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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Cucurbita maxima* Duchesne AND *Euphorbia royleana* Boiss

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Abstract: Phytochemical analysis of the ethanolic extract of the leaves of *Cucurbita maxima* indicated the presence of reducing sugars, alkaloids, saponins, tannin, steroid and glycosides whereas *Euphorbia royleana* indicated the presence of glycosides, alkaloids, steroids and tannin. The possible analgesic and anti-oxidant activities of *Cucurbita maxima* and *Euphorbia royleana* were investigated. *Cucurbita maxima* exhibited inhibition of writhing reflex 16.13% and 41.94% respectively ($P<0.001$ and $P<0.001$) and *Euphorbia royleana* exhibited inhibition of writhing reflex 19.35% and 45.16% respectively ($P<0.02$ and $P<0.001$) on animal model (Swiss Albino mice) at 250 and 500mg/kg body weight dose level. Diclofenac sodium was used as standard drug. Both plants showed little antioxidant activity, both qualitatively and quantitatively. In the quantitative assay, *Cucurbita maxima* and *Euphorbia royleana* displayed free radical scavenging activity in the DPPH assay IC_{50} = 218 µg/ml. and IC_{50} = 237 µg/ml respectively whereas that for ascorbic acid was IC_{50} = 17 µg/ml. The results provide a support for the use of this plant as analgesic and antioxidant.

Keywords: *Cucurbita maxima*, *Euphorbia royleana*, antioxidant activity, analgesic activity.

Introduction

Lipid peroxidation is an important deteriorate reaction in food during storage and processing. It not only causes a loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis (Yagi, 1987). The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized (Halliwell and Gutteridge, 1985). Cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage. Active oxygen, either in the form of superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), or singled oxygen (1 O_2), is a product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. When the mechanism of anti oxidant protection becomes unbalanced by exogenous factors such as smoking, ionising radiation, certain pollutants, organic solvents and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes may occur, resulting in above-mentioned diseases and accelerating ageing (Büyükokuroˇ glu et al., 2001). However, antioxidant supplements or foods rich in anti oxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen (Halliwell and Gutteridge, 1984; Mau et al., 2001; Gülçin et al., 2002b). Recently, various phytochemicals and their effects on health, especially the suppression

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of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (Ho et al., 1994). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertbutylhydroquinone (TBHQ) (Sherwin, 1990). However, they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Grice, 1986; Wichi, 1988). Therefore, the development and utilization of more effective antioxidants of natural origin are desired (Gülçin et al., 2002a; Oktay et al., 2003).

Cucurbita maxima is an annual climber growing at a fast rate. It is in flower from July to September, and the seeds ripen from August to October. It is found in Bangladesh, India, Burma, and North America (Ghani, 2003). A poultice of the crushed leaves has been applied to the head to treat headaches. Seeds are taken principally as a safe deworming agent. Seed oil is used as a nerve tonic. Extract of seed is also anthelmintic. The seed oil is also cooling, diuretic and tonic (Dubey, 2012). An anthelmintic effect was reported at the minimum inhibitory concentration of 23 g of *Cucurbita maxima* seed in 100 ml of distilled water in preclinical studies. (Srivastava *et.al* 1967). The antioxidative activity of a water soluble polysaccharide fraction (WSP) from *Cucurbita maxima* fruits was evaluated. In the WSP, DPPH radical scavenging and superoxide dismutase-like activity increased depending on the total sugar content. Furthermore, the WSP can serve as an inhibitor of ascorbic acid oxidation. The efficacy was also affected by the total sugar content. (Nara *et.al* 2009). The plant shows antioxidant activity in WSP, so it makes me interested to test the ethanolic extract for antioxidant activity.

Euphorbia royleana generally are 5-7 m tall, green, 5-7°-angled, many branching from upper parts. It is found in India, Pakistan, Nepal, Bangladesh and China (Ghani, 2003). The plant has piscicidal and anti-acetylcholinesterase activity (Tiwari et al., 2004), anti-inflamatory activity (Bani et al., 2000) and acute toxicity on catfish. As there is a relationship in between antioxidant and analgesic activity due to some molecule like tannins, flavonoids, steroids, planed to test the phytochemical and pharmacological test.

The aim of the present study was to investigate antioxidant activity of both plants by DPPH free radical scavenging activity. Another important goal of this research was to examine analgesic activity of both these plants.

Materials and method

Plant material collection and extraction: The plant *Cucurbita maxima* was collected from the Bhabanipur, Jhenidah, Bangladesh. The plant was collected in the 16 January, 2011 on the day time. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: DACB - 35557) and a voucher specimen was also deposited there. The leaves were separated and cleaned of any undesirable and washed with water. They were shade-dried for one week. The leaves were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 250gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95.6% ethanol. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered. Then it was filtered through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a water- bath at 45°C until dried. It rendered a gummy concentrate of greenish black color. The gummy concentrate was designated as crude extract of ethanol.

The fresh plant of *Euphorbia royleana* was collected from Kushtia, Bangladesh in December 2010 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. (Accession number-35573) and a voucher specimen was also deposited there. The plant was separated from undesired

part, washed and shed dried. The plant was then ground into coarse powder and rinsed with ethanol for cold extraction. The extract was filtered and dried under ceiling fan and water bath at 45°C and the gummy concentrate was designed as crude extract of ethanol.

Drug: Diclofenac sodium was collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Preliminary phytochemical screening: The ethanolic extract of the plant was subjected to preliminary phytochemical test for the detection of major chemical group. In each test 10% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test (Evans, 1989; Wagner et al., 1984, Ghani, 2003).

***Animals*:** Young Swiss-albino mice aged 4-5 weeks, average weight 28-35 gm were used for the experiment. The mice were purchased from the animal research branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRB). They were kept in standard environmental condition for one week in the animal house of the Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

Analgesic activity: The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice (Ghule et al., 2011). The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (diclofenac), negative control and test sample given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing. Experimental animals were randomly selected and divided into four groups denoted as Group I, Group II, Group III, Group IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples (250 and 500 mg/Kg), control and diclofenac were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered extracts and/or drugs. Then acetic acid solution (0.7%) was administered intraperitoneally to each of the animals of a group. After an interval of 5 minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15 minutes.

***Antioxidant activity*:** In case of qualitative analysis, a suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.,* 2003). DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants.

In case of quantitative analysis, the anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory conc.

50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta *et al*., 2003).

The formula used for % inhibition ratio is-
% inhibition = (Blank OD-Sample OD/ Blank OD) × 100

Results

Preliminary phytochemical screening: Phytochemical studies of *Cucurbita maxima* showed that reducing Sugars, alkaloids, saponins, tannin, steroid and glycosides are present in the ethanolic extract of *Cucurbita maxima* leaves (Table 1). And *Euphorbia royleana* showed the presence of glycosides, alkaloids, steroids and tannins.

Table 1: Results of preliminary phytochemical analysis of *Cucurbita maxima*.

Extract	Reducing Sugars	Saponins	Alkaloids	Glycosides	Flavonoids	Tannins	Gums	Steroids
Cucurbita maxima	+	+	+	+	-	+	-	+
Euphorbia royleana	-	-	+	+	-	+	-	+

+ = Presence - = Absence

Analgesic activity: Table 2a showed the effect of the ethanolic extract of *Cucurbita maxima* leaves on acetic acid-induced writhing model in mice. It produced 41.94% writhing inhibition in mice at oral doses of 500 mg/kg body weight and 16.13% at 250 mg/kg body weight of mice, while the standard drug diclofenac sodium inhibition was found to be 80.65% ($P<0.001$) at a dose of 25 mg/kg body weight. Whereas *Euphorbia royleana* produced 45.16% and 19.35% writhing inhibition in mice at oral doses of 500 mg/kg body weight and 250 mg/kg body weight of mice, while the standard drug diclofenac sodium inhibition was found to be 79.03% ($P<0.001$) at a dose of 25 mg/kg body weight (Table 2b).

Table 2a: Effect of *Cucurbita maxima* on acetic acid induced writhing in mice

Animal group	Treatment	Writhing count (% Writhing)	% Writhing inhibition
Group I n=5	1% tween-80 solution in water	12.4 (100)	0
Group II n=5	Diclofenac Sodium (25mg/kg)	2.4 (19.35)	80.65
Group III n=5	Ethanolic Extract (250 mg/kg)	10.4 (83.87)	16.13
Group VI n=5	Ethanolic Extract (500 mg/kg)	7.2 (58.06)	41.94

Table 2b: Effect of *Euphorbia royleana* on acetic acid induced writhing in mice

Animal group	Treatment	Writhing count (% Writhing)	% Writhing inhibition
Group I n=5	1% tween-80 solution in water	12.4 (100)	0
Group II n=5	Diclofenac Sodium (25mg/kg)	2.6 (20.96)	79.03
Group III n=5	Ethanolic Extract (250 mg/kg)	10 (80.64)	19.35
Group VI n=5	Ethanolic Extract (500 mg/kg)	6.8 (54.83)	45.16

Anti-oxidant activity: In the TLC-based qualitative antioxidant assay using DPPH assay, *Cucurbita maxima* and *Euphorbia royleana* showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate. In the quantitative assay, *Cucurbita maxima and Euphorbia royleana* displayed free radical scavenging activity in the DPPH assay IC_{50} =218 µg/ml and IC_{50} =237 µg/ml when compared to that of ascorbic acid (IC_{50} =17 µg/ml), a well-known standard antioxidant. (Table: 3a, 3b and Table: 4)

Table 3a: DPPH scavenging assay of *Cucurbita maxima* leaves

Conc. (extract) (µg / ml)	log Conc.	Average absorbance	% Inhibition
Blank		0.672	0
1.57	0.195	0.662	1.5
3.13	0.495	0.641	4.61
6.25	0.795	0.617	8.18
12.5	1.096	0.582	13.39
25	1.397	0.538	19.94
50	1.698	0.503	25.15
100	2	0.451	32.89
200	2.301	0.363	45.98
400	2.602	0.214	68.15

Table 3b: DPPH scavenging assay of *Euphorbia royleana* leaves

Conc. (extract) (µg / ml)	log Conc.	Average absorbance	% Inhibition
Blank		0.672	0
1.57	0.195	0.615	8.48
3.13	0.495	0.596	11.30
6.25	0.795	0.556	17.26
12.5	1.096	0.532	20.83
25	1.397	0.520	22.61
50	1.698	0.497	26.05
100	2.00	0.460	31.54
200	2.301	0.365	45.68
400	2.602	0.211	68.60

Table 4: DPPH scavenging assay of ascorbic acid

Conc. (ascorbic acid) µg / ml	log conc.	Average absorbance	% Inhibition
Blank		0.672	0
1.57	0.195	0.557	17.11
3.13	0.495	0.545	18.89
6.25	0.795	0.538	19.94
12.5	1.096	0.438	34.82
25	1.397	0.134	80.05
50	1.698	0.127	81.10
100	2	0.073	89.13

Discussion

In the TLC-based qualitative antioxidant assay using DPPH assay, *Cucurbita maxima* and *Euphorbia royleana* showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate. In the quantitative assay, both *Cucurbita maxima* and *Euphorbia royleana* displayed weak free radical scavenging activity in the DPPH assay. Some studies have revealed that the antioxidants melatonin and β-carotene potentiate the antinociceptive responses (Penn, 1995; Pang et al., 2001). It was indicated that vitamin E has beneficial effects in improvement of rheumatic disease, intermittent claudication or angina pectoris due to its antioxidant activity (Rapola et al., 1996; Sangha and Stucki, 1998; Kleijnen and Mackerras, 2000). According to the above information, it is said that there is a

relationship between antioxidant and analgesic activities. Analgesic activities may be related to antioxidant activity.

Analgesic activity of the ethanolic extract of the leaves of *Cucurbita maxima* and *Euphorbia royleana* was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represent pain sensation by triggering localized inflammatory response. Acetic acid which is used to induce writhing, cause algesia by liberation of endogenous substance, which in turn excite the pain nerve endings (Taesotikul et al., 2003). Increased level of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid has been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt et al., 1980). The ethanolic extract of *Cucurbita maxima* and *Euphorbia royleana* leaves produced significant writhing inhibition comparable to the standard drug Diclofenac sodium. The hydrosoluble fraction of *Euphorbia royleana* latex showed anti-inflammatory activity (Bani et al., 2000). On the basis of this result it can be concluded that the ethanolic extract of *Cucurbita maxima* possesses analgesic activity.

Preliminary Phytochemical studies showed that the ethanolic extract of *Cucurbita maxima* leaves showed the presence of the reducing sugars, alkaloids, saponins, tannins, steroid and glycosides and that for *Euphorbia royleana* showed the presence of glycosides, alkaloids, steroids and tannins.

Conclusion

The results obtained in the study indicate that the ethanolic extract of *Cucurbita maxima* and *Euphorbia royleana* possess considerable analgesic and mild antioxidant activity at the investigated doses on the experimental laboratory animal. This could provide a rationale for traditional uses of these plants and suggests further investigation and isolation of biologically active constituents responsible for the activity.

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