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ORIGINAL ARTICLE

Evaluation of Antioxidant Activity of Different Solvent-Based Extracts of *Cucurbita Maxima* Leaves

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ABSTRACT
Antioxidants are essential for protecting our bodies from oxidative stress. By eliminating Reactive Oxygen Species (ROS), oxidants minimize the chance of chronic illness that include cardiovascular diseases (CVD), cancer, along with neurological disorders. The article discusses methodologies to assess the '"antioxidant activity of Total Polyphenol Content (TPC), Total Flavonoids Content (TFC), DPPH (2,2-dipheny-l-picrylhydrazyl) and reports results. Phytochemical screening revealed flavonoids and phenolic acids' existence. The study ends by stating significance of Cucurbita maxima leaves as a potential natural antioxidant source and their potential application in health and industry. Based on the results, highest antioxidant activity and antioxidant activity present in the water extract as compared with other solvents extract. Maximum amount of extraction yield present in water extract (22%) as compared to other solvent extraction. The total polyphenol contents (TPC) show the highest in water extract. The TPC shows the highest in water extract while total flavonoid content (TFC) shows the highest in ethanol extract. The strong antioxidant activity present in water extract along with ethanol: water (1:1) extract in comparison with other solvent extracts. Further analysed the water extract utilizing LCUVMS for identifying marker-based antioxidant activity and identified the marker compounds are p-Coumaric acid or Hydroxycinnamic acid, Ferulic acid, and Gallo catechin gallate which shows antioxidant, anti-inflammatory, anti-Cinnamic acid, cancer as well as antimicrobial properties.
Keywords: *Cucurbita maxima, Solvent Extraction, Phytochemical Screening, Antioxidants*

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INTRODUCTION

Pumpkins belong to the *Cucurbitaceae* family [4]. They are cultivated and consumed globally for their rich nutritional content. These are very widely available belonging to some of the common species for example '"*Cucurbita moschata, Cucurbita maxima, Cucurbita pepo, and Cucurbita mixta* [1,3,8]. The crop, *Cucurbita maxima* is extensively cultivated in Asia, particularly Bangladesh and has a wide range of tolerance to climates. Aside from its economic importance pumpkins are utilized in traditional medicine in China, India, Korea, Mexico and Brazil [4, 5]. Traditionally, leaves of *Cucurbita maxima* are used for medicinal purposes owing to their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, immunomodulatory, antihypertensive, antihelminthic, anti-atherosclerotic and anti-hypercholesterolemic properties [3, 4, 5].

Antioxidants help cover cells against free radicals and oxidative damage, which has been associated with chronic diseases that include diabetes, cancer, along with CVD 4. The protective antioxidant effects of

extracts from plants emanate primarily from flavonoids and phenolic acids as bioactive substances [4]. Such compounds have been proven to have potent antioxidant activities that may lie behind the medicinal properties of *Cucurbita maxima* leaves. The increasing profile of the medicinal plant's utilization as natural sources of antioxidants has been taken seriously because of their bioavailability, safety and health benefits. Determination of the antioxidant potential of the leaves requires extraction of their bioactive components using different solvents. The selection of the solvent used in the extraction process is very critical as it determines the efficiency of the extraction and the nature of compounds obtained. In this study water, ethanol, hexane, ethyl acetate and hydroalcoholic were employed to target different phytochemical profiles. Thus, the goal of this investigation was to estimate-the antioxidant efficacy of several based on solvents extracts of *Cucurbita maxima* leaves. The goal of this investigation is to determine the antioxidant activity of different solvent-based extracts from *Cucurbita maxima* leaves using the assay DPPH, Total polyphenol content as well as and Total flavonoid content. Such analysis will make a great light on the possible worth of these leaves for a natural source of antioxidants useful in nutraceuticals, pharmaceutical and food preservation applications.

MATERIAL AND METHODS

Materials

The specified leaves of *Cucurbita maxima* are procured and authenticated from Krishi Vigyan Kendra (ICAR-Indian Council of Agricultural Research, New Delhi). The leaves are kept in water in order to get rid of impurities and later placed under the sun to dry. The dried leaves are then ground into powder using a grinder and then kept in an airtight container at the room temperature until the time of solvent extraction.

Chemicals

The HPLC grade solvents like Ethanol, Hexane, Ethyl Acetate and Water were purchased from Rankem, India. Other Analytical grade reagents like NaOH, Na_2CO_3, $AlCl_3$, along with $NaNO_2$ were acquired from Rankem, India. Standards used like Gallic acid, Rutin hydrate were bought from Sigma Aldrich and DPPH (2,2-diphenyl-1- picryhidrazyl) was purchased from Tokyo Chemical Industry, Japan. Folin & Ciocalteu's Phenol reagent was bought from Loba Chemie Pvt. Ltd., India. Every chemical and solvent utilized in this investigation is of analytical or HPLC quality.

Instrumentations

Sr. No.	Name of Instrument	Company Name
1.	LC-UV-MS Instrument	Agilent Technologies 6550 Q-TOF LC/MS System
2.	UV-Spectrophotometer	Shimadzu UV-1800
3.	Water Bath	PCI ANALYTICS
4.	Weighing Balance	Sartorius Analytical Balance
5.	Sonicator	PCI Ultrasonic Electronic Instrument
6.	Rotary Evaporator	BUCHI Switzerland
7.	Grinder	PHILIPS

Preparation of Extracts [6, 7]

Extracts were prepared in various solvents that include water, hexane, ethanol, ethyl acetate and ethanol-water (1:1) mixture. Exactly 10 gm of dried powder was taken and mixed up with 5 volumes of solvent. Reflux Extraction was done under the water bath at 70-90°C temperature for 30 minutes. After extraction remove flask, cool it to room temperature, decant and retain the solvent. Repeat four more cycles. Once extraction is complete, Combine the retained solvents in a flask and filter through Whatman 1 filter paper. By evaporating the solvent in a rotary evaporator at a reduced pressure, the filtered extract was concentrated, and the yield percentage was determined.

$$\text{Yield (\%)} = \frac{\text{Weight of solvent-free extract (gm)}}{\text{Dry extract weight (gm)}}$$

Determination of Total Polyphenol Content (TPC) [2, 3, 5,7,11]

To measure TPC, the Folin-Ciocalteu (FC) Method was utilized. Standard for this assay is Gallic acid, and water served as the blank. To explain briefly, separately, 0.4 mg/ml of the extracted sample and 0.16 mg/ml of the standard had been added to 2 ml of FC reagent and maintain to keep at the room temp. for five min. Subsequently, 5 ml of 30 percent Na_2CO_3 solution had been introduced into solution and it was mixed gently. The mixture was vortexed when the final volume of 25 ml was achieved utilizing distilled water. After that, the sample was kept at 60°C for 5 minutes in a water bath. After shaking, it was kept at 22 to 25°C for 30 minutes temperature in the dark. Following incubation, the UV-visible

spectrophotometer (UV-1800, Shimadzu) was employed to measure absorbance at 700nm as well as record the polyphenol sample.

$$TPC\ (\%\frac{w}{w}) = \frac{Absorbance\ at\ 700\ nm\ of\ sample \times Dilution\ of\ Standard \times \%\ Purity\ of\ Standard}{Absorbance\ at\ 700\ nm\ of\ Standard \times Dilution\ of\ Sample}$$

Determination of Total Flavonoid Contents (TFC) [3,5, 7,11]

To determine the TFC the aluminium chloride colorimetric approach had been utilized. For standard linearity preparation, weighed 0.5mg/ml of rutin hydrate standard was prepared in the methanol. From this stock solutions of standard, 0.5, 1, 1.5, 2, 4, and 10ml were pipetted out into separated 10ml volumetric flasks, then the volume had been fitted utilizing methanol to the appropriate level. For sample preparation, weighed 0.4mg/ml of different solvent-based sample extracts in CH_3OH.. For color development, in a separate 10ml volumetric flask take 2ml of water after that 1ml of the sample/standard/blank (water). 5 minutes later, put 3ml of 5 percent $NaNO_2$ solution along with 0.3ml of 10% aluminium chloride solution. Then 6 min. Later , add 2ml of 1 M NaOH, utilizing water the volume is attained. Mix properly and immediately record absorbance at 415nm on UV-Visible Spectrophotometer (UV-1800, Shimadzu).

$$TFC\ (ppm) = \frac{(Absorbance\ of\ sample\ solution - intercept) \times 5 \times 100 \times 1000}{Slope\ of\ Standard\ weight \times weight\ of\ sample\ (gm) \times 10^6 \times 0.1}$$

Determination of Antioxidant Activity [2,4, 6, 7]

Antioxidant potential of *Cucurbita maxima* leaves extracts in Ethanol, Water, Hexane, Ethyl Acetate and Ethanol: Water (1:1) medium was assessed by DPPH scavenging activity. Gallic acid was utilized as a standard to ascertain antioxidant potential based on DPPH radical scavenging activity.

0.12 mg/ml of DPPH was made in ethanol and stored in a dark environment for 1hour to activate the DPPH solution. In the standard preparation, 40 mg of gallic acid was weighed accurately as well as dissolved in 100ml of ethanol to make a solution with 400 µg/ml concentration. For sample preparation, 40 mg of extract had been weighed accurately, it dissolved in 100ml of ethanol to make a solution at a concentration of 400µg/mL. From the prepared above stock solutions of standard and sample, 0.5, 1, 2, 3, and 5ml were pipette out into individual 10ml volumetric flasks then to it add 2ml of DPPH solution, then made up to mark with ethanol to obtain final concentrations of 20 µg/ml, 40 µg/ml, 80 µg/ml, 120 µg/ml along with 200 µg/ml. The absorbance was measured at 517 nm utilizing a UV-visible spectrophotometer (UV-1800, Shimadzu) after the samples were left in the dark for 30 minutes. Ethanol was utilized as the blank. The subsequent equation was employed to determine radical scavenging activity:

$$\%\ Inhibition = \frac{(AB - AA)}{AB} \times 100$$

Where, AB represents the absorbance of the control and AA represents the absorbance of the extract solutions/standard solutions.

LC-UV-MS Analysis [13]

Sample preparation

For sample preparation, weighed 0.02mg/ml water extract sample dissolved in water. The filtered solution with a 0.45µm membrane filter was then transferred into HPLC vials.

LC-UV-MS conditions

LC-UV-MS analysis was carried out using Agilent Technologies 1260 Infinity UPLC system with Q-TOF LC/MS, 6540 SERIES of LCMS system. The system used a reverse-phase analytical C-18 column dimension is 250 × 4.6 mm, 5 µm, Purosphere (Make-Merck). System used a binary solvent elution with gradient. Used 0.1% acetic acid in the water as the mobile phase A and methanol as the mobile phase B.

Flow rate was set to 1.0 mL/min with the column temperature held at 40°C while wavelengths for detection at 274 nm, 300 nm and 330 nm were chosen. Metline Metabolite PCDL Library is used to identify the compounds.

RESULTS AND DISCUSSION

Extraction yield

Extraction of *cucurbita maxima* leaves in aqueous medium showed good yield. The highest extraction yields were employing water extract as compared to other solvents extract. The dry sample yields for water, ethanol, ethyl acetate, and hexane extract were 22.0, 18.8, 7.3, 8.6, and 7.7%, as indicated in Table 1. Thus, polarity of the various chemicals in the plant caused diversity in the varied extract yields.

Total polyphenol content

Polyphenols are natural antioxidants that are responsible for plants antioxidant activity; therefore, the TPC in extract indicates the extent of its antioxidant activity. The results show that water extracts contain

a significant amount of TPC. Highest TPC is observed in the water and ethanol extracts correspondingly, whereas the lowest TPC is recorded in the hexane extract, as shown in (Table 1).

Total flavonoids content

The findings indicate that a considerable amount of TFC is present in the water extract. According to Table 1, the water extract has the highest TFC while the hexane extract has the lowest TFC. Figure 1 compares the extraction yield, TPC, along with TFC of the *Cucurbita maxima* leaves extract.

Table 1: Extraction yield (%), Total polyphenol content (%) and Total flavonoids content (ppm).

Extraction Solvent	% Yields	TPC (%)	TFC (ppm)
Water	22	34.57	35.78
Ethanol : Water	18.8	16.11	44.63
Ethanol	7.3	35.75	57.00
Ethyl Acetate	8.6	19.44	36.54
Hexane	7.7	22.76	25.27

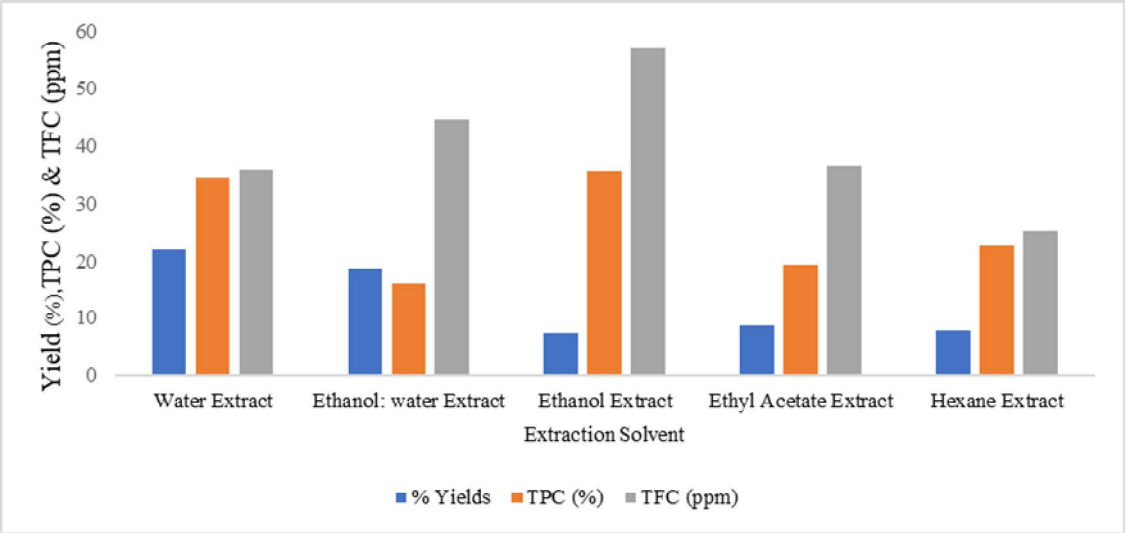

Figure 1. Effect of solvent type on extraction yield, total polyphenol content (TPC), and total flavonoid content (TFC)

Analysis of DPPH Scavenging Activity

The ability of antioxidant molecules to scavenge free radicals is regularly evaluated using the DPPH scavenging assay, which is regarded as one of the common and simple colorimetric techniques. When compared to other extracts, the water extract exhibits the strongest antioxidant activity. Overall, as the phenolic content rose, so did the DPPH scavenging capabilities. In comparison to other solvents, water is the most efficient way to extract *Cucurbita maxima* leaves for their DPPH scavenger activity. The antioxidant activity of various solvents extracted from *Cucurbita maxima* leaves is shown in the (Table 2). The antioxidant properties of different solvents extracted from *Cucurbita maxima* leaves at different concentrations are shown in (Figure 2).

Table 2. Antioxidant activity of different solvent extract of *Cucurbita maxima* leaves at different concentration.

Sample Concentration (ppm)	% Inhibition					
	Gallic acid Standard	Water extract	Ethanol : water extract	Ethanol extract	Ethyl acetate extract	Hexane extract
20	37	8	5	11	11	3
40	26	19	19	20	10	7
80	90	36	27	29	17	14
120	91	62	47	38	31	17
200	93	72	62	46	41	18

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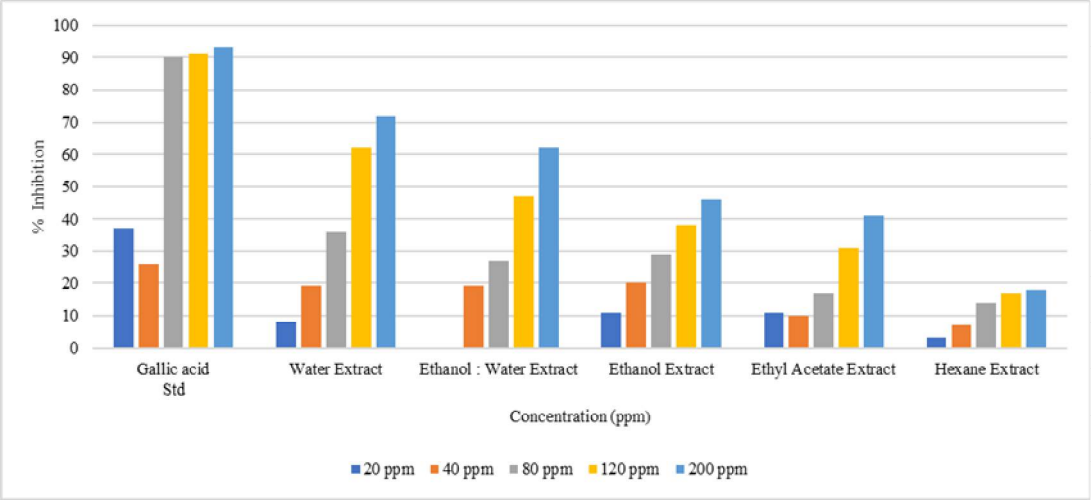


Figure 2. Antioxidant activity of different solvent extract of *Cucurbita maxima* leaves at different concentration.

Table 3: Probable markers identified by LC-UV-MS.

S.N.	Components	Molecular Formula	Molecular Mass	Retention Time	Mass adduct and % Area
1.	*p*-coumaric acid or 4-Hydroxycinnamic acid	$C_9H_8O_3$	164.0473	(Rt:17.688-18.154 min)	M+1 165.0539(57.37%) M+18 182.0808 (100 %)
2.	Ferulic acid	$C_{10}H_{10}O_4$	194.18	(Rt:21.349-21.865 min)	M+1 195.1218 (6.80 %) M+23 217.105 (84.97 %)
3.	Cinnamic acid	$C_9H_8O_2$	149.158	(Rt:22.132-22.232 min)	M+1 149.0588 (1.96%) M+18 166.085 (78.41%)
4.	Gallo catechin gallate	$C_{22}H_{18}O_{11}$	458.084	(Rt:24.645, 24.728 min)	M+1 459.2768 (1.18%) M+18 476.3049 (23.50%) M+23 481.2605 (100 %) M+39 497.2328 (2.5%)

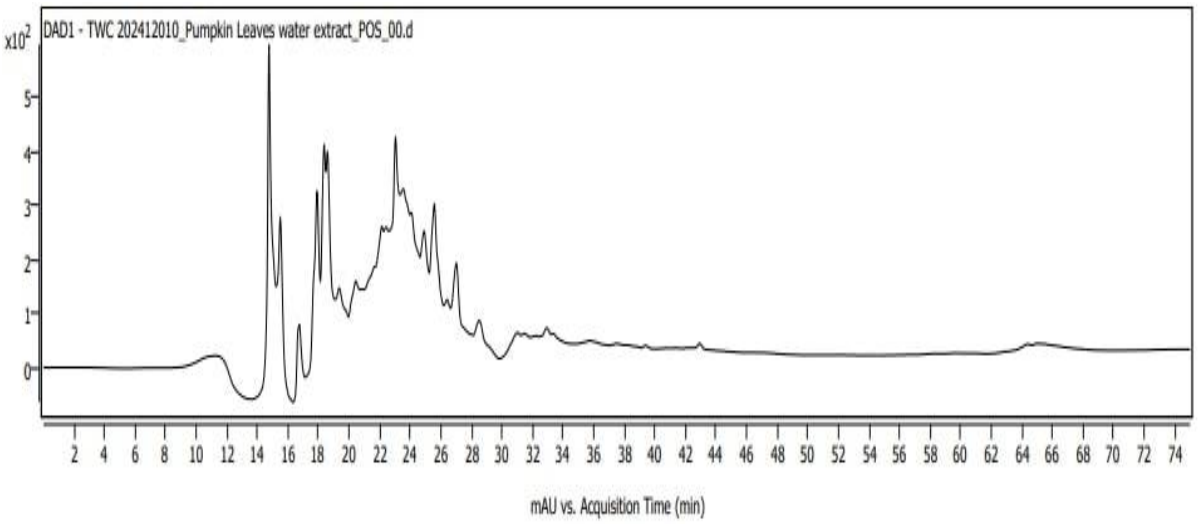
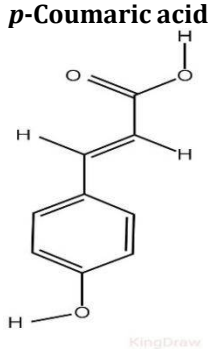
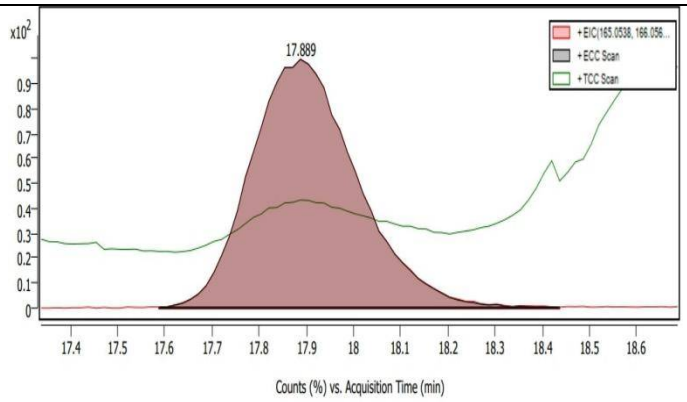
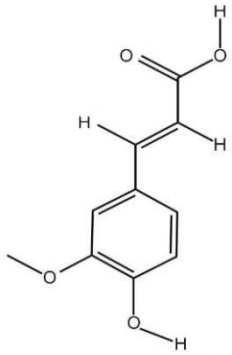
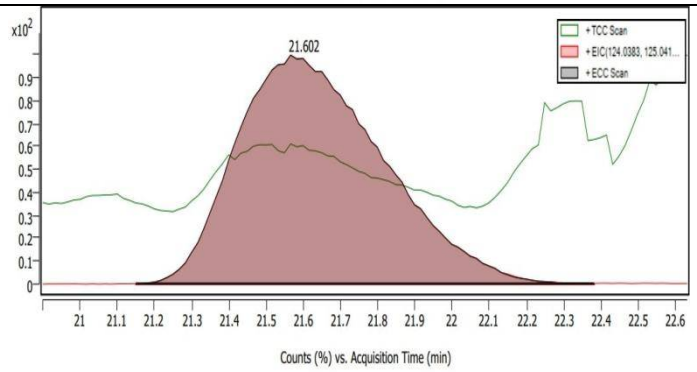
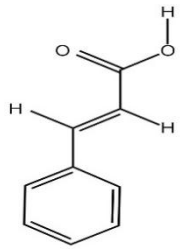
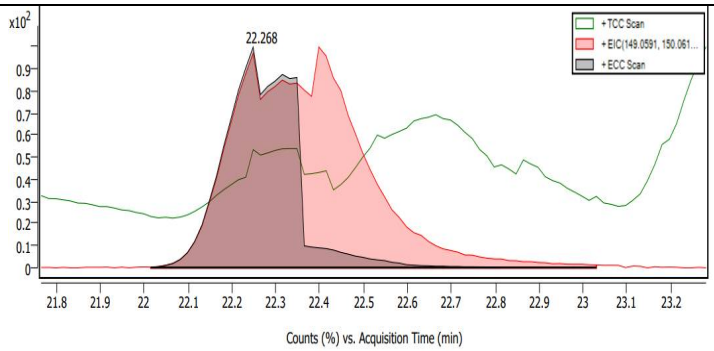
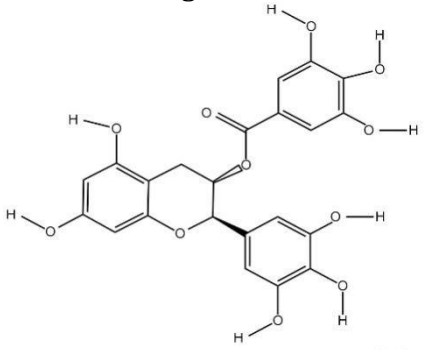
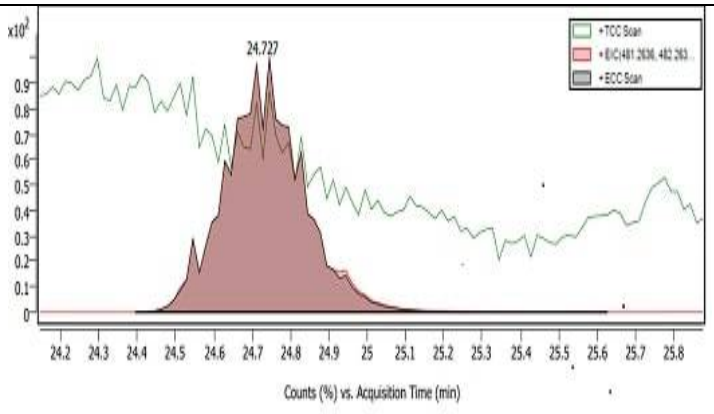


Figure 3: Chromatogram obtained by LC-UV-MS of water extract of *Cucurbita maxima* leaves.

Table 4: Name and structure of identified markers by LC-UV-MS.

Marker Identification	Retention time with Peak
***p*-Coumaric acid** 	
Ferulic acid 	
Cinnamic acid 	
Gallo catechin gallate 	

CONCLUSION

The optimization of extraction methods revealed that water, ethanol:water (1:1) and ethanol extracts of *Cucurbita maxima* leaves exhibited the highest antioxidant potential as compared to ethyl acetate and

hexane extracts. According to TPC, TFC along with DPPH assay highest activity was shown in water extracts. By analyzing the water extract by LC-UV-MS, the marker *p*-Coumaric acid or Hydroxycinnamic acid, Ferulic acid, Cinnamic acid as well as Gallo catechin gallate have been identified, which show antioxidant, anti-inflammatory, anti-cancer and antimicrobial properties. According to these results, *Cucurbita maxima* leaves are a rich source of naturally occurring antioxidants that could find application in functional foods, nutraceuticals and pharmaceutical goods.

CONFLICT OF INTEREST: Nil

FUNDING SOURCE: Nil

ETHICAL STATEMENT

The study was done in accordance with institutional and ethical guidelines; no human or animal subjects were utilized; all plant material was collected and handled carefully, and all federal and local regulations were observed.

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