

## DETERMINATION OF PHYSICOCHEMICAL PROPERTIES, ANTIOXIDANT CONSTITUENTS BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY FINGERPRINTING, AND ANTIOXIDANT ACTIVITY OF *CUCURBITA MAXIMA* SEEDS

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### ABSTRACT

**Objective:** The present study is designed to evaluate preliminary phytochemical constituents, physicochemical properties evaluation, free radical scavenging activity, and high-performance thin-layer chromatography (HPTLC) fingerprinting analysis of *Cucurbita maxima* seed oil.

**Methods:** Total phenol content, total flavonoid content, total tannin content, 1, 1-diphenyl, 2-picryl-hydrazyl (DPPH) radical scavenging method, and HPTLC fingerprinting analysis for compounds responsible for antioxidant activity were used.

**Result:** Phytochemical screening shows the presence of constituents such as alkaloids, glycosides, carbohydrates, fixed oil and fats, phytosterols, saponin, phenolic compounds, tannins, proteins, amino acids, gums, and mucilage. Physicochemical studies show that the oil contains an acid value, saponification value, iodine value of 9.53 mg KOH/g, 183 mg KOH/g, and 116.51 mg I<sub>2</sub>/100 g, respectively. Total phenol content was 3.9498±3.2 mg gallic acid equivalent/g, total flavonoid content was 56.115±4.6 mg rutin equivalent/g, and total tannin content was 19.4±0.40 mg tannic acid equivalent/g. The radical scavenging activity by DPPH Model shows that the methanolic extract has maximum antioxidant activity with IC<sub>50</sub> value of 238.31. HPTLC fingerprinting also proves the presence of phenol and flavonoid compounds in the seed oil.

**Conclusion:** The results in the paper show that the *C. maxima* seed oil is a natural antioxidant and can be used for the treatment of various disorders.

**Keywords:** Pumpkin, Antiradical scavenging, Carotenoids, Phytochemical studies.

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### INTRODUCTION

The use of food and food products as medicine has been in practice over centuries, and the extracts from the family Cucurbitaceae have been used in the preparation of medicines for a variety of diseases in Ayurveda and ancient Chinese medicine [1]. Cucurbitaceae, commonly known as gourd family, is a family of about 120 genera and more than 800 species, the family is distributed mainly in tropical and subtropical regions of the world. Some of its largely represented genera include Benincasa, Cucurbita, Cucumis, Lagenaria, Momordica, Luffa, and Trichosanthes [2].

Cucurbitaceae is divided into two subfamilies, which are well characterize: Zanonioideae and Cucurbitoideae. The subfamily Cucurbitoideae contains the food producing plants. Varieties of both pumpkin and squash fall under the species *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita mixta*.

Pumpkin plant has large leaves, sprawling veins with coiled, modified leaves called tendrils. The leaves are simple, alternate, and shallowly to deeply lobed. The root near the surface stem would be square, flowers will be bright yellow, and the fruit is more fibrous and less sweet than winter squash. Seed varies in size depending on variety and type. Pumpkin seed has a natural protective skin called the hull. The pumpkin plant has been grown since the earliest history of humankind. The annual species is probably native to Asia but now neutralized to temperate and warmer areas [3].

*C. maxima* also known as pumpkin, gourd, acorn squash, marrow, and summer squash and locally known as kaddu, konda, and kumra. It is grown for its fruit and edible seeds. It has white seeds enclosed in a

husk. These seeds are chewable and sweet with a little nutty flavor [3,4]. The pumpkin seeds yield approximately 50% oil, (mostly linoleic and oleic acid), but the main active constituents are  $\Delta^7$  sterols (avenasterol and spinasterol) and  $\Delta^5$  sterol (sitosterol and stigmasterol). It also contains triterpenoids, sesquiterpenoids, squalene, tocopherols ( $\alpha$ -tocopherol is predominant), carotenoids, minerals (particularly, phosphorus, potassium, magnesium, calcium, iron, zinc, and trace elements), proteins and amino acids, carbohydrates (6–10%), vitamins (thiamine, riboflavin, niacin, pyridoxine, and pantothenic acid), phenolic glycosides, and lignans. *C. maxima* has been traditionally used as diuretic and anthelmintic. The seeds and oil from pumpkin seeds have been used for many years for the relief of difficulties associated with an enlarged prostate gland and micturition problems related to irritable bladder.

The fruit is cooling and astringent to the bowels, increases appetite, cures leprosy, and purifies the blood. Seeds cure sore chests, hemoptysis, bronchitis, and fever. The seed extracts of *C. maxima* modulate immunobiochemical pathways induced by interferons [4]. Seeds are useful in the management of benign prostatic hyperplasia.<sup>5</sup> seed extract has antioxidant capacity [6].

### METHODS

#### Chemicals

All the chemical used like such as KOH, pPhenolphthalein indicator, HCl, carbon tetrachloride, Wijs reagent, potassium iodide, sodium thiosulfate, starch, Folin-Ciocalteus reagent, gallic acid, tannic acid, rutin, and 1, 1-diphenyl, 2-picryl-hydrazyl (DPPH) etc . were of analytical grade and purchased commercially from S D Fine, Mumbai.

Table 1: Preliminary phytochemical screening of seed extracts of *Cucurbita maxima*

Plant constituents test/reagent used	Hexane	Chloroform	Methanol	Ethanol	Water
Alkaloids	+	+	-	+	+
Meyer's reagent					
Dragendorff's reagent					
Carbohydrate and glycosides	-	-	+	+	+
Fehling solution					
Molisch solution	-	+	-	-	+
Benedict reagent	-	+	-	-	-
Phytosterols	+	-	-	-	+
Liebermann's test	-	-	+	-	+
Lieberman	-	-	-	-	-
Burchard's test	+	+	+	-	-
Fixed oil and fats	+	+	+	-	+
Spot test					
Saponification test					
Saponin	-	-	-	+	+
Foam test					
Hemolysis test					
Phenolic compound and tannin	+	+	+	+	+
Ferric chloride solution					
Gelatin solution					
Lead acetate solution					
Aqueous bromine solution					
Protein and amino acid	-	-	+	+	+
Millon's reagent					
Biuret test					
Ninhydrin reagent					
8-Gum and mucilage	-	-	-	+	+
Alcoholic test					
Precipitation test					
Molisch test					

Table 2: Physicochemical properties of seed oil of *Cucurbita maxima*

S. No.	Oil property	Unit	Value
1.	State of oil	-	Liquid
2.	Color of oil	-	Yellowish
3.	Oil content	%	28.1
4.	Acid value	Mg KOH/g	9.53
5.	Saponification value	mg KOH/g	183
6.	Iodine value	mg I <sup>2</sup> /100 g	116.51

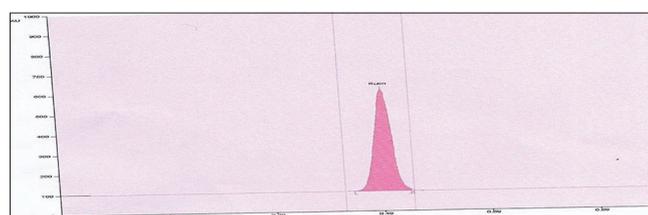


Fig. 1: High-performance thin-layer chromatography densitogram - Standard

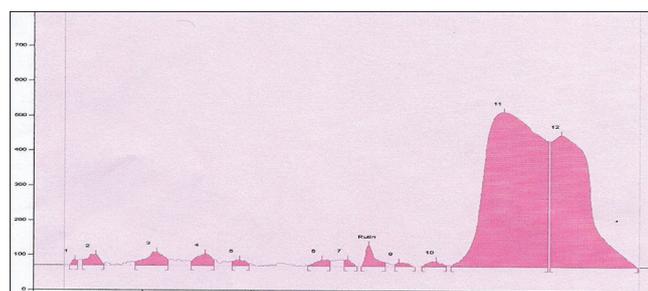


Fig. 2: High-performance thin-layer chromatography densitogram - Sample extract

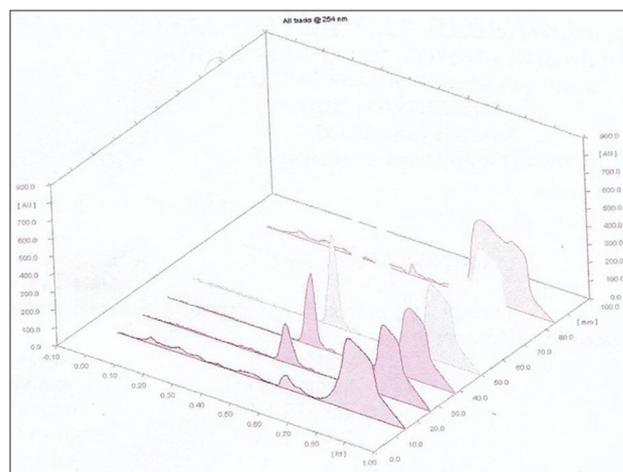


Fig. 3: High-performance thin-layer chromatography (HPTLC) densitogram - 3D diagram of HPTLC densitograms

**Plant material**

The dried seeds of *C. maxima* were purchased from local market of Lucknow, Uttar Pradesh, India. The purchased seeds are authenticated by a taxonomist from CSIR- NBRI Lab, Lucknow. A voucher specimen no (NBRI/CIF/531/2016) has been submitted.

**Preparation of extract**

The purchased seed material from market is screened for any impurities and dried. Dried seeds were grind to coarse powder using an electric grinder, sieved (80 mesh), and stored in airtight jars until use. The dried powder seeds were extracted by Soxhlet apparatus using hexane, chloroform, methanol, ethanol, and water successively. Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vacuum evaporator at

Table 3: Total phenolics, flavonoids, and tannins content of extracts of *Cucurbita maxima*

Plant extracts	Total phenolics (mg gallic acid equivalent/g)*	Total flavonoid (mg rutin equivalent/g)*	Total tannin (mg tannic acid equivalent/g)*
Methanolic extract	3.9498±3.2	56.115±4.6	19.4±0.40

\*n=3

Table 4: Results of DPPH radical scavenging activity

S. No.	Extract	Concentration (µg/ml) and % inhibition				
		20	40	60	80	100
1	Standard gallic acid	91.1	91.4	92.4	92.9	93.6
2	Hexane	20.3	20.9	25.2	29.0	30.9
3	Chloroform	18.8	19.2	20.8	21.2	22.3
4	Methanol	42.6	45.7	48.9	55.2	59.3
5	Ethanol	30.0	32.8	35.3	38.6	39.3
6	Water	30.2	33.7	39.4	40.1	42.5

DPPH: 1, 1-diphenyl, 2-picryl-hydrazyl

Table 5: IC<sub>50</sub> value for DPPH radical scavenging assay

S.No.	Plant extract	IC <sub>50</sub> value (µg/ml)*
1	Hexane	150.43
2	Chloroform	120.13
3	Methanol	238.31
4	Ethanol	181.31
5	Water	125.93

DPPH: 1, 1-diphenyl, 2-picryl-hydrazyl

Table 6: HPTLC - fingerprinting extract (mobile phase - ethyl acetate: butanone: formic acid: water - 5:3:1:1)

Peak	Rf value	Start height (AU)	End height (AU)	Area (AU)	Assigned
1	0.04	0.3	14.7	167.2	Unknown
2	0.00	15.9	3.3	781.5	Unknown
3	0.11	10.5	18.3	1345.5	Unknown
4	0.20	12.5	17.8	1007.5	Unknown
5	0.27	36.6	7.0	406.1	Unknown
6	0.42	28	16.3	491.2	Unknown
7	0.46	18.6	0.3	267.5	Unknown
8	0.50	0.8	12.0	1087.9	Rutin
9	0.56	6.8	0.2	274.0	Unknown
10	0.63	1.8	4.3	409.5	Unknown
11	0.75	3.9	58.3	45967.3	Unknown
12	0.86	358.3	1.2	27288.2	Unknown

HPTLC: High-performance thin-layer chromatography

40–50°C under vacuum, and dried residue was collected and stored in the refrigerator for further experimentation.

#### Phytochemical screening

Phytochemical screening is done according to method of C. K. Kokate, Practical Pharmacognosy, for hexane, chloroform, ethanol, methanol, and water extract [7] and results were tabulated in Table 1.

#### Physiochemical studies

The physiochemical parameters in hexane extract were determined using methods reported in AOAC 1990 [8], and results were tabulated in Table 2.

#### In vitro antioxidant activity

Estimation of total phenolic content, estimation of total flavonoid content [9], and estimation of tannin content, were expressed in terms of mg gallic acid equivalent/g, mg rutin equivalent/g, and mg tannic acid equivalent/g based on the calibration curves of standards, i.e. gallic acid, rutin, and tannic acid, respectively. The radical scavenging potential was analyzed through DPPH radical scavenging assay [10].

#### DPPH antiradical activity

DPPH method is performed using Gallic acid as standard. Extracts of all solvents are dissolved in methanol to achieve a concentration of 20, 40, 60, 80, and 100 µg/mL and were mixed with 400 µM DPPH methanol solution at a ratio 1:3. A control was prepared using methanol and DPPH solution. The mixture was mixed well and set in the dark at room temperature for 40 min. The change of color from violet to yellow of DPPH was determined by measuring the absorbance at 517 nm. The percentage of inhibition was calculated by the given formula and also IC<sub>50</sub> Value is calculated.

(%) Scavenging activity =  $\frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$

#### Chromatographic estimation

*Development of high-performance thin-layer chromatography (HPTLC) method for analysis of phenols and flavonoids*

HPTLC analysis was carried out using Camag HPTLC system equipped with Linomat-V applicator and 100 µl syringe. The samples were spotted in the form of bands using microliter syringe on pre-coated silica gel 60

F<sub>254</sub> HPTLC plates, and development of the applied plate was carried out in pre-saturated Camag twin-trough chamber. The mobile phase used was ethyl acetate: Butanone: Formic acid: Water in a ratio of 5:3:1:1. The developed plates were dried and analyzed at 254 nm and 366 nm, and the results are interrelated in Tables 3-6 and Figs.1-3.

## RESULTS

### DISCUSSION

As per Table 1, various important phytochemicals such as alkaloids, glycosides, carbohydrates, fixed oil and fats, phytosterols, saponin, phenolic compounds, tannins, proteins, amino acids, gums, and mucilage are present in hexanoic, chloroform, methanolic, ethanolic and water extracts of *C. maxima* which are very valuable for various therapeutic activities.

As per Table 2, the acid value was 9.53 mg KOH/g, this indicates that oil of *C. maxima* is safer for human consumption and also does not undergo deterioration. Saponification value was 183 mg KOH/g which shows that the oil is suitable for biodiesel processing. Iodine value was 116.51 mg I<sub>2</sub>/100 g which indicates that it is more suitable for biodiesel production and is a drying oil.

Antioxidant potential of many plants is due to total phenolic components which includes phenolic acid and phenolic diterpenes. As per Table 3, total phenol content was 3.9498±3.2 mg gallic acid equivalent/g, total flavonoid content was 56.115±4.6 mg rutin equivalent/g, and total tannin content was 19.4±0.40 mg tannic acid equivalent/g which shows that the plant has antioxidant properties.

Table 4 shows % inhibition by radical scavenging methods and Table 5 shows IC<sub>50</sub> value for DPPH radical scavenging assay which shows that the methanolic extract has maximum antioxidant activity and hence contains maximum antioxidant components.

Table 6 shows that the HPTLC fingerprinting of methanolic extract in mobile phase was ethyl acetate: butanone: Formic acid: Water in a ratio of 5:3:1:1 and densitogram shows the presence of rutin at R<sub>f</sub> value of 0.5.

## CONCLUSION

*C. maxima* belonging to family Cucurbitaceae is a plant which is also used as food, it is very easily available and can be used for the treatment of various disorders because the results in the paper show that the *C. maxima* seed oil is a very good natural antioxidant.

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