Journal of Experimental Biology and Agricultural Sciences, October - 2022; Volume – 10(5) page 1076 – 1092

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Bio Characterization via FTIR and GCMS Analysis of *Cucurbita* variety (Yellow and White Pumpkin)

Gowtham K[1], Saruniyadevi M[1], Thenmozhi M[2], Gopiesh Khanna V[2], Jayanthi M[2*]

[1]Plant Biotechnology Lab, Department of Biotechnology, BharathiarUniveristy, Coimbatore
[1]Department of Life Science, University of Greenwich, London, United Kingdom
[2] Department of Biotechnology, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai 117

Received – January 26, 2022; Revision – March 16, 2022; Accepted – March 20, 2022
Available Online – October 31, 2022

DOI: http://dx.doi.org/10.18006/2022.10(5).1076.1092

KEYWORDS

Cucurbita pepo L.

Peel

White pumpkin

Yellow pumpkin

Ethyl acetate

GC-MS

ABSTRACT

The current study aimed to conduct phytochemical screening, FTIR, and GCMS analysis in squash (*Cucurbita pepo* L.,) also known as a yellow and white selected pumpkin. It's one of the dicotyledonous vegetables consumed in daily diets that imparts high inhibitor properties of inflammation, cancer, and diabetes. Traditionally it is used as an anti-helminthic remedy. The phytochemical characterization can facilitate seeking out the substance with a therapeutic property. The peel, flesh, and seed sample of each pumpkin variety were used as sources and extracted consecutively with ethyl acetate and acetonitrile using the maceration method. Phytochemical screening and quantification were carried out by standard analytical methods. The functional groups of the sample extracts were analyzed using FT-IR methods. Further, phytochemical profiling was carried out utilizing the GCMS technique to identify the therapeutically important chemicals contained in the sample. Phytochemical analysis of ethyl acetate and acetonitrile extracts showed the presence of major components like alkaloids, phenol, carbohydrate, and proteins. The farthest alkaloid, phenol, carbohydrate, and protein varied consequently for different parts like peel, flesh, and seed. The FT-IR analysis of each extract in the peel, flesh, and seed revealed that the ethyl acetate extract had the most functional groups. The major peak was characterized at wavelength 3004.24 to 3421.05 nm which indicates O-H functional group. Further quantification and GC-MS analysis were performed in ethyl acetate extract. Remarkably, GC-MS analysis of yellow and white pumpkin ethyl acetate extracts showed the utmost 6 - 8 compounds within the flesh part. Further, employing these compounds for anti-inflammatory and anti-microbial assays may aid in the discovery of new drugs for therapeutic applications.

* Corresponding author
E-mail: rsjaya24@gmail.com (Jayanthi M)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Fruits and vegetables are vital components of a healthy daily diet because they provide a well-balanced plate of natural antioxidants. As a result, there has been an increased interest in screening natural antioxidants, preferably from fruits (Chandrasekara and Shahidi 2010, 2011; Deng et al. 2012; Li et al. 2007, 2008; Stangeland et al. 2009). Cucurbits are fruit and vegetable crops that belong to one of the most genetically diverse plant families (Bisognin 2002). It is a tropical and subtropical plant found worldwide. It is a member of the Cucurbitaceae family, which includes approximately 110 genera and 650–850 species (Pandey 1969). While the growth habitats above the ground level and at the root are comparable in grown cucurbits, they have a wide range of fruit characteristics. The fruits have dined as baked, pickled, candied, salads, or desserts. Members of the Cucurbitaceae family have always been thought about as a theme of analysis because of their anti-fungal, anti-bacterial, anti-viral, anti-diabetic, anti-tumour, and anti-AIDS activities (Mukherjee et al. 2022).

Cucurbita, commonly known as white and yellow pumpkin, is a popular vegetable in Tamilnadu due to its medicinal properties. The cytotoxicity, hepatoprotective, anti-inflammatory, and cardiovascular effects of the Curcurbitaceae family have been widely researched, and utilized in many pharmacopeias for millennia (Kaushik et al. 2015). Cucurbits are one of the most nutritious dietary for a healthy diet as they contain vitamins A, B1, B6, and C, a large number of alternative nutrients, and 96% of water. Due to its ethnopharmacological properties such as anti-inflammatory, anti-viral, analgesic, anti-ulcer, anti-diabetic, and anti-oxidant, *Cucurbita pepo* (yellow pumpkin) is one of the world's oldest cultivated species, according to the literature review (Smith 1997; Wang et al. 2001). Earlier phytochemical analysis on fruits of eighteen cultivars of Cucurbita found to possess low phenolic content (Kostecka-Gugała et al. 2020). At the same time seeds of this species are known to have an expensive supply of natural edible oil (fatty acid) reported to have a significantly high level of linoleic acid (Shelenga et al. 2020)

Cucurbita maxima (White pumpkin) which are grown in warm countries are popularly acknowledged vegetable crop for its nutritional and medicinal purposes. Peel and fruits of pumpkins contain sugars, volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, and minerals (Marquez Cardazo et al. 2021). Although numerous reports on phytoconstituents and their healthful properties are available the current investigation was to unveil the constituents present via solvent potency in every peel, flesh, and seed part of yellow and white pumpkin, individually.

2 Materials and Methods

2.1 Plant Material

The whole fruit of pumpkin white and yellow varieties was purchased from the general market located in Saidapet, Chennai. To remove the dirt and its specks of dirt, the fruits were vigorously washed with water followed by distilled water wash.

2.2 Sample Preparation and Extraction

From the cleaned sample, peel, flesh, and seed were separated from each species individually and left to shade dry to get rid of the equilibrium wet from the sample. The samples were ground to powder form and stored for further study. Extraction was carried out through the maceration method as described by Muralidharan et al. (2018). About 5 g of peel, flesh and the seed of each species on an individual basis was extracted with 100 ml of ethyl acetate and acetonitrile on a shaker with 150 rpm at 30°C for 24 hours. The extracts were filtered using Whatman No. 1 filter paper.

2.3 Qualitative Phytochemical Screening

Qualitatively phytochemicals screening was carried out according to the method described by Morsy (2014).

2.4 Metabolites Quantification

2.4.1 Total Carbohydrate Determination

Hedge and Hofreiter (1962) described a method for estimating carbohydrate content. In a boiling water bath, 100 mg of the sample was hydrolyzed for 3 hrs with 5.0 mL of 2.5N HCl and neutralized with solid sodium carbonate until the effervescence has stopped. It was made up to 100 mL with distilled water and centrifuged at 5000 rpm for 15 min and the supernatant was collected. About 0.2 mL of supernatant of each sample, 0.8 mL of distilled water, and 4.0 mL of anthrone reagent were mixed. The reaction mixture was heated for 8 min, in a boiling water bath and cooled rapidly. Using a spectrophotometer, the colour intensity was measured at 630 nm. Standard glucose was prepared by dissolving 100 mg in 100 mL of distilled water. Further working standard was prepared from 10 mL of stock diluted to 100 mL with distilled water. The glucose content was measured using D-glucose as a standard and expressed as (µg/mL).

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{mg of glucose x 100}}{\text{Volume of test sample}}$$

2.4.2 Total Protein Determination

The total protein was determined using Lowry's method (1951). About 4.5 mL of reagent I having 96 mL of solution A (2 %

Content	Test	Observation	Inference
Alkaloid	**Mayer's Test** A few drops of Mayer's reagent were applied to a 2 mL extract along the test tube's side	Appearance of white creamy precipitate	Presence of alkaloid
Protein	1 mL concentrated H_2SO_4 and a few drops of concentrated Nitric acid were added to 1ml of extract	Formation of yellow colour	Presence of protein
Flavonoid	1 mL of 10% lead tetra acetate was added to 1 mL of extract	Indication of yellow hue	Presence of Flavonoid
Coumarins	A total of 3 mL of 10% sodium hydroxide was added to a 2 mL extract	Yellow colour formation	Presence of Coumarins
Carbohydrate	**Fehling's Test** 5 mL Fehling's A reagent was added to 1 mL extract, and the mixture was boiled for 5 minutes in a boiling water bath	Shift in yellow colour to red precipitate	Presence of carbohydrate
Glycosides	**Liebermann's Test** 2 mL ferric chloride and 2 mL H_2SO_4 were added to a 2 mL extract	Brown ring formation	Presence of glycosides
Steroid's	**Salkowski Test** 2 mL acetic anhydride was added to a 2 ml extract, and 2-3 drops of concentrated H_2SO_4 were added slowly along the sides of the test tube	Change in colour from violet to blue to green	Presence of Steroid
Terpenoid's	2 mL acetic anhydride was added to a 2 mL extract, and 2-3 drops of concentrated H_2SO_4 were added slowly along the sides of the test tube	Indication of deep red hue	Presence of Terpenoid
Saponin's	**Emulsion Test** Few drops of olive oil were added to a 5 mL of extract	Formation of emulsion	Presence of Saponin
Tannin	**Braymer's Test** 2 mL extract was dissolved in 2 mL distilled water, and 2-3 drops of ferric chloride (5%) were added	Presence of green precipitate	Presence of Tannin
Phlobatannin	**Precipitate Test** 2 mL extract and 2 mL of 1% HCl, heated in a boiling water bath for 5 minutes	Indication of red colour precipitate	Presence of Phlobatannins
Emodins	2 mL of 0.25% Ninhydrin, and 3 mL of benzene were added to a 2 mL of extract, which was then boiled for a few minutes	Formation of blue colour	Presence of Emodins
Anthraquinones	**Borntrager's Test** 3 mL of benzene and 5 mL of 10 % ammonia were added to 3 mL of extract.	Indication of pink, violet, or red coloration in the ammonical layer.	Presence of Anthraquinones
Anthocyanin	Add 2 mL of 2N HCl and a few drops of ammonia to 2 mL of extract	Colour change from pinkish red to bluish violet	Presence of Anthocyanin
Leucoanthocyanin	Add 5 mL of Isoamyl alcohol to 5 mL of extract	Change of organic layer into red	Presence of Leucoanthocyanin
Phenol	**Ferrichloride Test** In 5 mL of distilled water, the extract (50 mg) is dissolved. A few drops of a neutral 5% ferric chloride solution were added to this	Dark green colour formation	Presence of Phenol

Na_2CO_3 in 0.1N NaOH) + 2.0 mL of solution B (1% NaK Tartarise in H_2O) + 2.0 mL of solution C (0.5 % $CuSO_4$ in H_2O) was added to 1.0 mL of sample extract and incubated in a boiling water bath for 10 min. After 30 min of incubation, 0.5 mL of reagent II (Folin Phenol) was added and incubated in the dark for 30 min. At 660 nm, the intensity of the colour was measured. From the standard graph, the amount of protein in the samples was calculated.

2.4.3 Total Alkaloid Determination

The alkaloid content was determined using the Shamsa et al. (2007) method. Tannic acid was tested at different concentrations, with results expressed as mg tannic acid equivalents/g dry matter (TAE/g DM). The dried samples of about 1 mg each part were dissolved with 1 mL of 2N HCl and filtered. The filtered samples were transferred to a separating funnel; to this 5.0 mL of phosphate

buffer (pH4.7) and 5.0 mL of bromocresol green solution were added. The mixture was shaken by sequentially adding 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of Chloroform. The chloroform layer was collected in a 10 mL volumetric flask and diluted with chloroform up to the mark. The samples were absorbed against blank at 470 nm.

2.4.4 Total Phenol Determination

The total phenol content of the extract was determined using the Folin-Ciocalteau reagent, according to Bhuvaneshwari et al. (2016). The Folin-Ciocalteau reagent (500 µL) was added to both 100 µL of phenol standard solutions (Gallic acid was tested at various concentrations and the result was expressed as mg Gallic acid equivalents/g dry matter – GAE/g DM). To achieve a total volume of 1.5 mL, distilled water was added to each tube. After 8 minutes of incubation, 2.5 mL of 20% sodium carbonate was added to each test tube. After thoroughly shaking the mixture, the total phenolic content was measured at 765 nm against a blank.

2.5 Fourier Transform-Infrared (FT-IR) Spectroscopy

FT-IR is a useful tool for the identification and characterization of functional groups (chemical bonds) in a compound. Furthermore, FT-IR spectra are unique in that they are similar to a molecular "fingerprint." Between the cells, the drop creates a thin layer of film. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet using a hydraulic press before being analyzed. FTIR spectroscopy IR-affinity was performed on a yellow pumpkin sample extracted with ethyl acetate and acetonitrile extracts (Shimadzu, Japan). The samples were run in the infrared region between 400 nm and 4000 nm, and a standard DLATGS detector with a mirror speed of 2.8 mm/sec was used.

2.6 Gas Chromatography-Mass Spectroscopy (GC-MS)

A Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) with a Perkin Elmer Auto sampler XLGC was used for the GC-MS analysis. Perkin Elmer Elite - 5 capillary columns measuring 30 m x 0.25 mm with a film thickness of 0.25 mm composed of 95 % dimethylpolysiloxane were used. Helium was used as the carrier gas, with a flow rate of 0.5 mL/min. And a sample injection volume of 1 µL was used. The inlet temperature was maintained constant at 250°C. And the oven temperature was set to 110°C for 4 min and then increased to 240°C. Temperatures were then programmed to rise to 280°C at a rate of 20°C per minute for 5 min. The total running time was 90 min. The temperature of the MS transfer line was kept at 200°C. The source temperature was kept constant at 180°C. For compound identification and quantification, GC-MS was analyzed using electron impact ionization at 70eV, and data were analyzed using total ion count (TIC). The spectrums of the components were compared to a database of known component spectrums stored in the GC-MS library (Seemakkani and Thangapandian 2012).

2.7 Statistical Analysis

The mean and standard deviation of triplicate determinations was calculated using Microsoft Excel.

Table 1 Phytochemical constituents in ethyl acetate extract

S. No	Constituents	Yellow Pumpkin			White Pumpkin		
		Peel	Flesh	Seed	Peel	Flesh	Seed
1.	Tannins	+	-	-	+	-	-
2.	Flavanoids	+	+	+	+	-	-
3.	Terpenoids	-	+	+	-	-	+
4.	Saponins	-	-	-	-	-	-
5.	Steroids	+	++	+	++	+	+
6.	Phlobatannins	+	+	+	-	-	-
7.	Carbohydrates	++	++	++	++	+	+
8.	Glycosides	++	+	+	+	-	+
9.	Coumarins	+	+	+	-	-	-
10.	Alkaloids	+	+	+	+	-	-
11.	Proteins	+	+	+	+	-	-
12.	Emodins	-	-	-	-	-	-
13.	Anthraquinones	-	-	-	-	-	-
14.	Anthocyanins	-	-	-	-	-	-
15,	Leucoanthocyanins	-	-	-	-	-	-
16.	Phenol	+	-	-	+	-	-

Here ++ indicate: Highly presence, + indicate: Presence, - indicate: absence

Table 2 Phytochemical constituents in acetonitrile extract

S. No	Constituents	Yellow Pumpkin			White Pumpkin		
		Peel	Flesh	Seed	Peel	Flesh	Seed
1.	Tannins	+	-	+	+	-	-
2.	Flavanoids	+	+	+	+	-	-
3.	Terpenoids	-	-	+	+	-	-
4.	Saponins	+	+	+	+	++	+
5.	Steroids	+	-	+++	+	+++	-
6.	Phlobatannins	-	-	+	-	-	-
7.	Carbohydrates	+	+	+	+	+	+
8.	Glycosides	+	+	+	+	-	+
9.	Coumarins	+	+	+	-	-	-
10.	Alkaloids	+	+	+	+	+	-
11.	Proteins	+	+	+	+	-	-
12.	Emodins	-	-	-	-	-	-
13.	Anthraquinones	-	-	-	-	-	-
14.	Anthocyanins	-	-	-	-	-	-
15,	Leucoanthocyanins	-	-	-	-	-	-
16.	Phenol	+	-	+	+	-	-

Here ++ indicate: Highly presence, + indicate: Presence, - indicate: absence

Table 3 Quantitative analysis of primary metabolites in ethyl acetate extract

Primary Metabolites	Yellow Pumpkin			White Pumpkin		
	Peel	Flesh	Seed	Peel	Flesh	Seed
Total Carbohydrate (µg/ml)	1.3±0.1	2.2±0.2	2.1±0.1	2.6±0.2	3.1±0.2	3.23±0.2
Total Protein (µg/ml)	10.4±0.3	13.2±0.2	15.4±0.4	8.4±0.3	6.7±0.4	11.3±0.4

3 Results

3.1 Qualitative Phytochemical Screening

The phytochemical screening in the yellow and white pumpkin variety was carried out using acetonitrile and ethyl acetate extracts. The screening test disclosed the presence of constituents in varied proportions within the peel, flesh, and seed as described in Tables 1 and 2. The result showed that tannin, flavonoid, steroid, saponin, carbohydrate, protein, alkaloid, and phenol were present in each extract of the two pumpkin varieties. The acetonitrile extract of each part of both kinds had a substantial presence of metabolites, but the ethyl acetate extract had a lower number of metabolites. Among the fruit parts studied, flesh showed a reduced range of metabolites like saponin, steroid, carbohydrate, and alkaloid, compared to peel and seed parts. Though the samples were analyzed by polarity-based different solvents, ethyl acetate extract of each yellow and white pumpkin variety showed an inflated range of metabolite presence, therefore for additional study ethyl acetate extract samples were solely analyzed.

3.2 Determination of Primary Metabolite

Plant growth requires the presence of primary metabolites, which are essential components and play important roles in pharmaceutical compounds like antipsychotic drugs as precursors or pharmacologically active metabolites (Jayaraman 1981). The results of the measurement of primary and secondary metabolites observed in the study are shown in Table 3. The carbohydrate content was found to be high within the flesh part compared to the peel and seed part, with an amount of 2.2±0.2 and 3.1±0.2 µg/mL in the yellow and white variety respectively. Whereas a lesser amount of carbohydrate was found in the yellow peel part compared to other parts and peel parts of the white variety. Consequently, total protein analysis showed the extreme presence of 15.4±0.4 µg/mL and 11.3±0.4 µg/mL in seed parts of yellow and white varieties respectively. Whilst a low amount of 6.7±0.4 µg/mL was shown in white flesh extract. The amount of protein in the yellow variety parts was found to be higher than in the white variety parts.

Table 4 Quantitative analysis of secondary metabolites in ethyl acetate extract

Secondary Metabolites	Yellow Pumpkin			White Pumpkin		
	Peel	Flesh	Seed	Peel	Flesh	Seed
Total Alkaloid (µg/ml)	2.8±0.3	4.9±0.2	4.8±0.3	7.4±0.3	2.0±0.2	2.0±0.3
Total Phenol (µg/ml)	39.1±0.7	54.0±0.5	23.2±0.4	22.2±0.4	43.6± 0.8	26.6±0.5

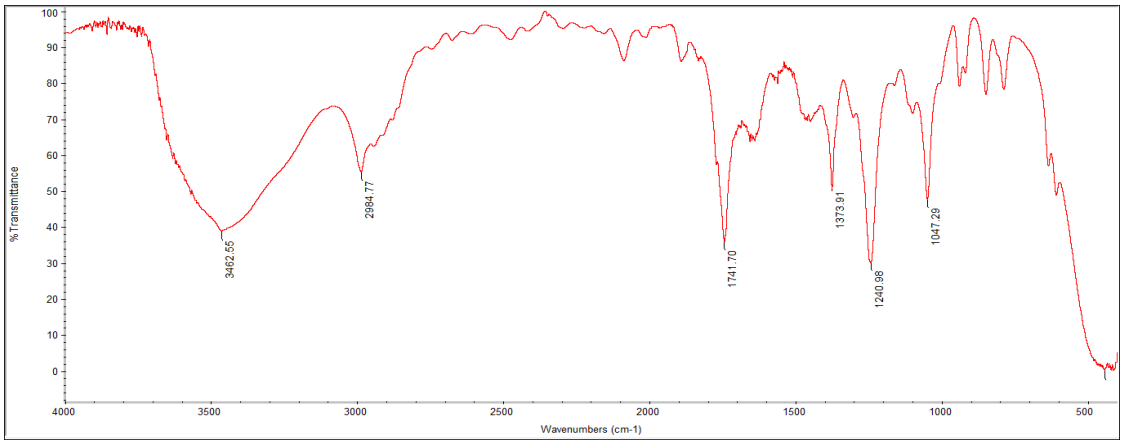

Figure 1 FTIR spectrum of Yellow pumpkin variety peel

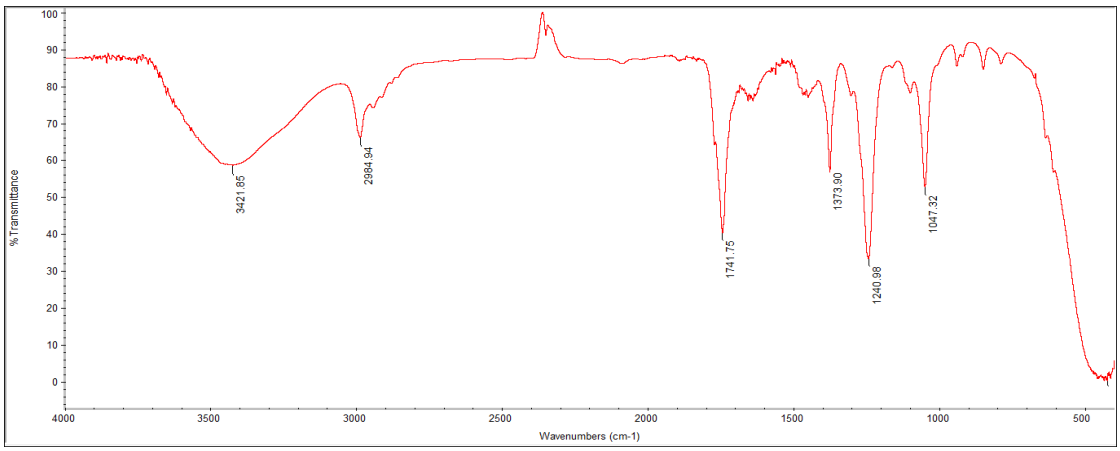

Figure 2 FTIR spectrum of Yellow pumpkin variety flesh

3.3 Determination of Secondary Metabolite

Plant secondary metabolites are important to treat various diseases and pharmacological applications in recent decades. It conjointly plays a task as a troubleshooter in the ecological interaction between plants and their environment. White variety peel had a significantly higher alkaloid concentration (7.4±0.3 g/mL) compared to its seed which showed lower alkaloid concentrations. While in the case of the yellow variety, the amount of alkaloid was found to be similar in flesh and seed (2.0±0.2 µg/mL) and the peel showed 2.8±0.3 µg/mL**.** Subsequently, the analysis of phenol content in flesh parts showed a considerably higher amount of 54.0±0.5 µg/mL and 43.6±0.8 µg/mL in yellow and white pumpkin varieties (Table 4).

3.4 Fourier Transmittance-Infrared Spectrometry (FT-IR)

The functional groups in ethyl acetate extract of yellow and white variety peel, flesh, and seed, were separated based on absorption using an infrared spectrum and are displayed in Figures 1 to 6. The results of the FTIR spectrum helped to spot the active components within the extract-supported magnitude relation within the region of IR radiation. The FTIR result analysis showed the absorption peaks of alcohol, amine, alkane, esters, nitro compounds, carboxylic acid and phosphines are the major functional groups observed in both varieties of pumpkin ethyl acetate extract. FTIR spectrometry proved to be a reliable and sensitive methodology for detecting biomolecular composition based on the functional groups present (Tables 5 and 6).

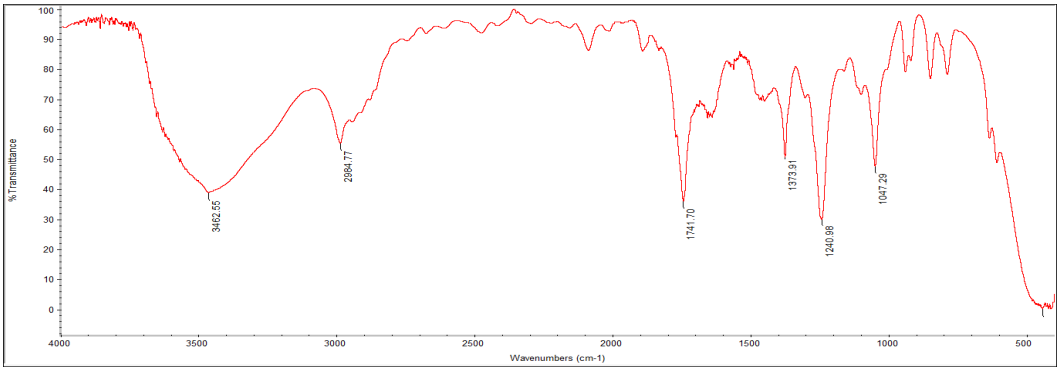

Figure 3 FTIR spectrum of Yellow Pumpkin variety seed

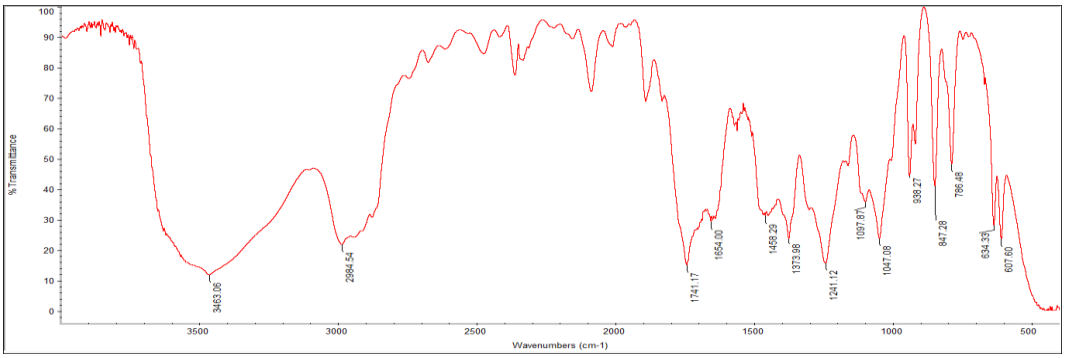

Figure 4 FTIR spectrum of White Pumpkin variety peel

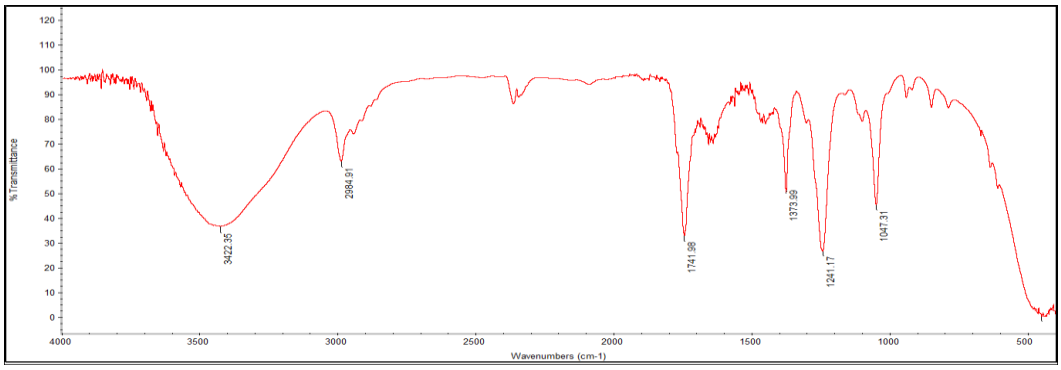

Figure 5 FTIR spectrum of White pumpkin variety flesh

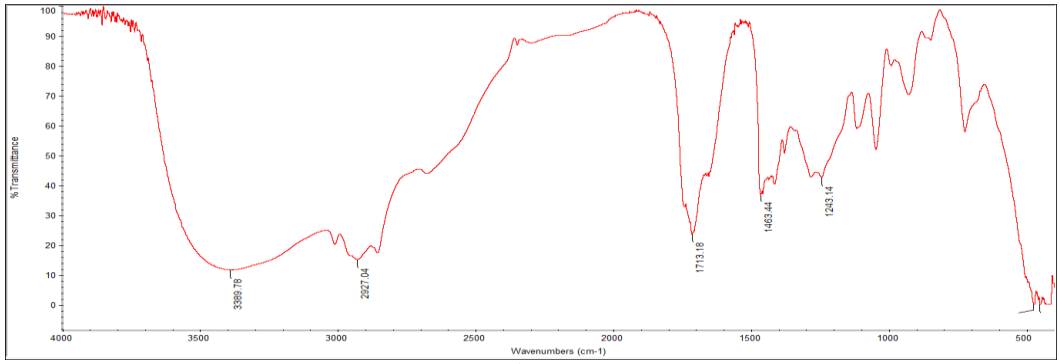

Figure 6 FTIR spectrum of White pumpkin variety seed

Table 5 Peak value, bond type, and functional group for FTIR spectra ethyl acetate extract of Yellow Pumpkin Variety

Sample Analysed	Frequency Cm^{-1}	Functional Group	Class
Peel	3402.55	O-H	Alcohol
		N-H	amine
	2984.77	C-H	Alkane
	1741.70	C=O	Esters
	1373.91	N=O	Nitro compounds
	1240.98	C-O	Carboxylic acids
		C-N	Amines
		P-H	Phospines
		P=O	Phosphonate, phosphoramide
		$Si-CH_3$	Silane
	1047.29	O-C	Anhydrides
		C-N	Amines
		S=O	Sulfoxide
		P-OR	Esters
		Si-OR	Silane
Flesh	2984.94	C-H	Alkane
	1741.75	C=O	Esters
	1373.90	N=O	Nitro
		S=O	Sulfate
	1240.98	C-O	Carboxylic acid
		C-N	Amines
		N-O	Aromatics
		P=O	Phosphonate, Phosphoramide
		$Si-CH_3$	Silane
	1047.32	O-C	Anhydride
		C-N	Amines
		S=O	sulfoxide
Seed	3420.07	OH	Alcohol
		N-H	Amines
	1646.93	C=C	Alkene
		C=O	Amines
		NH_2	Amines
	1241.15	C-O	Carboxylic acid
		C-N	Amines
		P-H	Phosphines
		P=O	Phosphonate, Phosphoramide
		$Si-CH_3$	Silane
	668.47	NH_2 & N-H	Amines

Table 6 Peak value, bond type, and functional group for FTIR spectra ethyl acetate extract of White Pumpkin Variety

Sample Analysed	Wavelength Cm^{-1}	Functional Group	Class
Peel	2984.54	C-H	Alkane
	1741.17	C=O	Esters
	1654.00	C=C	Alkene
		C=N	Oxime (Oxidized Nitrogen)
		C=O	Amides
	1458.29	C=C	Aromatic
		N=O	Nitoso, Nitro
	1373.98	N=O	Nitro
		S=O	Sulfate
	1241.12	C-O	Carboxylic Acids
		C-N	Amines
		N-O	Aromatic
		P=O	Phosphonate, Phosphoramide
		$Si-CH_3$	Silane
	1097.87	C-O	Alcohol
		O-C	Anhydrides
		C-N	Amines
		C=S	Thio carbonyl
	1047.08	O-C	Anhydrides
		C-N	Amines
		S=O	Sulfoxide
		Si-OR	Sulfoxide
	938.27	C-C	Alkane
		P-OR	Esters
		N-O	Oxime
	847.28	C-C	Alkane
		NH_2& N-H	Amines
		S-OR	Esters
	786.48	NH_2-NH	Amines
		S-OR	Esters
Flesh	2984.91	C-H	Alkane
	1741.17	C=O	Esters
	1373.99	N=O	Nitro compounds
	1241.17	C-O	Carboxylic
		C-N	Amines
		P-H	Phosphines
		P=O	Phosphonate
		P=O	Phosphoramide
		$Si-CH_3$	Silane
	1047.31	O-C	Anhydrides
		C-N	Amines
		S=O	Sulfoxide
		P-OR	Esters
		Si-OR	Silanes

Sample Analysed	Wavelength Cm^{-1}	Functional Group	Class
Seed	2927.04	C-H	Alkane
		O-H	Carboxylic Acids
	1713.18	C=O	Carboxylic Acids
		C=O	Ketone
	1463.44	C=C	Aromatics
	1243.14	$Si-CH_3$	Silane
		P=O	Phosphoramide, Phosphonate
		P-H	Phosphine
		N-O	Aromatic
		C-N	Amines

Table 7 Phytocomponents identified in ethyl acetate extracts of peel, flesh, and seed of Yellow Pumpkin var. by GC-MS peak

S. No	Sample Name	RT (Min)	Mol. wt	Mol. formula	Name of the compound	Compound Nature	Biological activity
1	*Peel*	20.44	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (z,z)	Polyunsaturated fatty acid (Linoleic acid)	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic
		22.95	390	$C_{24}H_{38}O_4$	Di-N-Octylphthalate	1,2-Benzenedicarboxylic acid, dioctyl ester	Antifouling, Antimicrobial
		24.95	474	$C_{30}H_{50}O_4$	1,2-Benzenedicarboxylic acid, Diundecyl ester	Phthalic acid	Antioxidant, Anemiagenic
		29.34	468	$C_{32}H_{52}O_2$	Lup-20(29)-E,N-3-ol, Acetate,3-Beta	Lupeol acetate	Antinoceptive, Anti-inflammatory
		30.03	468	$C_{29}H_{40}O_5$	Phthalic acid, 3-methoxybenzyl tridecyl ester	Phthalate	Antioxidant, Anemiagenic
2	Flesh	19.81	280	$C_{20}H_{40}$	9-eicosene – E	Essential oil	Antimicrobial and Cytotoxic properties
		22.23	418	$C_{26}H_{42}O_4$	Phthalic acid, Bis(7-methyloctyl) ester	Plasticizer compound	Antimicrobial, Antifouling
		22.56	334	$C_{16}H_{31}BrO_2$	Pentadecanoic acid, 15-bromo-, methyl ester	Fatty acid esters	Antifungal, antibacterial, antimicrobial, emulsifier,
		22.80	278	$C_{16}H_{22}O_4$	1,2-benzene dicarboxylic acid, BIS (2-methyl propyl) ester	Isobutyl phthalate	Antimicrobial, antifouling
		24.66	222	$C_{12}H_{14}O_4$	1,2-benzene dicarboxylic acid, diethyl ester	Diethyl phthalate	Cosmetics, insecticides, plasticizers
		24.88	446	$C_{24}H_{38}O_4$	1,2-benzene dicarboxylic acid, diisodecyl	Diisoctyl phthalate	Antimicrobial antifouling
		29.51	426	$C_{30}H_{50}O$	Lupeol	Triterpenoids	Anti-inflammatory, anticancer, antiprotozoal, chemopreventive
	Seed	18.40	256	$C_{17}H_{36}O$	n-Heptadecanol-1	Fatty alcohol	Antiarthritis, skin diseases
		20.18	213	$C_{12}H_{23}NO_2$	1-Hexyl-2-Nitrocyclo hexane	Ketone	Neuroactive, anti-inflammatory, analgesic properties
		22.84	390	$C_8H_6O_4$	1,2-benzene dicarboxylic acid	Phthalic acid	Antioxidant, antimicrobial, antifouling

3.5 Gas Chromatography-Mass Spectroscopy Analysis (GC-MS)

The key to revealing the constituents of volatile matter, long-chain, branched-chain hydrocarbons, alcohols, acids, esters, and so on is GC-MS. Yellow and white variety pumpkins to date are used for various medicative functions to grasp the constituents present within the peel, flesh, and seed, therefore ethyl acetate extract of plant sample was analyzed. The compound was analyzed based on the retention time present in the total ionic chromatogram. Results presented in Tables 7 and 8 revealed the list of active principle compounds along with their retention time, molecular formula, chemical name, and common name.

The active principle compounds identified in our present study within the peel, flesh, and seed parts of both yellow and white varieties (Figures 7 and 8) are 9,12-octadecadienoic acid (z,z), 1,2-benzene dicarboxylic acid, Di-undecyl ester, 9-eicosene–E, 1,2-benzene dicarboxylic acid, diethyl ester, lupeol, and eicosane.

Table 8 Phytocomponents identified in ethyl acetate extracts of peel, flesh, and seed of White Pumpkin variety by GC-MS peak

S. No	Sample Name	RT (Min)	Mol wt	Mol. formula	Name of the compound	Compound Nature	Biological activity
1.	Peel	20.12	213	$C_{12}H_{23}NO_2$	1,-Hexyl-2-Nitrocyclohexane	Ketone	Neuroactive, anti-inflammatory, analgesic properties
		22.78	390	$C_8H_6O_4$	1,2-benzene dicarboxylic acid	Phthalic acid	Antioxidant, antimicrobial, antifouling
		24.65	390	$C_{24}H_{38}O_4$	1,2-benzene dicarboxylic acid, diisodecyl	Diisoctyl phthalate	Antimicrobial antifouling
		28.93	470	$C_{32}H_{54}O_2$	9,19-cyclonostan-3-ol,acetate, (3,-Beta)	Cycloartenol	Steroid precursor
		29.31	290	$C_{20}H_{34}O$	Thunbergol	Diterpene alcohol	Antibacterial
		30.15	282	$C_{20}H_{42}$	Eicosane	Alkane	Antifungal, antitumor, antibacterial, larvicidal, antimicrobial, cytotoxic
2	Flesh	19.18	280	$C_{20}H_{40}$	9-eicosene – E	Essential oil	Antimicrobial and Cytotoxic properties
		20.41	278	$C_{16}H_{22}O_4$	1,2-benzene dicarboxylic acid, BIS (2-methyl propyl) ester	Isobutyl phthalate	Antimicrobial, antifouling
		22.69	390	$C_8H_6O_4$	1,2-benzene dicarboxylic acid	Phthalic acid	Antioxidant, antimicrobial, antifouling
		22.71	390	$C_{24}H_{38}O_4$	1,2-benzene dicarboxylic acid, diisodecyl	Diisoctyl phthalate	Antimicrobial antifouling
		22.74	278	$C_{16}H_{22}O_4$	1,2-benzene dicarboxylic acid, BIS (2-methyl propyl) ester	Isobutyl phthalate	Antimicrobial, antifouling
		19.18	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (z,z)	Polyunsaturated fatty acid (Linoleic acid)	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic
3	Seed	18.11	256	$C_{17}H_{36}O$	n-Heptadecanol-1	Fatty alcohol	Antiarthritis, skin diseases
		19.75	138	C_9H_{14}	Isophorone	Cyclic ketone	Anti-platelet
		19.81	248	$C_{12}H_{25}Br$	2-Bromo dodecane	Alkane	Antibacterial activity

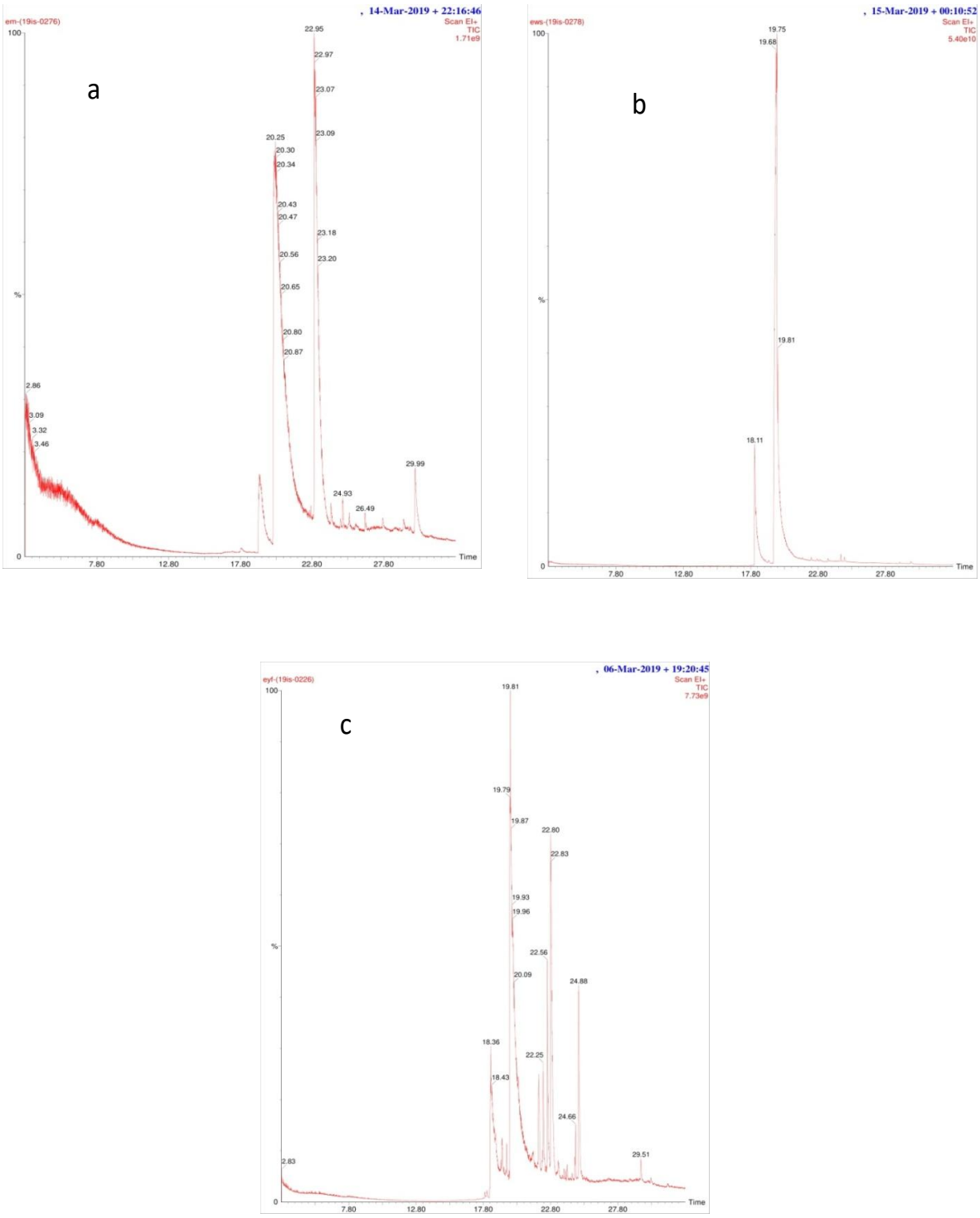

Figure 7 TIC of GC-MS analysis of Yellow pumpkin var. Ethylacetate Extract a: Peel; b: Flesh; c: Seed

Following our study, similar results are ascertained in some medicative plants (Banakar and Jayaraj 2018; Naz et al. 2020). The results indicated the ethyl acetate extract's capacity to extract more active components, which may be responsible for a variety of biological functions when comparing the biological activity of the compounds to literature data from previous research on medicinal plants (Swamy et al. 2015; Velmurugan and Anand 2017; Banakar and Jayaraj 2018; Rajadurai et al. 2018; Naz et al. 2020).

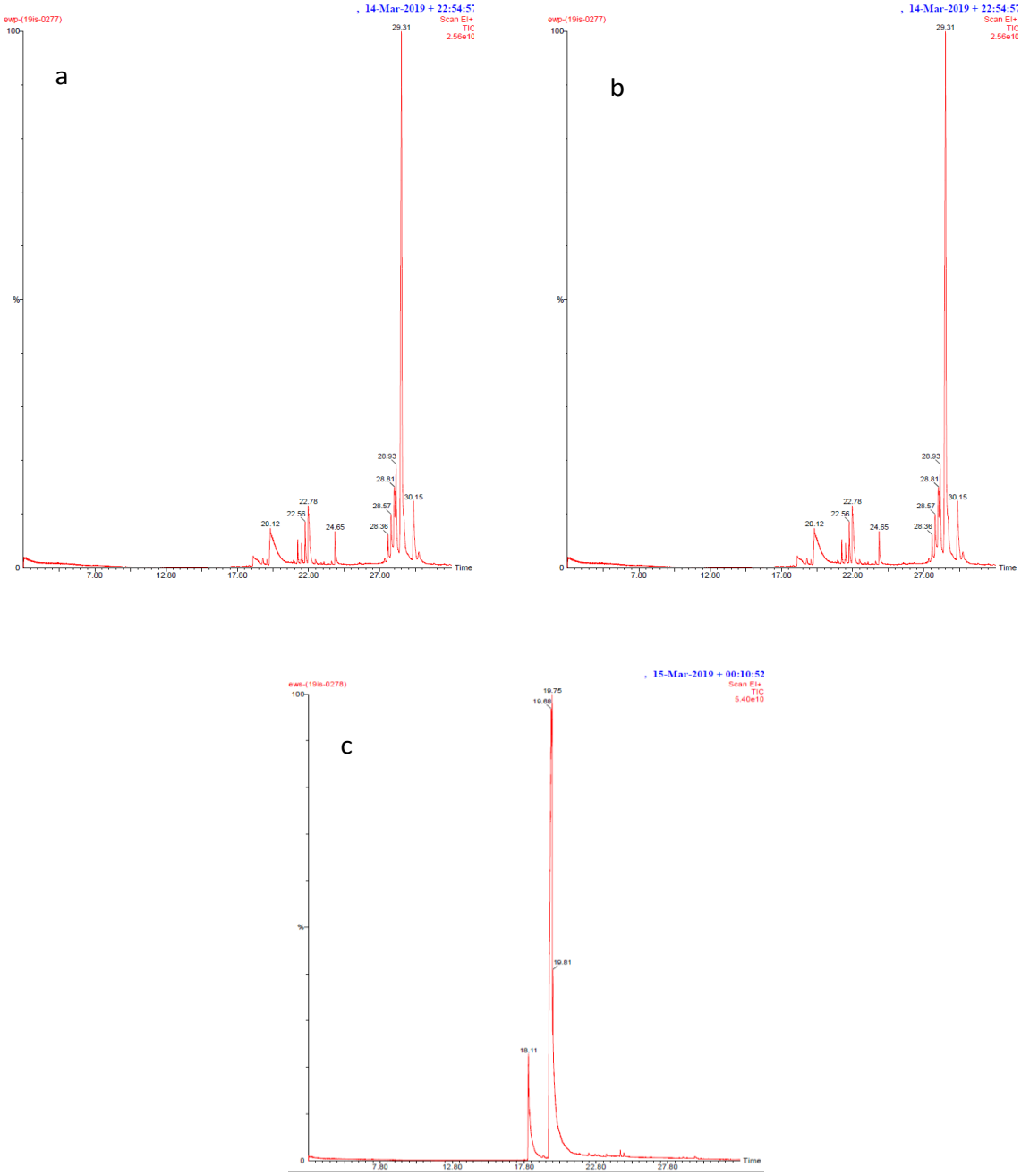

Figure 8 TIC of GC-MS Analysis of White pumpkin var. Ethylacetate extract a: Peel; b: Flesh; and c: Seed

4 Discussions

4.1 Qualitative Phytochemical Screening

Preliminary phytochemical screening aids in the development of new drugs (Doss 2009). In the present study, tannins are found to be lacking in the flesh and seed parts in both varieties. Similar to the previous studies on Cucurbita varieties, our study showed the presence of saponin, quinones, glycosides, phenol, alkaloids, and terpenoids in seed extract (Sumathi and Janarthanam 2016). The phytochemicals reported in the ethyl acetate extract of white variety fruit was similar to the study report of Vijayalakshmi (2014). In this study, researchers identified significant differences in the presence of phytochemical constituents in the peel, flesh, and seed of both varieties. The variation within the phytochemical constituent might be due to the solvent utilized for the extraction, location, and genetic variation (Wadood et al. 2013).

4.2 Determination of Primary Metabolite and Secondary Metabolite

In general, traditional or ayurvedic medicines are made from a single plant or a mixture of plants. The knowledge of taxonomic features revealed the potency of plant parts and the biological property of medicinal plants that are found to be dependent on the presence of primary and secondary metabolites, according to Vinoth et al. (2011). Primary metabolites play a direct role in the growth, whereas secondary metabolites appear to serve a secondary role and are only used as an accelerator. Following the above phytochemical screening in yellow and white varieties, ethyl acetate extract of peel, flesh, and seed revealed maximum numbers of metabolites, thus further ethyl acetate extract alone was employed for the quantification of major metabolites.

The current study on the estimation of primary metabolites (total soluble carbohydrate and total protein) and secondary metabolites (alkaloid and phenol) present in ethyl acetate extract of peel, flesh, and the seed of yellow and white varieties aids in understanding the source for isolation of therapeutically and industrially necessary compounds. The quantitative analysis of primary metabolites given in Table 3 showed that the carbohydrate content was found to be high in yellow flesh (2.2±0.2 µg/mL) and white variety seed (3.2±0.2 µg/mL). Subsequently, protein content was found to be high in seed parts of yellow (15.4±0.4 µg/mL) and white (11.3±0.4 µg/mL) varieties compared to the flesh and peel of each species. Carbohydrates are one of the building blocks of the cell, exploited for potential energy supplements. Similarly, proteins are most significantly important for maintaining the structure or function of all life and for growth development. The presence of higher protein levels in fruit parts of our current study has the potential to increase food value or to be used in the future for the isolation of protein-based bioactive compounds (Thomsen et al. 1991). Plant sugars are used as synthetic sweeteners and can even help patients with diabetes by assisting the body in its rebuilding process (Freeze 1998). Raj et al. (2018) performed similar work on the quantification of primary metabolites within the peel, flesh, and seed of yellow and white varieties and concluded that a sample with low carbohydrate content is appropriate for diabetic and hypertensive patients requiring a low-sugar diet. Likewise compared to our study alkaloid and phenol content has been analyzed in white and yellow variety peel, flesh and seed have been reported (Raj et al. 2018). Young et al. (2002) suggested that including or excluding polyphenol-enriched or fortified foods may have unfavorable effects. Due to their analgesic, spasmolytic, and disinfectant properties, pure isolated alkaloids or synthetic derivatives are used as basic health agents (Stray 1998).

4.3 Fourier Transmittance-Infrared Spectrometry (FT-IR)

FT-IR spectrum confirmed the presence of alcohols, alkene, aromatic, ketone, carboxylic, aromatic amine, nitro compounds, silane, and phosphine. The presence of aromatic and aliphatic amine has been reported on the aqueous extract of *Gymnema sylvestre* FT-IR analysis (Sangeetha et al. 2014). Similarly, OH-group potential expression was observed in different solvent extracts like petroleum ether, chloroform, methanol, and ethyl acetate in FT-IR analysis of four medicinal plants (Ashok Kumar and Ramaswamy 2014).

4.4 Gas Chromatography-Mass Spectroscopy Analysis (GC-MS)

The total ionic chromatogram of GC-MS analysis in peel, flesh, and seed ethyl acetate extract of yellow variety revealed a total of 15 compounds (Figure 7) that might contribute to the medicinal property of this particular species. Further, the total ionic chromatogram of ethyl acetate extract of white variety peel, flesh, and seed showed 14 compounds (Figure 8). The bioactive compounds known by GC-MS analysis in the present study are medicinally vital as they possess a novel structure with specific biological activities. Lupeol is one of the pharmacologically active triterpenoids that has been identified in yellow flesh and has been extensively studied for its anti-inflammatory property. Lupeol is abundant in vegetables such as white cabbage, pepper, cucumber, and tomato (Saleem 2009). Another study on a lupeol-rich extract of the *Pimenta racemosa* plant exhibited significantly high anti-inflammatory activity in animal models (Lima et al. 2007). Moreover, lupeol has a complicated pharmacology in humans, with anti-protozoal, anti-microbial, anti-inflammatory, anti-tumor, and chemopreventive properties (Gallo and Sarachine 2009; Saleem 2009). Thunbergol is another major metabolite that has been observed in the peel of white variety, which has been previously reported to be found in flower buds of tobacco (Xu et al. 2015) and tuberous roots of *Ampelocissus latifolia* (Theng and Korpenwar 2015). Similar to our study, Di-2-ethylhexyl phthalate had been reported in the fruit of the white variety (Du et al. 2011). GC-MS analysis revealed the presence of polyunsaturated fatty acids such as linoleic acid in both yellow and white varieties. Linoleic acid, a precursor of arachidonic acid, is important in the inflammatory cascade (Wendt 2005). The compounds identified in the different parts of both yellow and white variety ethyl acetate extracts support the potential for many medicinal applications of this vegetable or fruit. In the present study, the biological activity of the compounds detected was analyzed from Duke's phytochemical and Ethno botanical database. The identification of these compounds in various parts would serve as the foundation for determining the potential health benefits of this vegetable.

Conclusion

Yellow and white pumpkin varieties are commonly used vegetables in Tamilnadu. Due to the change in lifestyle in recent years, people get affected by many diseases; hence they turned towards healthy diets. Although both the species of our study are used as a food source, the understanding of these species is less known. Though many studies on these species have been reported, the goal of this study was to learn about the biologically significant elements of the plant part as a nutritional source. In general, the whole fruit of pumpkin is taken as a dietary source, hence to reveal the biological potential of each part of the present study was carried. Simultaneously, the solvent efficiency was also analyzed. From the qualitative analysis, it has been observed that ethyl acetate solvents are efficient in extracting a maximum number of principal compounds in different parts of the study plant material. Flesh parts are found to have linoleic acid, and essential oil, which are essential sources of cancer-preventive, anti-inflammatory, and antimicrobial properties.

Acknowledgments

The authors would like to thank the Sophisticated Instrumentation Facility Centre of VIT University, Vellore for GC-MS analysis and FT-IR work.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

References

Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FT-IR spectroscopic analysis of leaf extracts of selected Indian Medicinal Plants. *International Journal of Current Microbioloy and Applied Science*, *3*(1), 395 – 406.

Bankar, P., & Jayaraj, M. (2018). GC-MS analysis of Bioactive compound from ethanolic leaf extract of *Waltheria indica* Linn., and their Pharmacological activities. *International Journal of Pharmaceutical Sciences and Research*, *9*(5), 2005 – 2010.

Bhuvaneshwari, K., Gokulanathan, A., Jayanthi, M., Govindasamy, V., et al. (2016). Can *Ocimum basilicum* L. and *Ocimum tenuiflorium* L., *in vitro* culture be a potential source of secondary metabolite. *Food Chemistry*, *194*, 55-60.

Bisognin, D.A. (2002). Origin and Evolution of cultivated curcurbits. *Ciencia Rural*, *32*, 715 – 723.

Chandrasekara, A., & Shahidi, F. (2010). Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Functional Foods*, *58*, 6706–6714.

Chandrasekara, A., & Shahidi, F. (2011). Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC–DAD–ESI–MS. *Journal of Functional Foods*, *3* (3), 144–158.

Deng, G.F., Xu, X.R., Guo, Y.J., & Xia, E.Q., et al., (2012). Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *Journal of Functional Foods, 4*, 906-914.

Doss, A. (2009). Preliminary phytochemical screening of some Indian medicinal plants. *Ancient Science of Life*, *29*, 12 – 6.

Du, B., Song, Y., Hu, X., Liao, X., Ni, Y., & Li. Q. (2011). Oligosaccharides prepared by acid hydrolysis of polysaccharides from pumpkin (*Cucurbita moschata*) pulp and their prebiotic activities. *International Journal of Food Science Technology, 46* (5), 982–987.

Freeze, H.H. (1998). Disorders in protein glycosylation and potential therapy: Tip of an iceberg. *Journal of Pediatrics*, *133*, 593 – 600.

Gallo, M.M.B., & Sarachine, J. (2009). Biological Activities of Lupeol. *International Journal of Biomedical and Pharmaceutical Sciences, 3*, 46-66.

Hedge, J.E., & Hofreiter, B.T. (1962). Determination of reducing sugars and carbohydrates. In R.L.Whistler, and M.L. Wolfrom (Eds.), *Methods in Carbohydrate Chemistry* (pp. 380 - 394). Academic Press, New York.

Jayaraman, J. (1981). *Laboratory Manual in Biochemistry*. New Delhi: *Wiley Eastern Limited*.

Kaushik, U., Aeri, V., & Mir, S.R. (2015). Cucurbitacins – An insight into medicinal leads from nature. *Pharmacognosy Review*, *9*(17), 12 – 18.

Kostecka Gugala, A., Kruczek, M., Ledwozyw-Smolen, I., & Kaszycki, P., (2020) Antioxidants and Health-Beneficial Nutrients in Fruits of Eighteen Cucurbita cultivars: Analysis of Diversity and Dietary Implications. *Molecules, 25*, 1-20.

Li, H.B., Cheng, K.W., & Wong, C.C. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*, 771–776.

Li, H.B., Wong, C.C., Cheng, K.W., & Chen, F. (2008). Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Science and Technology*, *41*, 385–390.

Lima, L.M., Perazzo, F.F., Tavares Carvalho, J.C., & Bastos, J.K. (2007). Anti-inflammatory and analgesic activities of the ethanolic extracts from *Zanthoxylum riedelianum* (Rutaceae) leaves and stem bark. *Journal of Pharmacy and Pharmacology*, *59*, 1151 – 1158.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein Measurement with the Folin Phenol Reagent. *Journal Biological Chemistry, 193*, 265 – 275.

Marquez Cardazo, C.J., Molina Hernandez, D., Caballero, B.L., Ciro Velasquez, H.J., Restrepo Molina, D.A., & Correa Londono, G.A. (2021) Physical, physiological, physiochemical and nutritional characterization of pumpkin (*Cucurbita maxima*) in postharvest stage cultivated in Antioqua-Colombia. *Revista Facultad Nacional de Agronomia Medellin, 74* (3), 9735 – 9744.

Morsy, N. (2014). Phytochemical analysis of biologically active constituents of medicinal plants. *Main Group Chemistry*, *13*, 7 – 2.

Mukherjee, P.K., Singha, S., Kar, A., Chanda, J., Banerjee, S., Dasgupta, B., Haldar, P.K., & Sharma N. (2022). Therapeutic importance of Cucurbitaceae: A medicinally important family. *Journal of Ethnopharmacology*, *10* (282), 114599.

Muralidharan, K., Jayanthi, M., Surendran, R., Balasubramanian, M., et al. (2018). Effect of sample extraction, preparation methods on HPLC quantification of Plumbagin in *in vivo* and *in vitro* plant parts of *Plumbago zeylanica* L. *African Journal of Biotechnology*,*17*(33), 1021 – 1030

Naz, R., Roberts, T.H., Bano, A., Nosheen, A., Yasmin, H., & Hassan, M.N., et al. (2020). GC-MS analysis, antimicrobial, antioxidant, antilipoxygenase and cytotoxic activities of *Jacaranda mimosifolia* methanol leaf extracts and fractions. *PLoS ONE 15*(7), e0236319.

Pandey, B.P. (1969). *Taxonomy of Angiosperms*. S. Chand and Company Ltd. New Delhi, India.

Raj, L.S., Indira, G., Jeyanisha, S., & Punitha Kumari, M. (2018). Quantification nutritional and anti-nutritional content of Selected Cucurbitaceae members, Proceedings of the National Conference on Climate Change and Sustainable Environment, Published by Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, ISBN: 978 – 93 -5300-509-2, 88 - 95.

Rajadurai, M., Maithilli, V., Arunazhagi, R., & Yogesh, V. (2018). Phytochemical profiling of medically significant crude extracts using GC-MS analysis. *International of Journal Current Pharmaceutical Research, 10* (6), 16 – 20.

Saleem, M. (2009). Lupeol, A Novel anti-inflammatory and anti-cancer, dietary triterpene. *Cancer Letter*, *285* (2), 109 -115.

Sangeetha, S., Archit, R., & Sathiavelu, A. (2014). Phytochemical testing, Antioxidant activity, HPTLC, and FTIR analysis of antidiabetic plants *Nigella sativa*, *Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre*. *Research Journal of Biotechnology*, *9*(9), 66 – 72.

Seamakkani, M., & Thangpandian, V (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*, *2*, 90-94.

Shamsa, F., Monsef, H.R., Ghamooshi, R., & Verdian Rizi, M.R. (2007) Spectrophotometric Determination of Total Alkaloids in *Peganum harmala* L. using Bromocresol Green. *Research Journal of Phytochemistry*, *1*, 79 – 82.

Shelenga, T.V., Piskunova, T.M., Malyshev, L.L., Taipakova, A.A., & Solovyeva, A.E., (2020). Seed Oil Biochemical Composition of Cultivated *Cucurbita* L. Species from the VIR Collections Grown in the Astrakhan Province of the Russian Federation. *Agronomy*, *10*, 2-12.

Smith, B.D. (1997). The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. *Science*, *276*, 932 – 934.

Stangeland, T., Remberg, S.F., & Lye, K.A. (2009). Total antioxidant activity in 35 Ugandan fruits and vegetables. *Food Chemistry*, *113*, 85–91.

Stray, F. (1998). *The natural guide to medicinal herbs and plants*. London, Tiger books international.

Sumathi, E., & Janarthanam, B. (2016). Phytochemical and Biological Activity of Cucurbita Seed Extract. *Journal of Advances in Biotechnology*, *6*, 813-821.

Swamy, M.K., Sinniah, U.R., & Akhtar, M.S. (2015). *In vitro* pharmacological activities and GC-MS analysis of different solvent extracts of *Lantana camara* Leaves collected from Tropical Region of Malaysia. *Evidence-Based Complementary and Alternative Medicine*, 1-9.

Theng, K.B., & Korpenwar, A.N. (2015). Phytochemical analysis of ethanol extract of *Amphelocissus latifolia* (Roxb.) Planch tuberous root using UV-VIS, FTIR, and GC-MS. *International Journal Pharmaceutical Science Research*, *6* (9), 3936 – 3942.

Thomsen, S., Handen, H.S., & Nyman, V. (1991). Ribosome inhibiting proteins from *in vitro* cultures of *Phytolaceadodecandra*. *Planta Medica*, *57*, 232 – 236.

Velmurugan, G., & Anand, S.P. (2017). GC-MS analysis of Bioactive compounds on Ethanolic leaf extract of *Phyllodium pulchellum* L. Desv. *International Journal of Pharmacognosy and Phytochemical Research*, *9* (1), 114 – 118.

Vijayalakshmi, K. (2014). Pharmacognostic, phytochemical and pharmacological evaluation of the leaves of *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Cucurbitaceae), Dissertation submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Vinoth, S., Rajesh khanna, P., Gurusaravanan, P., & Jayabalan, N. (2011). Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of *Indigofera trita L.f. spp., Subulata* (Vahl ex poir). *International Journal of Agricultural Research*, *6*, 358 – 67.

Wadood, A., Ghufran, M., Jamal, S.B., & Naeem, M., et al. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, *2*, 2-4.

Wang, X.L., Liu, J., Chen, Z.H.B., & Gao, F., et al. (2001). Preliminary study on pharmacologically effect of *Curcurbita pepo* cv *Dayanggua*. *Journal of Traditional Chinese Veterinary Medicine*, *20*, 6-9.

Wendt, S.B. (2005). Comparison of the effectiveness of marigold and sunflower oil in healing by second intention of wounds in small animals Master's Degree Program (Veterinary Sciences), Federal University of Parana, Curitiba, Brazil.

Xu, C., Zeng, Y., Li, M., Zhao, S., & Hu, Z. (2015). Composition, antioxidative, and antimicrobial activities of tobacco flower bud oil. *Tobacco Science and Technology*, *48(1)*, 76 – 80.

Young, W.B., James, R., & Montgomery, I. (2002). Is muscle power related to running speed with changes of direction?. *Journal of Sports Medicine and Physical Fitness*, *43*, 282 – 288.