

Determination of Antioxidant Activity and Phytochemical Screening of *Cucurbita maxima* Duch. fruit extracts in non polar to polar solvents

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Abstract: Antioxidant compounds in food play an important role as a health-protecting factor. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. *Cucurbita maxima* Duch. (Cucurbitaceae) known as pumpkin, winter squash. The aim of this study was to analyze the presence of different phytochemical constituents and to evaluate antioxidant activity of *C. maxima* fruit in petroleum ether, benzene, chloroform, acetone, ethanol and water extracts. All the extracts was tested for 1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity and compared with L-Ascorbic acid as standard. The antioxidant activity of these extracts was investigated based on their ability to scavenge (DPPH) stable free radical. Phytochemical screening of *C. maxima* revealed the presence of carbohydrate, protein, amino acid, alkaloid, tannins, saponins, flavonoids. A higher percentage free radical scavenging activity was found for aqueous extract as compared to all other extracts.

Key Words: *Cucurbita maxima*, 1-diphenyl-2-picryl hydroxyl.

Introduction

Naturally occurring antioxidants in vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases.^{1,2}

Oxidative stress has been recognized to have a pathological role in many types of chronic diseases such as diabetes, heart diseases and cancer. Oxidative stress occurs when the formation of free radicals increases.³ In oxidative stress, the balance between the formation of reactive oxygen species and amount of antioxidants is destroyed. Oxidative stress causes damage to cell components, such as proteins, lipids and nucleic acids.^{4,5} and eventually leads to cell death.⁶

Cucurbita maxima Duch. (Cucurbitaceae) commonly known as pumpkin⁷, Red Gourd in English, Dadhiphala in Sanskrit, Kashiphala in Hindi, cultivated throughout India⁸. The chemical constituents from seeds contain 30% unsaturated fixed oil (linoleic and oleic fatty acids) triterpenoids, flavonoids, coumarins, saponins, cucurbitacins, vitamins, minerals, amino acids⁹.

C. maxima is an excellent source of beta carotene and ascorbic acid which acts as antioxidant to neutralize harmful free radicals in our skin thereby helping to prevent wrinkles, resist infection, to keep the skin moist and youthful¹⁰. Seeds are anthelmintic, carminative, tenecide, aromatic, stomachic, stimulants, astringent and diuretic^{11, 12}. It is beneficial for conditions such as acne and eczema. It contains enzymes that exfoliate the skin and have anti-inflammatory properties.¹³

Present study was carried out to analyze the presence of different phytochemical constituents and to evaluate antioxidant activity of *C. maxima* fruit in petroleum ether, benzene, chloroform, acetone, ethanol and water extracts.

Experimental

Plant materials and extraction

The fruits of *C. maxima* (Cucurbitaceae) were procured from the local market of Nagpur (Maharashtra) and authenticated in Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur.

Preparation of extracts

Fruits of *C. maxima* were washed and cut into very small pieces and then dried under the shade at room temperature for 8 days and later dried in an oven at 45°C for complete removal of moisture to obtain constant weight then subjected to size reduction. 200g of air dried powdered fruit material was successively extracted in soxhlet assembly by using series of solvents in increasing order of polarity viz. petroleum ether, benzene, chloroform, acetone, ethanol, water.¹⁴ Each extract was then concentrated by distilling off the solvent and then evaporating the solvent to dryness and weighed¹⁵. Their percentage extractive values were recorded.

Preliminary Phytochemical Screening

All the extracts were subjected to preliminary phytochemical screening for evaluation of phytochemical constituents such as carbohydrate, protein, amino acid, alkaloids, tannins, fats and oil, flavonoids using standard procedure of analysis.^{16,17,18}

Determination of antioxidant activity of *C. maxima* fruit extract by DPPH method.¹⁹

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compound. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non radical form DPPH-H*²⁰. The DPPH is reacted with methanol or absolute ethanol to yield purple color. The presence of antioxidants in the sample scavenge the formed DPPH radical and decrease in color is observed which is Spectrophotometrically measured at 517nm.^{21,22} In one cuvette 3ml of methanol was taken and kept as a standard for all the extracts. In other cuvette 3ml of DPPH was taken. Absorbance for the blank samples at 517 nm was determined.²³ Cuvette of methanol was not disturbed. Now in another cuvette 3ml of DPPH was kept aside for 5min. To this cuvette ascorbic acid was added in microlitre in various concentrations. Absorbance at 517nm was read for each concentration. Scavenging activity was expressed as the % inhibition. Now ascorbic acid was replaced by extracts and followed same procedure.

The percentage of inhibition can be calculated using the formula

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test

Results

Extractive Value

Table No.1. Extractive Value % (W/W)

Sr.N.	<i>C. maxima</i> Fruit Extracts	% (W/W)
1	PECM	2
2	BECM	1.33
3	CECM	1.35
4	AECM	1.65
5	EECM	2.66
6	WECM	3.46

The extractive value of petroleum ether, benzene, chloroform, acetone, ethanol and water were found to be 2% w/w, 1.33% w/w, 1.35% w/w, 2.21% w/w, 3.26% w/w and 3.53% w/w, respectively as recorded in Table no.1. Percentage yield of water extract (WECC) was found to be maximum i.e. 3.53% w/w as compared to other extracts.

Preliminary phytochemical screening

All the extracts were screened for presence of carbohydrate, protein, amino acid, alkaloid, tannin, fat and oil, flavonoid. Preliminary phytochemical screening showed the presence of carbohydrate, protein, tannin and flavonoid in water extracts which is recorded in table no.2

Table No.2. Preliminary phytochemical screening of *C.maxima* fruit extracts.

S. N.	Phytochemical	Test	PECC	BECC	CECC	AECC	EECC	WECC
1	Carbohydrate	Fehling test	—	-	-	-	+	+
2	Protein	Biuret test	—	-	-	-	-	+
		Xanthoprotein	—	-	-	-	-	+
3	Amino acid	Ninhydrin test	-	-	-	-	-	+
4	Alkaloid	Hager's Reagent	-	-	+	-	-	-
		Wagner's Reagent	-	-	-	-	+	-
5	Tannins	Ferric chloride reagent	-	-	-	-	-	+
		Lead acetate Test	-	-	-	-	+	+
		Potassium dichromate Test	-	-	-	-	-	-
6	Fat and Oil	Spot Test	+	-	-	-	-	-
7	Flavonoid	Shinoda Test	-	-	-	-	-	+

DPPH free radical scavenging activity

DPPH free radical scavenging activity of PELC, BELC, CELC, AELC, EELC, and WELC is depicted in fig.1. It was observed that water extract of *C.maxima* showed highest DPPH free radical scavenging activity than other extracts. Different concentrations of L-ascorbic acid were used as standard antioxidant.

DPPH free radical scavenging activity of *C.maxima* fruit in Non-Polar to Polar solvents

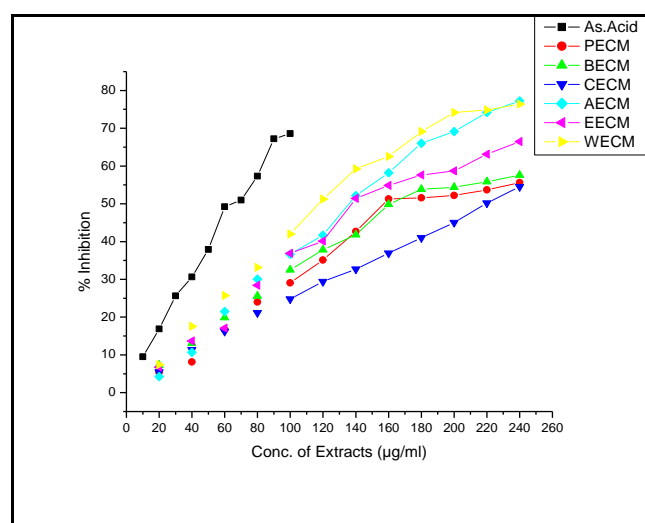


Fig. No.1. DPPH free radical scavenging activity of *C.maxima* fruit in Non-Polar to Polar solvents

IC₅₀ Value for Antioxidant activity

IC₅₀ value (Table no.3) states the amount of concentration of extract required to produce 50% free radical scavenging activity. Hence IC₅₀ value is inversely related to the free radical scavenging activity. Here result clearly states that water extract of *C.maxima* fruit showed highest whereas chloroform extract showed lowest DPPH free radical scavenging activity.

Table No.3: DPPH free radical scavenging activity of *C.maxima* fruit in Non-Polar to Polar Solvents

Conc. of Extracts (µg/ml)	PELC % Inhibition	BELC % Inhibition	CELC % Inhibition	AELC % Inhibition	EELC % Inhibition	WELC % Inhibition
Control	-	-	-	-	-	-
20	5.39	7.3	5.48	4.23	6.74	7.40
40	8.13	13.09	11.39	10.68	13.66	17.56
60	16.70	19.85	16.24	21.48	17.07	25.71
80	23.99	25.55	21.15	30.05	28.43	33.12
100	29.06	32.52	24.78	36.61	36.88	42.01
120	35.09	37.8	29.43	41.69	40.14	51.21
140	42.70	41.81	32.70	52.16	51.42	59.25
160	51.26	49.84	36.91	58.20	54.89	62.53
180	51.58	53.85	41.03	66.03	57.63	69.10
200	52.21	54.38	45.04	69.10	58.69	74.17
220	53.69	55.86	50.21	74.17	63.11	74.81
240	55.60	57.55	54.53	77.24	66.49	76.40
IC ₅₀	156 µg/ml	160 µg/ml	224 µg/ml	134 µg/ml	124 µg/ml	116 µg/ml
IC ₅₀ (Std.) Ascorbic acid - 60 µg/ml						

Abbreviation : CM- *Cucurbita maxima* ; PE-Petroleum ether extract ; BE-Benzene extract ; CE-Chloroform extract ; AE-Acetone extract; AE-Alcohol extract; WE- Water extract

Discussion

The phytochemical study of different extracts of *C.maxima* showed the presence of flavonoids and tannins in water extracts. Antioxidant activity of different extracts was found to be Water>Ethanol>Acetone>Petroleum ether>Benzene>Chloroform. Maximum antioxidant activity of water extracts could be contributed to presence of flavonoid and tannins.

Conclusion

Thus it can be concluded that *C.maxima* possesses antioxidant activity. Water extract possesses maximum antioxidant activity while chloroform possesses minimum activity.

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