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Anti-oxidant and anti-inflammatory activity of *Cucurbita maxima* flowers (Pumpkin)

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A B S T R A C T

Cucurbita maxima, belongs to family Cucurbitaceae, is commonly known as pumpkin. Several literature reports suggest it to be antidiabetic, antihypertensive, anticancer, immune modulators, and antibacterial and antihyperlipidaemic. Fresh flowers of Cucurbita maxima were extracted and evaluated for antioxidant activities by 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH), 2,2'-azino-bis (3-ethyl benzthiazoline-6-sul- phonic acid (ABTS) assay and anti inflammatory activities by human blood cell (HRBC) membrane stabilization method and Inhibition of albumin denaturation method. The results obtained showed that the ethyl acetate fractions of Cucurbita maxima flowers can be considered as good sources of anti-oxidants, anti-inflammatory and can be incorporated into the drug formulations. This study justifies the anti-oxidants, anti-inflammatory activity of the compound isolated from ethyl acetate fractions of Cucurbita maxima flowers. Further detailed analysis of this sample is required to identify the presence of bioactive compounds responsible for anti-oxidants activity and anti-inflammatory activities.

Keywords: Cucurbita maxima, Anti-oxidant activity, Anti-inflammatory activity, Albumin denaturation, DPPH, Total antioxidant activity etc.

A R T I C L E I N F O

CONTENTS

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1. Introduction

Cucurbita maxima belong to the family Cucurbitacea is traditionally used in treatment of several problems of stomach, wounds and tapeworms. C. maxima are native of Malaysia but it is now found throughout the tropical countries. It is distributed throughout India, China, Burma, Thailand and Vietnam and is widely spread throughout the tropical and subtropical countries of the world. Pumpkin is one such plant that has been frequently used as functional food or medicine. C. maxima reported to have antidiabetic [1], hepatoprotective [2], anthemintic [3], immune modulatory, antihypertensive, anti-cacer, anti-bacteria, anti-hypercholesterolemic and anti-inflammatory [4].

It has received considerable attention in recent years because of the nutritional and health benefits of the bioactive compounds obtained from its seeds and fruits. Many studies demonstrated that pumpkin has extensive bioactivities, such as hepatoprotection [5], anti-diabetes [6], anti-cancer [7], and anti-obesity properties [8]. Medicinal plants are now becoming more widely used by people all over the world people understands the gentle strength of these natural remedies. There are approximately 1250 indian medicinal plants,which are used in formulating therapeutic preparations according to ayurveda and other traditional system of medicine [9].

The plant Cucurbita maxima is widely cultivated throughout the world for use as vegetable as well as medicine. Both of its fruits and aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual (or) perennial [10]. Plant has been used traditionally as medicine in many countries such as china, India, Yugoslavia and America [11]. Traditionally it is used in most countries as anti diabetic, antitumor, anti-inflammatory, anti-ulcer,anti-bacterial agent [12]. Cucurbita maxima fruit was an old world “pumkin” native inindia having important medicinal uses [10]. Cucubitamazima fruits contain flavonoids, Tannins, Steroids, Terpine aldehyde, Inhibit PG synthesis [13]. Literature reveals no scientific data on anti inflammatory activity of Cucurbita maxima fruits. The present study has been undertaken to investigate the anti-oxidants, anti-inflammatory potential of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

2. Experimental

Extraction and fractionation

Fresh flowers (1kg) of Cucurbita maxima were collected at O. Koothur village, Ariyalur district, during the month of August and identified by Dr. John Britto, Director, Rabinat Herbarium and Center for Molecular Systematics, St. Joseph’s College (Campus), Trichirappalli-2, Tamilnadu. India. The flowers were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction was taken for screening of anti-oxidants, anti-inflammatory potential of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

***In-vitro* Antioxidant Activity**

DPPH (2,2-Diphenyl 1-picryl hydrazyl) assay method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

Reagents:

A. **2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100 «M)**:
   22mg of DPPH was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100μM DPPH solution.
B. **Preparation of test solutions**:
   21 mg of the solid obtained from test sample extract was dissolved in distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21mg/ml concentration. This solution was serially diluted to obtain lower concentrations.
C. **Preparation of standard solutions:**
   10mg each of ascorbic acid and rutin were weighed separately and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to get 10mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.
D. **Procedure:**
   The assay was carried out in a 96mm well microtitre plate. To 200μl of DPPH solution, 10μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 μg/ml. The plates were incubated at 37^o C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

Evaluation of total antioxidant

Capacity of the extract

The total antioxidant capacity was determined by phosphor molybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695nm.

Preparation of test and standard solutions

Weighed accurately 55mg of the sample and the standard ascorbic acid and dissolved in 5ml of DMSO. The lower dilutions were made serially with Dimethyl sulfoxide (DMSO).

Procedure

An aliquot of 0.1ml of the sample solution containing a reducing species in Dimethyl sulfoxide (DMSO) was combined in an Eppendorff tube with 1ml of reagent solution (0.6mM sulphuric acid, 28mM sodium phosphate,

and 4mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 minutes. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid (Mojca et al., 2005).

Total antioxidant activity = 259.8µg/ml

ABTS

((2,2'-azinobis (3-ethyl benzthiazoline-6-sul-phonicacid) radical scavenging activity:

ABTS radical scavenging activity was performed as described by Re et al. (1999) with a slight modification. 7.0 mM ABTS in 14.7 mm ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution dissolved in ethanol (20.0 µl) were added to 980.0 µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µl of ethanol and processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

Anti- Inflammatory Activity

The human red blood cell (HRBC) membrane Stabilization method

The method as prescribed by Gopalkrishnan et al., 2009; Sakat et al., 2010 was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of test sample were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the test sample. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula,

Percentage of Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

Albumin denaturation method

The method as prescribed by Sakat et al., 2010 was followed with some modifications. The reaction mixture was consisting of test sample and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows,

Percentage of inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100

3. Results and Discussion

Anti-oxidant activity:

In the present study, ethyl acetate fractions of Cucurbita maxima flowers exhibited significant anti-oxidant activity when compared with DPPH assay. It is evident from the data presented in Table -I that the sample possesses DPPH assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as 74.12 %, 500 µg/ml as 57.09 %for 125 µg/ml as 36.48 % and for 31.25 µg/ml as 27.15 %, respectively when compared with ABTS assay activity. It is evident from the data presented in Table II that the sample possesses ABTS assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as 79.15 %, 500 µg/ml as 63.28 %for 125 µg/ml as 54.92%, and for 31.25 µg/ml as 34.06%. Total antioxidant activity of the plant is 259.8µg/ml.

Anti-inflammatory activity:

Ethyl acetate fractions of Cucurbita maxima flowers exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) membrane stabilization exhibited data presented in Table III. The result showed the percentage of inhibition in Membrane Stabilization for 100 µg/ml as 37.12 ± 0.28 %, for 200 µg/ml as 42.05 ± 0.61%, for 400 µg/ml as 57.31 ± 0.86%, for 600 µg/ml as 69.83 ± 0.25% and for 800 µg/ml as 73.18 ± 1.66 % respectively when the Inhibition of Albumin denaturation activity exhibited data presented in Table IV. The result showed the percentage of inhibition in membrane Stabilization for 100 µg/ml as 42.19 ± 0.42 %, for 200 µg/ml as 56.74 ± 0.26 %, for 400 µg/ml as 63.51 ± 0.59 %, for 600 µg/ml as 72.6 ± 1.47 % and for 800 µg/ml as85.03 ± 1.36 %.

Table 1: DPPH assay activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

S.No	Concentration (µg/ml)	% IC_{50} Cytotoxicity	IC_{50}
1	1000	74.12	403.21µg/ml
2	500	57.09	
3	125	36.48	
4	31.25	27.15	

The anti-inflammatory effect of the compound isolated from ethyl acetate fraction (test sample) of Cucurbita maxima may be due to presence of active constituent flavonoids. Based on the results described, it may be concluded that the compound isolated from ethyl acetate fraction of (test sample) Cucurbita maxima shown above the result may be due to the presence of flavonoids. The results strongly suggest anti-inflammatory effect and anti-oxidant effects by percentage of inhibitions, which are explained in the Table 1,2,3,4. The medical use of

Cucurbita maxima as a useful remedy in various disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential. However, chemical constituents and mechanism responsible for the pharmacological activities remain to be investigated.

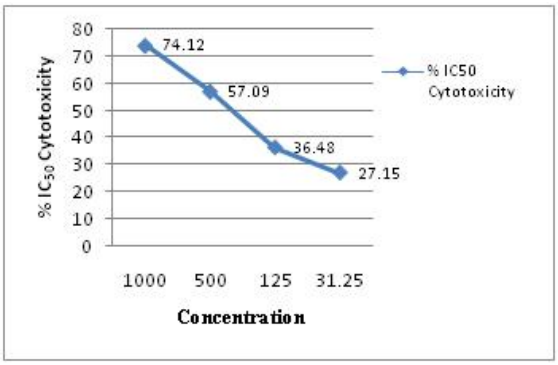

Figure 1: Graphical representation of DPPH activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

Table 2: ABTS assay activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

S.No	Concentration (µg/ml)	% IC_{50} Cytotoxicity	IC_{50}
1	1000	79.15	108.27 (µg/ml)
2	500	63.28	
3	125	54.92	
4	31.25	34.06	

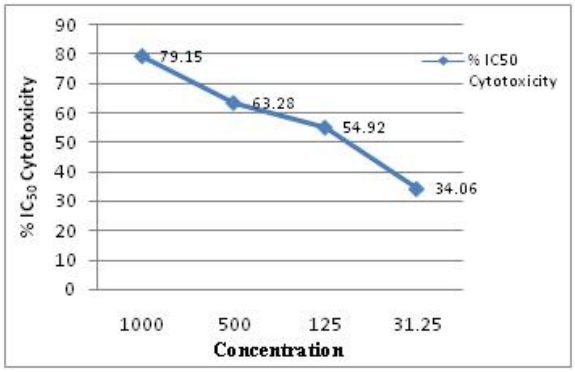

Figure 2: Graphical representation of ABTS radical scavenging activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

Table 3: The human red blood cell (HRBC) membrane stabilization activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M(S-I)
1	100	37.12 ± 0.28
2	200	42.05 ± 0.61
3	400	57.31 ± 0.86
4	600	69.83 ± 0.25
5	800	73.18 ± 1.66

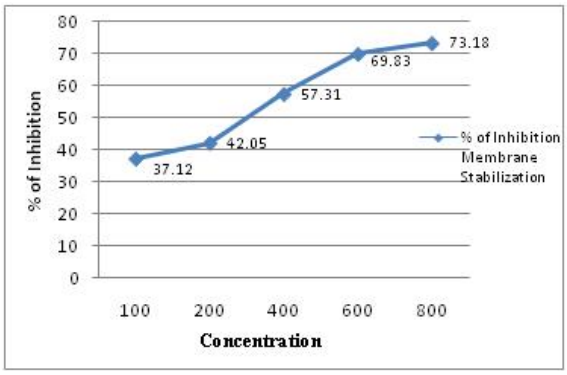

Figure 3: Graphical representation of human red blood cell (HRBC) membrane stabilization activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

Table 4: The Inhibition of Albumin denaturation activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M(S-I)
1	100	42.19 ± 0.42
2	200	56.74 ± 0.26
3	400	63.51 ± 0.59
4	600	72.6 ± 1.47
5	800	85.03 ± 1.36

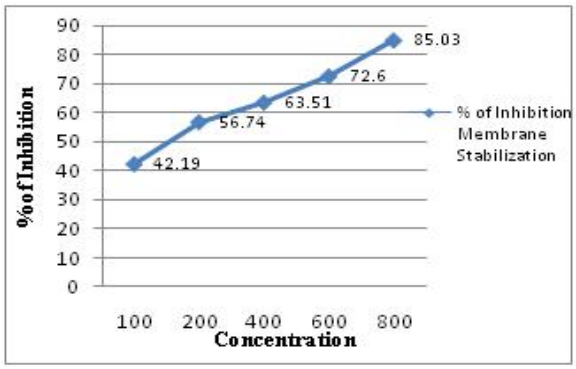

Figure 4: Graphical representation of Inhibition of Albumin denaturation activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers.

4. Conclusion

In conclusion, the present study has confirmed that DPPH assay, total anti-oxidant capacity and ABTS have showed a strong antioxidant activity and also the human red blood cell (HRBC) membrane stabilization, Inhibition of albumin denaturation indicated the anti-inflammatory activity. The present investigations have demonstrated a strong association between the anti-inflammatory and antioxidant activities of the compound isolated ethyl acetate fractions of Cucurbita maxima flowers. The prevention of oxidative damage to tissue could therefore be one of the mechanisms responsible for the anti-inflammatory effect shown by this plant. The medical use of Cucurbita maxima flowers as a useful remedy in arthritic disorders could possibly be because of its excellent anti-inflammatory and antioxidant

potential. The purification and interpretation flavonoidal components of Cucurbita maxima flowers will be carried out in future studies.

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