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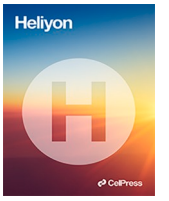

Research article

Phytochemical screening, FTIR and GCMS analysis of *Cucurbita pepo* seeds cultivated in Kiambu county, Kenya

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ABSTRACT

Bioactive compounds and other constituents of plants have been shown to vary by cultivation region, species, environmental conditions and method of extraction among others. Phytochemical analysis of *Cucurbita pepo*s farmed in Kiambu County, Kenya, or their seeds has not been documented. The present research aimed to bridge this knowledge gap by screening phytochemicals and characterizing the seed extracts of *Cucurbita pepo* cultivated in Kiambu County, Kenya. *Cucurbita pepo* seeds extracted using organic solvent extraction method employing methanol and preconcentrated in a vacuum rotatory evaporator. The extracts were characterized by GCMS and Fourier transform infrared (FTIR) techniques. Phytochemical analysis of the seeds revealed the presence of flavonoids, alkaloids, saponins, cardiac glycosides, and steroids. FT-IR analysis showed significant peaks for C–N, N–H, C–O, C–H, and CH_3 functional groups. The GCMS studies revealed a significant number of fatty acids and their derivatives with 12-cis-octadecadienoate being the most abundant in the oil (53.93 %). A significant amount of the macrocyclic lactone 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione (0.58 %) in the seeds was reported. Macrocyclic lactones are generally a class of anthelminthic drugs. These reported biologically active compounds have a wide range of medicinal and nutritional value. One interesting compound from the GCMS analysis of the seed extracts analyzed was the macrocyclic lactone providing a basis for further research on the anthelminthic actions of the seeds.

1. Introduction

The seeds of *Cucurbita pepo* are generally discarded as trash during processing. It has been reported, however, that these seeds are of considerable use. The pharmacological activities reported for the seeds include antidiabetic, antibacterial, antioxidant, antifungal, and anti-inflammatory properties [1]. *Cucurbita pepo* seeds which were previously regarded as waste now can find an important role in the food industry [2]. This is due to their nutritious composition mainly zinc, selenium, phosphorous, potassium, and magnesium [3]. The seeds are consumed either in raw or in roasted form.

Seymen and company reported that the high unsaturated fatty acid also increases the nutritious benefits of the same [4]. Findings by Oladele and Ondigi showed that farmers ate *Cucurbita pepo*s for their nourishments with the flowers, leaves, and fruits used as

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vegetables [5,6]. Reported also is that *Cucurbita pepo* seeds are an excellent nutrient source having zinc, phosphorous, magnesium, potassium, and selenium responsible for diseases such as arthritis, prostate diseases, and inflammation among others [7]. The seeds can be consumed regularly without any side effects on human health [8].

The seed oil is accepted as edible oil and also as nutraceutical [9]. Research has proved the seeds to be a rich source of sterols [10]. Reported also that *Cucurbita pepo* seeds are an excellent nutrient source having zinc, phosphorous, magnesium, potassium, and selenium responsible for fighting diseases such as arthritis, prostate diseases, and inflammation among others [7]. The seeds can be consumed regularly without any side effects on human health. The seeds have been reported to contain an abundance of sterols responsible for their use in treatment of prostate diseases [11].

Phytochemicals are bioactive plant components meaning that they have a wide range of biological activities [12]. The extraction of phytochemicals depends on the polarity of extracting solvent and the polarity of the chemical being extracted thus increasing the polarity of the solvent is done to ensure the extraction of a wide range of bioactive compounds [13]. They can be obtained from the whole plant or some specific parts of the plant by extraction with different types of non-polar and polar solvents [14].

Some phytochemicals include saponins, flavonoids, terpenes, tannins, cardiac glycosides and Saponins are phytochemicals that are freely soluble in organic solvents and water [15]. They are potential mosquito larvicidal compounds [16]. Flavonoids are phenolic substances linked to an aromatic ring the plant synthesizes in response to microbial infection [17]. Terpenes give plants fragrances which are a class of molecules that typically contain building blocks called isoprene. Tannins are phenolic substances capable of turning leather and precipitating gelatines from solutions [18]. Hydrolysable tannins have antimicrobial properties associated with the hydrolysis of ester linkages [19]. Cardiac glycosides are associated with some parricidal activities [20]. Phlobatannins are antioxidants and wound healing [21].

Phytochemical contents and amounts reported in *Cucurbita pepo* different parts of the plant have been found to differ from one farming region to another. For instance, a recent research by Hagos et al. [22], compared total phenolic, flavonoids and antioxidants activity of *Cucurbita pepo* samples from five locations in Ethiopia. The researchers reported the antioxidant activity of *Cucurbita pepo* seed from Debire Birhan higher compared to other samples from other areas sampled. In their investigation into the bioactive compounds of *Cucurbita maxima* grown in Ethiopia, Bisrat et al. [23], found that the *Cucurbita pepo* seed oil they examined contained 12.4 % stearic acid, 18.8 % oleic acid, 17 % palmitic acid (17.9 %), and 50.7 % linoleic acid. In another research on phytochemicals in *Cucurbita maxima* cultivated in Ethiopia, Hagos et al. [24], reported that the main constituents in the *Cucurbita pepo*'s seed, peel, and flesh analyzed were heneicosane, 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine, and 2,6-dimethyl-5,6-dihydro-2H-thiopyran-3-carbaldehyde respectively.

The bioactive compounds of the *Cucurbita pepo seeds* cultivated in Kiambu County, Kenya have not been analyzed or documented in literature. In the present research, we report the bioactive compounds from methanol extracts of *Cucurbita pepo* seeds and further analysis of the extracts employing FT-IR and GCMS. FT-IR helps in assessing the functional groups present in a compound which can be related to the chemical and physical characteristics of the compound [25]. GCMS is applied widely in forensics, drug discovery and medicine, and environmental monitoring by examining each component independently after gas chromatography has separated the individual components of the mixture [26].

2. Research methodology

2.1. Requirements

Acetic acid, sodium hydroxide, ethanol, methanol, chloroform, cyclohexane, hydrochloric acid (70 %), Conc. sulphuric acid (H_2SO_4, 98 %), Marquis reagent, zinc sulphate, calcium carbonate, magnesium chloride, Iron (II) chloride ($FeCl_2$), formaldehyde, distilled water, alcoholic ferric chloride, Sodium hydroxide (NaOH), iodine, Molisch's reagent, n-hexane, methanol, Fehling's solution, benzene solution, propan-2-ol, ammonia solution, sodium tetraborate ($Na_2B_4O_7$, pH = 9.2 buffer solution), potassium bromide (KBr) powder. All reagents and chemicals were analytical grades obtained from Germany Sigma-Aldrich Co. and Lobba Chemicals Co. Microsoft Office 2016 Suite (Microsoft), ChemDraw 7.0.1(Cambridge Soft Corp.) and OriginPro 2023 (OriginLab Corp.)

2.2. Sample preparation

Fresh *Cucurbita pepo* seeds were collected from Juja Market Kiambu County, Kenya. The C. Pepo seeds were dried under shade for 14 days to reduce the large initial moisture content to enable prolonged storage life. The dried sample seeds were then size-reduced using a heavy-duty mechanical sample blender. Coarsely powdered crude 10 g samples were placed in two different stoppered containers with the solvent 250 mL Methanol and 250 mL n-hexane respectively and allowed to stand at room temperature for a period of 72 h with frequent agitation until the soluble matter had dissolved. The mixtures were then filtered by gravitation using fluted Whatman #41 filter papers, the marc (the damp solid material) was also pressed, and the extracts were decanted and further filtered. The extracts were then concentrated using a vacuum rotatory evaporator and the concentrate was refrigerated at 4 °C for analysis.

2.3. Phytochemical analysis of the extracts

The phytochemicals screened in this research were flavonoids, alkaloids, terpenoids, tannins, phlobatannins, cardiac glycosides, steroids, saponins, and quinones. In this procedure, 10 g of the coarsely powdered sample (50 μm) were placed in three different stoppered containers with the solvent methanol and hexane respectively, and allowed to stand at room temperature for at least 3 days with frequent agitation until the soluble matter had dissolved. The mixtures were then filtered by gravitation using fluted Whatman #41 filter papers, the marc was also pressed, and the combined liquids decantation decanted after 20 min. The filtrate was divided into portions of 2 mL and subjected to various phytochemical tests as described by Shaikh and Patil [27].

2.4. FT-IR functional groups analysis

Fourier Transform Infrared (Shimadzu-119) in the range 350 cm^{-1} to 4700 cm^{-1} was used for this analysis. The instrument was calibrated after 30 min of warming using the inactive potassium bromide. Approximately 5 g of the blended *C. pepo* seeds were placed in a mortar and ground finely. This sample was oven-dried for 30 min at 105 °C and thereafter allowed to cool in a lidded crucible. About 1 mg of the dried sample was placed on the FTIR mortar and the inert potassium bromide sample was added in the ratio of 1:50 for the sample to KBr and ground to attain homogeneity. The mixture was then compressed with the FT-IR hand-press equipment to prepare a translucent pellet. The pellet was placed in the sample holder and mounted on the FTIR machine ready for analysis. The FT-IR spectrum for transmittance against the wavelength was taken and interpretation to get various pronounced peaks of interest was done. The FT-IR functional groups determination was carried out at the Maasai Mara University Chemistry Laboratories, Kenya.

2.5. GC-MS analysis of the methanolic extracts

The individual sample of the powdered Cucurbita pepo seeds (5 g) was dissolved in methanol (100 mL) for 24 h. This procedure was repeated thrice on ice. The extracts were filtered through a 45 μm filter and the resulting solvent 100 mL was concentrated under a vacuum rotatory evaporator. Concentrated samples were re-suspended in 1 mL Methanol, filtered through a 0.2 μm filter, and stored at 4 °C until GC-MS analysis. The samples were analyzed on a GC-MS QP2010 Plus instrument (Shimadzu, Kyoto, Japan) with a standard BPX5-MS capillary non-polar column (dimension: 30 Mts, ID: 0.25 mm, film thickness: 0.25 μm). The flow rate of the mobile phase (carrier gas: helium) was set at 1.0 mL/min. For the GC component, the oven temperature increased from 60 °C to 250 °C at 10 °C/min and the injection volume was 1 μL. Samples were dissolved in isooctane and run to completion at a range of 50–650 *m/z* and the results were compared with data from the National Institute of Standards and Technology (NIST) database [28,38–41]. The GCMS analysis was performed at the Jomo Kenyatta University of Science and Technology Analytical Chemistry Laboratories, Kenya.

3. Results and discussions

3.1. Phytochemical screening

Cucurbita pepo seed extracts showed a significant presence of different phytochemicals with respective solvent systems. Methanol extracts showed the presence of flavonoids, alkaloids, saponins, cardiac glycosides, and steroids as shown in Table 1 below. Alkaloids are compounds that have proved to be very important secondary metabolites in humans. Morphine is one of the oldest alkaloids to be isolated and is a strong narcotic that has been utilized as a pain reliever although very addictive [29]. Flavonoids have been proven to exhibit many pharmacological activities such as anticancer and wound healing properties [30,31]. Steroids in the seeds have been linked to the seeds' use as a remedy for prostate cancer [32].

Table 1
Phytochemicals test results for methanolic extracts of the *Cucurbita pepo* seeds.

Phytochemical	Conclusion
Flavonoids	+
Alkaloids	++
Saponins	++
Tannins	–
Phenols	–
Cardiac glycosides	+
Terpenoids	–
Steroids	++
Anthraquinones	–

*Negative (−) - Not Present.
Positive (+) -Present in lesser magnitude.
(++)- Present in high magnitude.

The methanol extracts also were found to contain saponins. There is evidence that saponins have antiviral, antibacterial, antifungal, and anticancer effects [33]. There is evidence of saponins having antiviral, antibacterial, antifungal, and anticancer effects. Cardiac glycosides are a class of phytochemicals that prove a great significance in curing heart muscle problems such as heart failure [34]. Anthraquinones, terpenoids, and phenols were absent in all extracts. Tannins were present only in water extracts and flavonoids were present only in methanol extracts. From the results alkaloids, saponins, cardiac glycosides, and steroids were found to be the major phyto-constituents of the Cucurbita pepo seeds. The presence of these secondary metabolites in the Cucurbita pepo seeds that are biologically important are key contributors to the seeds' medicinal value and their physiological effects [35].

3.2. FTIR analysis for the Cucurbita pepo seed

From the FTIR results the sample showed some significant peaks at about 3400 cm^{-1}, 2924.09 cm^{-1}, 2854.65 cm^{-1}, 2314.58 cm^{-1}, 1743.65 cm^{-1}, 1651.07 cm^{-1}, 1535.34 cm^{-1}, 1458.18 cm^{-1}, 1365.60 cm^{-1},1234.44 cm^{-1}, 1157.29 cm^{-1} and some small peaks at 1087.85 cm^{-1}, 1049.28 cm^{-1} and 702.09 cm^{-1} as in the FTIR spectrum in Fig. 1. Peaks at 3400 cm^{-1},2924.09 cm^{-1}, and 1365.60 cm^{-1} are associated with O–H bonds, 1087.85 cm^{-1},1157.29 cm^{-1}, and 1234.44 cm^{-1} peaks are associated with C–O bonds in secondary, tertiary alcohol, and alkyl aryl ethers respectively, peaks at 1234.44 cm^{-1} and 2854.65 cm^{-1} can also be linked to C–N bonding in amines, N–H bonding in amine salts and N–H bonding in secondary amines respectively as shown in Table 2.

Peaks at 2314.58 cm^{-1}, 1743.65 cm^{-1},1651.07 cm^{-1},1535.34 cm^{-1}, 1458.18 cm^{-1},1049.28 cm^{-1}, and 702.09 cm^{-1} are associated with O═C═O carbon dioxide, C═O bonds in lactones, N–O bonding in nitro compounds, C–H bonding in alkanes, S═O bonding in sulfoxides, C═C bonding in alkenes respectively. The results agreed with the phytochemicals tests to some extent as alkaloids contain at least one nitrogen atom in an amine-type structure, with presence peaks of 3400 cm^{-1}, 2854.65 cm^{-1}, and 1234.44 cm^{-1} [36].

3.3. GC-MS analysis of the methanolic extracts of the seeds

The components of the *Cucurbita pepo* oil were also analyzed by GC-MS. The first component observed was hexadecenoic acid methyl ester, with a retention time of 18.549 min, while the last component observed was octadecanoid with a retention time of 24.445 min. The most abundant compound in the oil was Methyl 10-trans,12-cis-octadecadienoate with a 53.93 % peak area while the least abundant compound was Methyl 18-methylnonadecanoate, with a 0.25 % peak area. Low concentrations of the fatty acids were noted despite the use of the ultrasonic-assisted extraction method. Research by Applequist et al. (2006) to compare four fatty acids contents from four varieties of cucurbits revealed that *C. pepo* had the highest content of saturated fatty acids [37]. Rodríguez-Miranda et al. (2013) reported that for *Cucurbita pepo* seeds the concentration of the fatty acids detected and the speed of extraction was high when the ultrasonic-assisted extraction method was employed [38]. Fig. 2 shows the GC-MS molecular mass fragments of the sample with the compounds highlighted in Table 3.

The extraction was done after sample preconcentration using a vacuum rotatory evaporator, the high temperatures in the pre-concentration step may have damaged some of the polynuclear unsaturated fatty acids [39] (see Table 4). The *Cucurbita pepo* seeds oil sample was found to contain nine compounds. Fatty acids of C8–C20 like palmitic acid, linoleic acid, and oleic acid among others exhibit anthelminthic properties. Palmitic acid, oleic acid, and linoleic acid were present in the sample the palmitic acid and its derivatives being more abundant. These fatty acids belong to the omega-3 and omega-6 classes which portray significant metabolic and nutritional benefits [40].

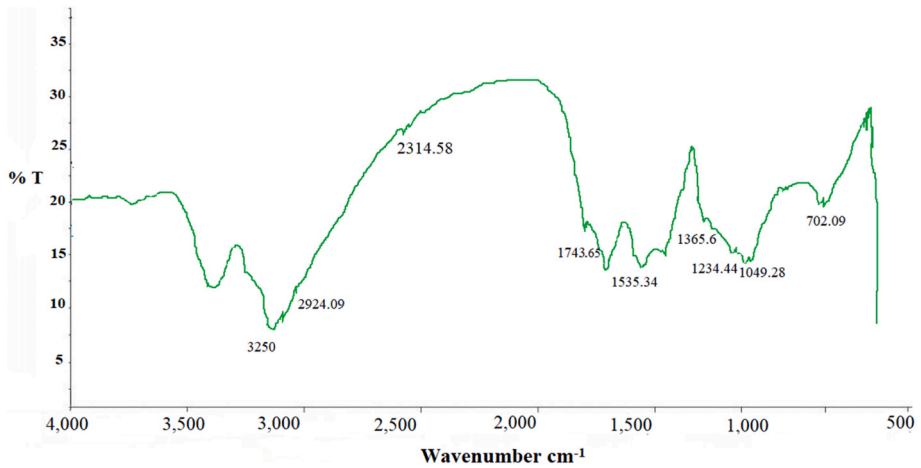


Fig. 1. FT-IR spectrum of *Cucurbita pepo seeds*.

Table 2
Significant functional groups from the FT-IR spectrum.

Wavenumber (cm^{-1})	%Transmittance	Functional group	Reference Compound
3250	15	O–H	Intermolecular bonded alcohol
		N–H	Secondary amines
2924.09	3.74	O–H	Primary alcohol
2854.65	5.6	N–H	amine salt
2314.58	19.23	O═C═O	Carbon dioxide
1743.65	10.8	C═O	α-lactones or ethers
1651.07	6.12	C═C	Alkene
1535.34	8	N–O	Nitro compound
1458.18	10.24	C–H	Alkane
1365.6	13.15	O–H	Alcohol
1234.44	10.04	C–N	Amines
1157.29	9.3	C–O	Tertiary alcohol
1087.85	9.23	C–O	Secondary alcohol
1049.28	9.17	S–O	Sulfoxide
702.09	18.22	C═C	Alkene

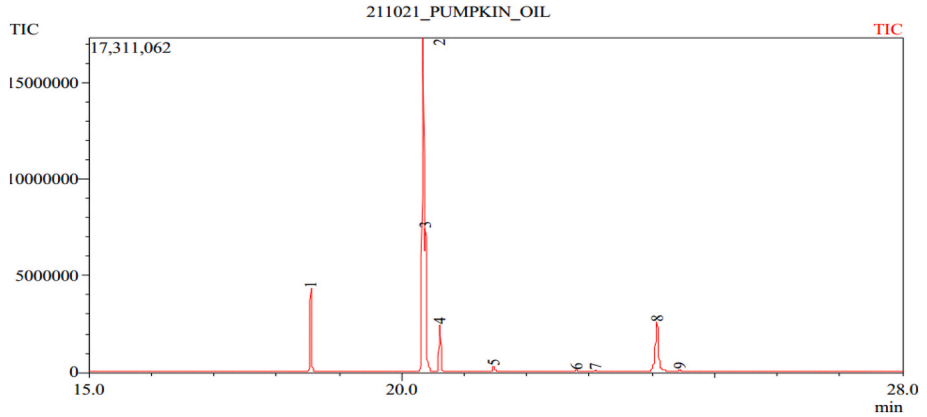


Fig. 2. GC-MS spectrum of the oil extracts.

The methanol extracts of the seeds without oil were also analyzed by GCMS where eight compounds were reported. The first component to be observed was undecane with a retention time of 7.488 min the last being octadecanamide with a retention time of 24.441 min as shown in Fig. 3. The most abundant component in the methanol extracts was observed to be 9-Octadecenamide, (Z)- methyl ester with an area of 77.96 % with the least being 9,12-Octadecadienoic acid (Z, Z)-, methyl ester with a 0.58 % area. 7,9-Ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione can be recognized as one of the bioactive ingredients in Euphorbia pulcherrima extracts, which is used as a traditional therapy for lesions, gonorrhea, migraines, parasitic infections, and skin conditions [41]. Research by Hagos et al. (2022) comparing flesh, peels, and seed parts of *Cucurbita maxima* species of Ethiopia reported that hydrocarbon composition was the highest accounting for 43 % of the seed's composition [24].

In addition, 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione can also be found in marine algae, which have been highly acknowledged to possess noticeable pharmacological activities, including antineoplastic, antimicrobial and antiviral activities due to this specific functional compound [42]. It is an oxaspiro compound with a lactone, an enone, and a cyclic ketone. Macrocyclic lactone drugs such as ivermectin have been reported to eliminate worms by paralyzing them worms first before death [43].

4. Limitations

This research has several limitations. First, the analysis was done on *Cucurbita pepo* seeds from Juja market only hence, the research results are limited to *Cucurbita pepo* seeds only from the specific locality. Secondly is that our research did not seek to quantify the phytochemicals in the samples and was precisely qualitative. Thirdly, the environmental conditions of Kiambu county that could affect the composition of the *Cucurbita pepo* seeds were not assessed.

Table 3
Compounds identified in the *Cucurbita pepo* seeds oil.

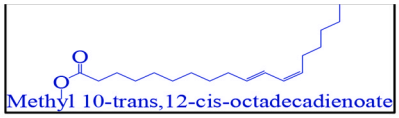
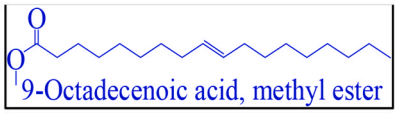
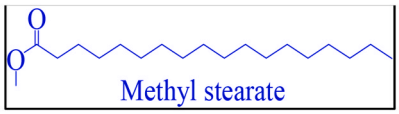
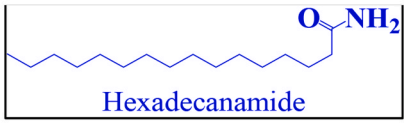
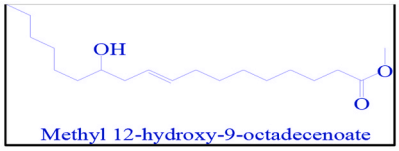
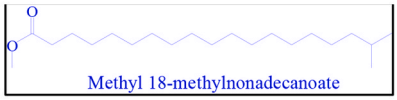
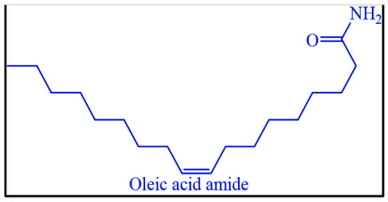
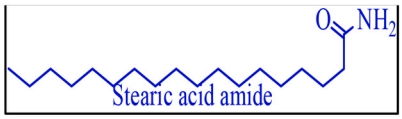
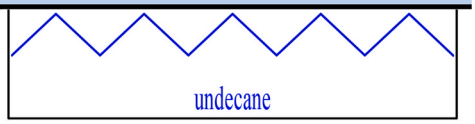
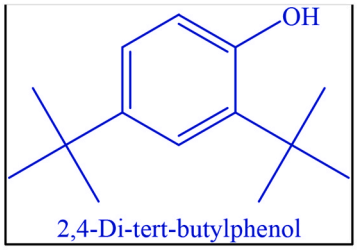
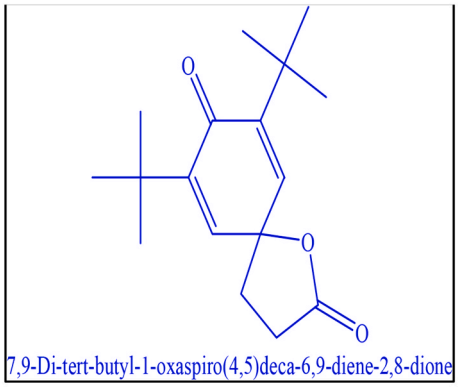
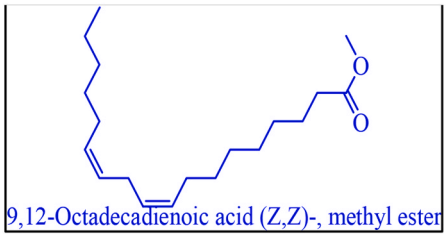
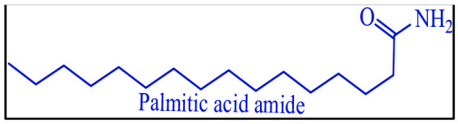
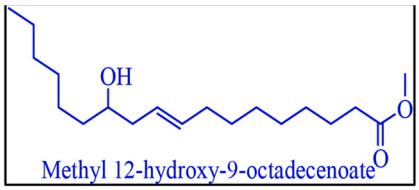
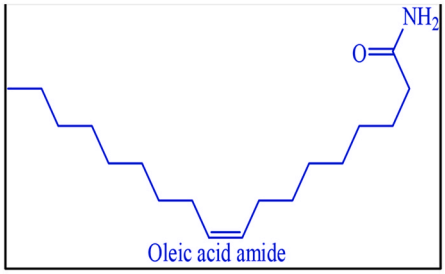
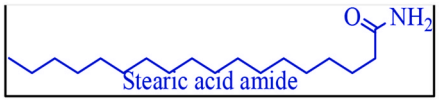
2	13.772	53.95	294	$C_{19}H_{34}O_2$	
3	18.622	14.33	296	$C_{19}H_{36}O_2$	
4	20.331	6.72	298	$C_{19}H_{38}O_2$	
5	21.477	1.02	256	$C_{16}H_{33}NO$	
6	22.794	0.30	312	$C_{19}H_{36}O_3$	
7	23.100	0.25	326	$C_{21}H_{42}O2$	
8	24.070	0.25	281	$C_{18}H_{35}NO$	
9	24.441	12.22	283	$C_{18}H_{37}NO$	

Table 4
Compounds identified by GCMS analysis of the seed's methanol extracts.

Sn #	RT	% Area	Molecular Weight	Chemical Formula	Compound name and structure
1	7.488	0.43	156	$C_{11}H_{24}$	
2	13.772	2.18	206	$C_{14}H_{22}O$	
3	18.622	1.00	276	$C_{17}H_{24}O_3$	
4	20.331	0.58	294	$C_{19}H_{34}O_2$	
5	21.477	5.26	255	$C_{16}H_{33}NO$	

6	22.794	11.01	312	$C_{19}H_{36}O_3$	
7	24.070	77.96	281	$C_{18}H_{35}NO$	
8	24.441	1.58	283	$C_{18}H_{37}NO$	

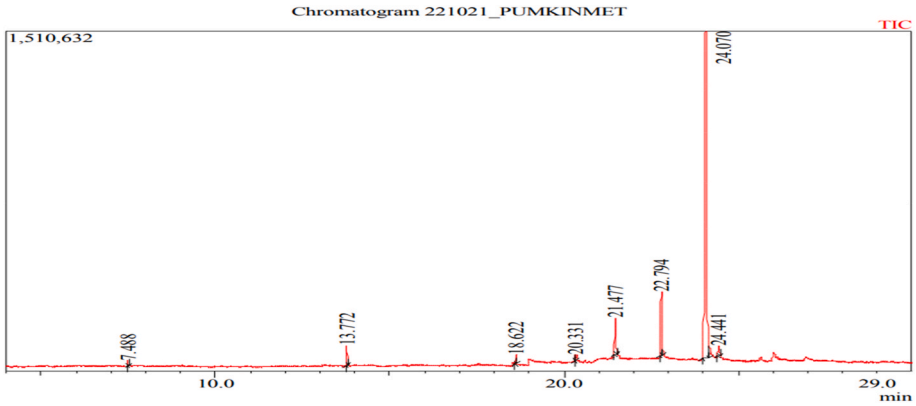


Fig. 3. GCMS chromatogram for methanolic extracts of the *Cucurbita pepo* seeds.

5. Conclusion

In summary, *Cucurbita pepo* seeds from *Cucurbita pepo* farmed in Kiambu County, Kenya studied in this research, can be a significant source of many useful organic compounds such as macrocyclic lactones and fatty acids, as well as their derivatives which are interesting compounds that can be further studied to qualify the seeds as natural medicinal sources such as anthelminthic alternative. Essential fatty acids linoleic acid and oleic acid are of great importance in human health. The findings of this research warrant further research on the potential medicinal and nutritional properties of the *Cucurbita pepo* seeds from pumpkin variety farmed in Kiambu County, Kenya.

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Data availability statement

All the data associated with this research has been provided herein except for the raw data that can be provided upon request without withholding.

CRediT authorship contribution statement

John Wamumwe Mwangi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Denis Kiragu:** Writing – review & editing, Writing – original draft, Software. **Bakari Chaka:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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