. The rats were purchased from the Animal Resources Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24°C temperature, 55-65% relative humidity and 12 h light/12 h dark cycle) for one week for acclimation after their purchase and fed with ICDDR,B formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Drugs and chemicals: The active drugs indomethacin, was a generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Formalin and carrageenan was obtained from CDH, India. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Ammonium molybdate (Merck, Germany), sodium phosphate (BDH, England), potassium ferricyanide [$K_3\{Fe(CN)_6\}$] trichloroacetic acid (CCl_3COOH), Folin-Ciocalteu reagent, gallic acid [$C_6H_2(OH)_3COOH$], ascorbic acid and other chemicals used were of analytical reagent grade.

Instruments: The molecular absorption spectra and absorbance at specific wavelengths were recorded with a HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of 1-cm light path.

Oral toxicity tests: Long Evans rats (135 - 150 g) were randomly divided into nine groups of five animals each. The animals were starved for 12 h prior to testing. Eight doses of the extract, were administered by oral

administration to eight groups of the animals, separately. The animals in the control group received 0.2 ml distilled water. All animals were observed for 24 h and general symptoms of toxicity and mortality were recorded (Amida *et al.*, 2007).

In vivo anti-inflammatory activity:

Carrageenan-induced paw edema: The animals were divided into five groups containing five rats in each. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 h after oral administration of MCS at doses of 150 and 250 mg/kg, b.w. The paw volume was measured at 1, 2, 3, 4 and 5 h after the carrageenan injection using micrometer screw gauge. The difference between the two readings was taken as the volume of edema. Indomethacin suspended in 2% gum acacia was used as the standard drug. The percentage inhibition of inflammation was computed using the following formula (Adedapo *et al.*, 2008):

$$\% \text{ inhibition} = \{(D_o - D_t) / D_o\} \times 100\%$$

where, D_o = the average inflammation (hind paw edema) of the control group; D_t = the average inflammation (hind paw edema) of the treated group.

Formalin-induced paw edema: Following the method described by Sharma *et al.* (2010), paw edema was induced by formaldehyde to assay the anti-inflammatory activity of MCS. The experimental rats in control group received 5% formalin. 20 μ l of 5% formalin was injected into the dorsal surface of the right hind paw at 60 min after administration of two different doses of MCS (150 and 250 mg/kg, b.w.) and indomethacin (10 mg/kg, b.w.). The increase in paw diameter was measured using vernier caliper. The difference in edema of the right hind paw and the left hind paw indicates inflammation. Measurement was done immediately before and after 1 - 5 h following formalin injection.

In vitro antioxidant study: *In vitro* antioxidative abilities of MCS were screened in terms of total phenol content, total antioxidant capacity, DPPH radical scavenging activity and reducing power.

Assay for total phenolic content in MCS: The total phenolic content of the extract was determined using Folin-Ciocalteu reagent (Yu *et al.*, 2010). The content of total phenol in the extracts was calculated from regression equation of the calibration curve ($y = 0.0138x + 0.1276$,

$R^2 = 0.988$) and was expressed as gallic acid equivalents (GAE).

Assay for total antioxidant capacity: The antioxidant activity of the extract was evaluated by the phosphomolybdenum method (Prieto *et al.*, 1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic, pH. The total antioxidants in the extracts was calculated from regression equation of the calibration curve ($y = 0.0043x + 0.1503$, $R^2 = 0.8874$) and was expressed as ascorbic acid equivalents (AAE).

DPPH free radical scavenging activity: The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al.* (2001). The percentage inhibitory activity was calculated from the following equation:

$$\% \text{ of inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration ($\mu\text{g/ml}$) versus % inhibition.

Reducing power: The reducing power of the extract was determined according to the method described by Oyaizu *et al.* (1986). Here the transformation of Fe^{3+} to Fe^{2+} was investigated using ascorbic acid as standard. Increase in absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis: All the values in the test are expressed as mean \pm standard deviation (SD). The data were statistically analyzed by ANOVA (one way of analysis of variance) and post-hoc Dunnett's tests with the

Statistical Package for Social Sciences (SPSS 16.0, USA) program. Dissimilarity between the means of the various groups were measured significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Results and Discussion

Oral administration of the methanolic extract of leaf of *C. sativus* produced no mortality, even no visible signs of toxicity in the animals except for an initial huddling observed at the highest dose of 4 g/kg body weight. In addition, no toxic symptoms and reduction in food and water intake were observed during the period of experiment.

In the carrageenan-induced edema test, the higher dose (250 mg/kg of body weight) produced significant ($P < .001$) inhibition (48%) of the paw edema compared to the control after 3 h of carrageenan injection. Both the doses of the extract significantly inhibited the edema at 5 h after carrageenan injection. At 150 mg/kg dose, 50 % inhibition and at 250 mg/kg dose, 72.06% inhibition was observed. Indomethacin (10 mg/kg of body weight) significantly ($P < .001$) reduced paw edema after 2 h from carrageenan injection compared to MCS (Table 1).

Oral administration of MCS showed dose dependant inhibition ($P < 0.05$) of edema in formalin test. It significantly inhibited the edema in rats at both doses (150 and 250 mg/kg) from the 3rd -5th observation period and indomethacin showed maximum inhibition of edema as presented in Table 2.

The total phenolics content of MCS was found to be 262.31 mg/g equivalent of gallic acid. On the other hand, the total antioxidant capacity was observed as 267.2 ± 0.22 mg/g equivalent of ascorbic acid for MCS.

Table 1. Anti-inflammatory activity of methanolic extract of *C. sativus* leaves (MCS) in carrageenan-induced paw edema in rats.

Group	Paw volume increase (cm) after 3h	Paw volume increase (cm) after 5h	Inhibition (%)	
			After 3h	After 5h
Control (vehicle)	0.50 \pm 0.03	0.68 \pm 0.05	-	-
Indomethacin (10 mg/kg bw)	0.23 \pm 0.01***	0.14 \pm 0.01***	54.00	79.41
MCS (150 mg/kg bw)	0.35 \pm 0.02	0.29 \pm 0.01***	30.00	57.35
MCS (250 mg/kg bw)	0.26 \pm 0.01***	0.19 \pm 0.01***	48.00	72.06

All values are expressed as mean \pm SD, (n=5); One way analysis of variance (ANOVA) followed by Dunnett's test. *** $P < 0.001$, significant compared to control.

Table 2. Anti-inflammatory effect of methanolic extract of *C. sativus* leaves (MCS) in in formalin induced edema test in rats.

Groups	Paw edema volume (cm) in hours				
	1h	2h	3h	4h	5h
Control	0.20 ± 0.03	0.17 ± 0.02	0.15 ± 0.01	0.13 ± 0.01	0.11 ± 0.04
Indomethacin (10 mg/kg bw)	0.13 ± 0.05*	0.13 ± 0.03	0.11 ± 0.00***	0.08 ± 0.04***	0.07 ± 0.02***
MCS, 150 (mg/kg bw)	0.19 ± 0.01	0.15 ± 0.04	0.13 ± 0.02*	0.12 ± 0.05*	0.10 ± 0.01*
MCS, 250 (mg/kg bw)	0.16 ± 0.03	0.14 ± 0.01	0.12 ± 0.00*	0.11 ± 0.01***	0.08 ± 0.01***

All values are expressed as mean ± SEM, (n=5); One way analysis of variance (ANOVA) followed by Dunnet's test. *P < 0.05, *** P < 0.001, significant compared to control.

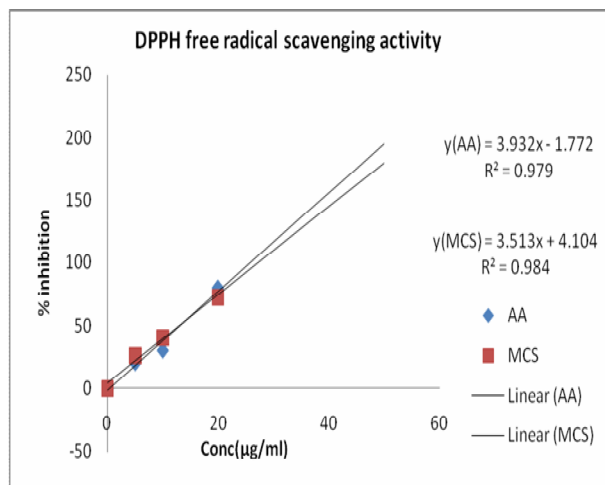


Figure 1. DPPH free radical scavenging activity of MCS and ascorbic acid.

DPPH is most stable radical commonly used in antioxidant assays. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent, i.e. concentration of the extract between 5-100 µg/ml greatly increased the inhibitory activity (Figure 1) with the IC₅₀ value of 13.06 µg/ml for MCS while for standard ascorbic acid it was found to be 13.17 µg/ml. For the measurement of the reductive ability, we investigated the Fe³⁺ to Fe²⁺ transformation in the presence of MCS compared with standard ascorbic acid as shown in figure 2. The reducing power of extracts was found to be concentration dependent.

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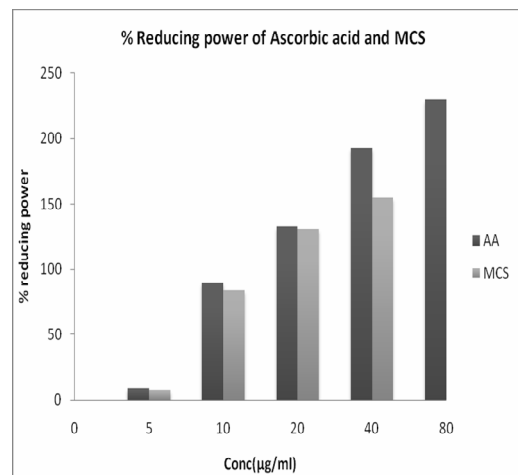


Figure 2. Comparison of reducing power of MCS and ascorbic acid.

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