Research Paper

Isolation, Structural Elucidation and Therapeutic Potentials of Root of *Cucurbita pepo*

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The roots of *Cucurbita pepo* were investigated with the aim of identifying bioactive constituents with therapeutic potentials. To achieve this, the roots of *Cucurbita pepo* were harvested, washed, air dried and grounded into the powder. The ground material was extracted using hexane, ethyl acetate and methanol. The extracts were fractionated using column chromatography and fractions were monitored using thin layer chromatography. The fractions were characterised using proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance spectroscopy. Phytochemical screening showed that the extracts contained sterols and terpenoids, alkaloids, resins, flavonoids, saponins and carbohydrates. Nuclear magnetic resonance analysis led to the identification of hexadecanoic acid or palmitic acid, alpha-spinasterol and squalene. Some of these compounds may represent a new pharmacological approach in the development of novel and adjuvant therapy for several medical conditions.

Key words: Hexadecanoic acid, alpha-spinasterol, squalene, *Cucurbita pepo*, nuclear magnetic resonance spectroscopy

Plants are rich in bioactive compounds that have various medicinal properties[1-3]. Developments in technology have made it possible to identify and purify these secondary metabolites which have served as the backbone in the formulation of many pharmaceutical drugs[4]. In addition to the various side effects and limited efficacy of orthodox drugs, there is an increase in the development of resistance by disease causing organisms to orthodox drugs with the tendency of the disease to reoccur[5]. This has led to a growing interest in the search for alternative therapeutic drugs derived from plants.

Phytochemical studies are driven by the need for the discovery of inexpensive, safer and new drugs which is performed by screening different parts (root, leave, stem and seed) of local medicinal plants in order to develop drugs to combat various health challenges faced by man[6]. Pumpkin belongs to the family Cucubitaceae and is comprised of *Cucurbita moschata*, *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita ficifolia* and *Telfairia occidentalis*. The economically important species cultivated worldwide are *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*[7,8]. The use of pumpkin in various traditional medicines for the treatment of several ailments has attracted scientific attention to this plant[9]. Pumpkin contains biologically active components that include polysaccharides, para-aminobenzoic acid, fixed oils, sterol, proteins and peptides[10] . While the fruits are a good source of carotenoid and gamma-aminobutyric acid[11,12], the presence of anti-nutrients in pumpkin seeds have limited the usefulness of fresh pumpkin seed as a protein source for human food[13-15]. Phytochemicals such as polysaccharides, phenolic glycosides, 13-hydroxy-9Z, 11E-octadecatrienoic acid from the leaves of pumpkin and proteins from germinated seeds have been isolated[16-18].

The upsurge in techniques involved in the isolation and identification of novel natural compounds from plant materials have made the discovery of novel compounds which have the potential to become drugs more feasible. Isolation of bioactive compounds from different parts of plants is performed using chromatographic

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techniques such as Thin Layer Chromatography (TLC), Preparative Thin Layer Chromatography (PTLC), Column Chromatography (CC), Medium Pressure Liquid Chromatography (MPLC) and High Performance Liquid Chromatography (HPLC) amongst others[19]. Structure elucidation provides the molecular structures of compounds through the use of techniques such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) analysis[20].

This study focused on the roots of *Cucurbita pepo* (pumpkin). Ethno-medicinal reports indicate the traditional use of different parts of the plant (seed, fruit, vegetative part) in the treatment of different health problems and as functional food[21-24] but no work has been carried out on the roots. There is a growing interest in determining the scientific basis for the usage of plants in primary health care and in discovering novel compounds of pharmaceutical importance[25]. In this study, column chromatography was used to isolate some bioactive compounds and NMR used to identify the isolated compounds from the roots of *Cucurbita pepo*.

MATERIALS AND METHODS

Collection of plant material:

The roots of *Cucurbita pepo* were collected from a farm within the University of Nigeria, Nsukka. The plant was authenticated by a taxonomist in the Department of Plant Sciences and Biotechnology, of the University and a voucher specimen was deposited at the herbarium as UNH no. 552. The roots were washed and air dried. Once completely dry, the roots were ground to powder using a blender. The ground roots were stored in closed containers at room temperature until required.

Extraction and phytochemical screening:

The powdered material (350 g) was introduced into a Winchester bottle and macerated successively for 48 h with 700 ml of hexane, ethyl acetate and methanol. The extracts were filtered into clean glass jars using Whatman No.1 filter papers and allowed to dry in fume hood. All extracts were subjected to phytochemical tests using the procedure outlined by Trease *et al.*[26] to detect the presence of secondary metabolites. Test for the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, resins, steroids terpenoids, reducing sugars and tannins were carried out.

Column chromatography of the ethyl acetate extract:

The ethyl acetate extract was fractionated using column chromatography as described by Hostettmann *et al.*[27]. The extract was adsorbed onto silica gel and dried until a free flowing powder was obtained. It was then loaded onto a column packed with 150 g silica gel. The column was eluted gradient wise with increasing amounts of ethyl acetate in hexane until 100 % ethyl acetate. Column fractions were monitored using thin layer chromatography and further purified by repeated CC. Similar fractions were combined on the basis of TLC and allowed to dry.

NMR spectroscopy:

The ^{1}H and ^{13}C NMR spectra were acquired using a Bruker DPX 400 MH_Z spectrophotometer. Deuterated chloroform ($CDCl_3$) was used as solvent while Tetramethylsilane (TMS) was used as internal standard. Spectra were processed using MestReNova (Mestrelab Research S.L., Santiago de Compostela, Spain) Software.

RESULTS AND DISCUSSION

Phytochemical screening of extracts showed that the root extracts of *Cucurbita pepo* contained sterols and terpenoids, alkaloids, resins, flavonoids, saponins and carbohydrates. NMR analysis led to the identification of the below mentioned compounds.

White crystalline solid characterized as follows: ^{1}HNMR (400 MHz; $CDCl_3$): 2.32 (2H, t, J=7.3 Hz, H-2), 1.60 (2H, m, H-3), 1.28 (2H, m, H-15), 1.26 (22H, br, s, H-4 to H-14), 0.89 (3H, t, J=7.3 Hz, H-16).

^{13}C-NMR (100 MHz, $CDCl_3$): 178.3 (C-1), 33.0 (C-2), 31.8 (C-14), 29.0-29.7 (C-4) to C-13); 24.6 (C-3), 21.6 (C-15), 14.0 (C-16) (Table 1).

The identity of this compound was confirmed to be hexadecanoic acid or palmitic acid (fig. 1). The ^{1}HNMR spectrum showed the features of an aliphatic carboxylic acid at region between δ^{H} 0.81-2.41. The spectrum

TABLE 1: ^{1}H NMR, ^{13}C NMR CHEMICAL SHIFTS OF HEXADECANOIC ACID (400 MHz, $CDCL_3$)

Position	13C (δ)	H (δ)	HMBC
1	178.3	-	-
2	34	2.32 (t, J=7.3 Hz)	C-1, C-3, C-4
3	24.7	1.60, m	C-1, C-2, C-4
4-13	29.0-29.5	1.26, br s	-
14	31.9	-	C-15
15	22.7	1.28, m	C-13, C-14, C-16
16	14.1	0.89 (t, J=7.3 Hz)	C-14, C-15

showed the presence of a set of long chain methylene protons representing 11 methylene groups overlapped as a broad peak at 1.25 ppm. The ^{13}C NMR spectrum showed seven signals including a carbonyl signal at δ^C 178.30, a terminal methyl (C-16) at δ^C 14.1 and methylene carbons (C-4 to C-13) overlapped between 29.0-29.5 ppm (fig. 2).

The ^1H spectrum (fig. 3) was typical of a pentacyclic triterpene and similar to stigmasterol. It showed three multiplets; one at δ^H 5.18 (typical of an olefinic H-5 or H-7 observed for steroids) and two others at 5.35 and 5.04 for protons H-22 and H-23 respectively. The spectrum also showed two methyl doublets at 0.81 and 0.84 for an isopropyl unit in the steroid. Two other methyl groups were observed at 0.55 and 1.08 and were assigned to tertiary methyl groups at C-18 and C-19, while the rest of the methyl signals were assigned to C-21 and C-29. The ^{13}C-NMR spectrum indicated resonances for twenty nine carbons including a carbonyl, six methyl, four olefinic and seven quaternary carbons. These signals and comparison with literature reports allowed for the identification of the steroid as spinasterol[28](fig. 4).

^1H NMR (400 MH_Z, $CDCl_3$), δ^H 0.55, 0.80, 0.81, 0.81, 0.84, 1.08, 1.20, 1.20, 1.20, 1.26, 1.30, 1.32, 1.39, 1.40, 1.50, 1.53, 1.77, 1.84, 2.02, 2.02, 3.53, 4.96, 5.08, 5.11, 33.91.

^{13}C NMR (100MH_Z $CDCl_3$) 139.57, 138.16, 129.45, 117.46, 71.45, 55.92, 55.14, 51.25, 49.46, 43.3, 40.8, 40.28, 40.28, 39.47, 38.00, 37.15, 33.91, 31.9, 31.48, 29.70, 28.50, 25.40, 23.02, 21.56, 21.38, 21.1, 19.00, 13.05, 12.2, 12.06 (Table 2).

The ^1H NMR Spectrum indicated resonances for three olefinic protons at δ^H 5.03 (1H, dd, J=8.6, 15.2 Hz, H-23), 5.15. (1H br, s, H-7), 5.16 (1H, dd, J=9.0, 15.5 Hz, H-22); 3.60 (1H, M, H-3) and six methyl protons at δ^H 0.55 (3H, s, H-18), 0.79 (3H, s, H-19), 0.81 (3H, t, J=7.0 Hz, H-29), 0.83 (3H, d, J=6.0 Hz, H-27), 0.85, (3H, d, J=6.0 Hz, H-26), 1.03 (3H, d, J=6.0 Hz , H-21).

The ^1H NMR showed methyl groups at δ^H1.57 (18H, s) and 1.65 (6H, s), methylene groups at 1.97-2.07 (20 H, m) and vinylic signals between 5.05-5.14 (m, 6H). The ^{13}C NMR showed eight methylene carbons at δ^C 16.0-25.7, ten methylene carbons between 26.69-39.75 and 12 olefinic carbons between 124.3-134.9 ppm. The protons spectrum (fig. 5) and chemical shift assignments (Table 3) are in agreement with literature reports and identified the compound as squalene (fig. 6).

^1HNMR (400 MHz $CDCl_3$); δ5.05-5.14 (6H,=CH-), 1.57 (18H, allylic CH_3, Cis), 1.65 (6H, allylic CH_3 trans), 1.97-2.07 (20H, allylic CH_3). $^{13}CNMR$ (100 MHz - $CDCl_3$) δ25.70 (C-1), 131.8 (C-2), 124.32 (C-3), 26.73 (C-4), 39.76 (C-5), 134.88 (C-6), 124.28 (C-

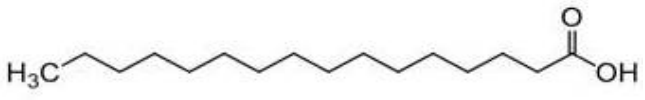

Fig. 1: Structure of hexadecanoic acid

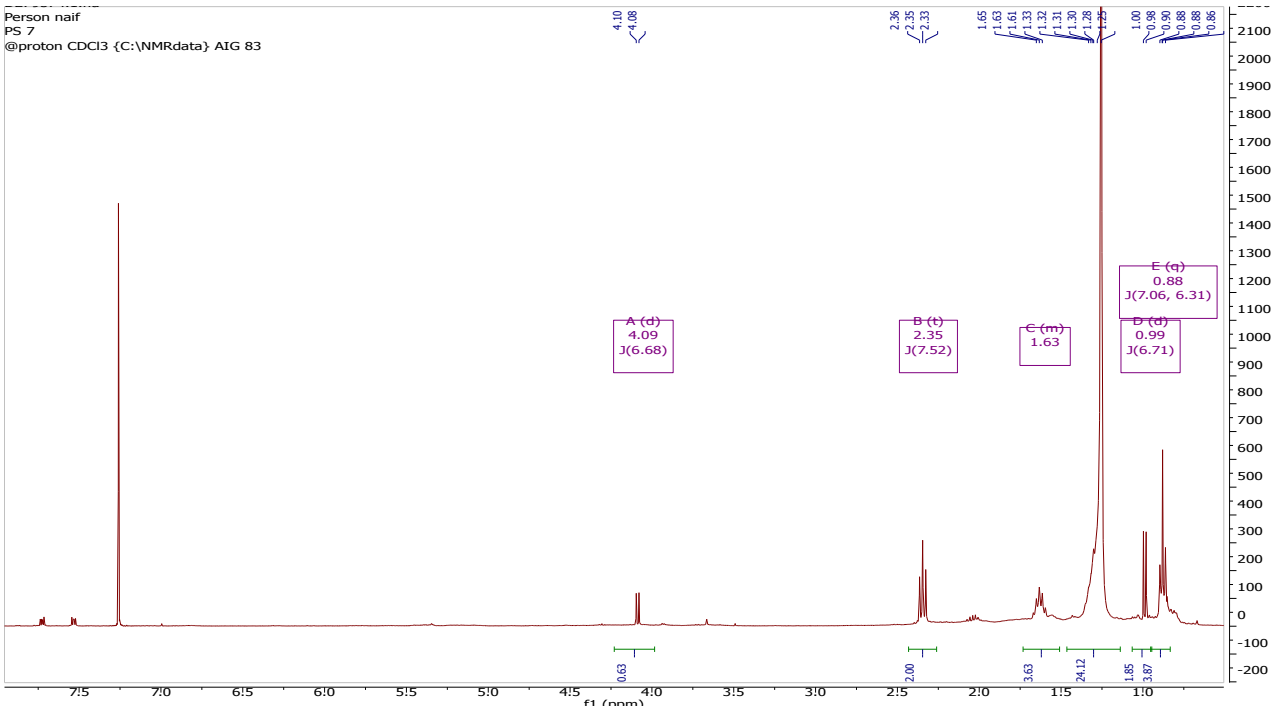

Fig. 2: Proton NMR spectrum of hexadecanoic acid

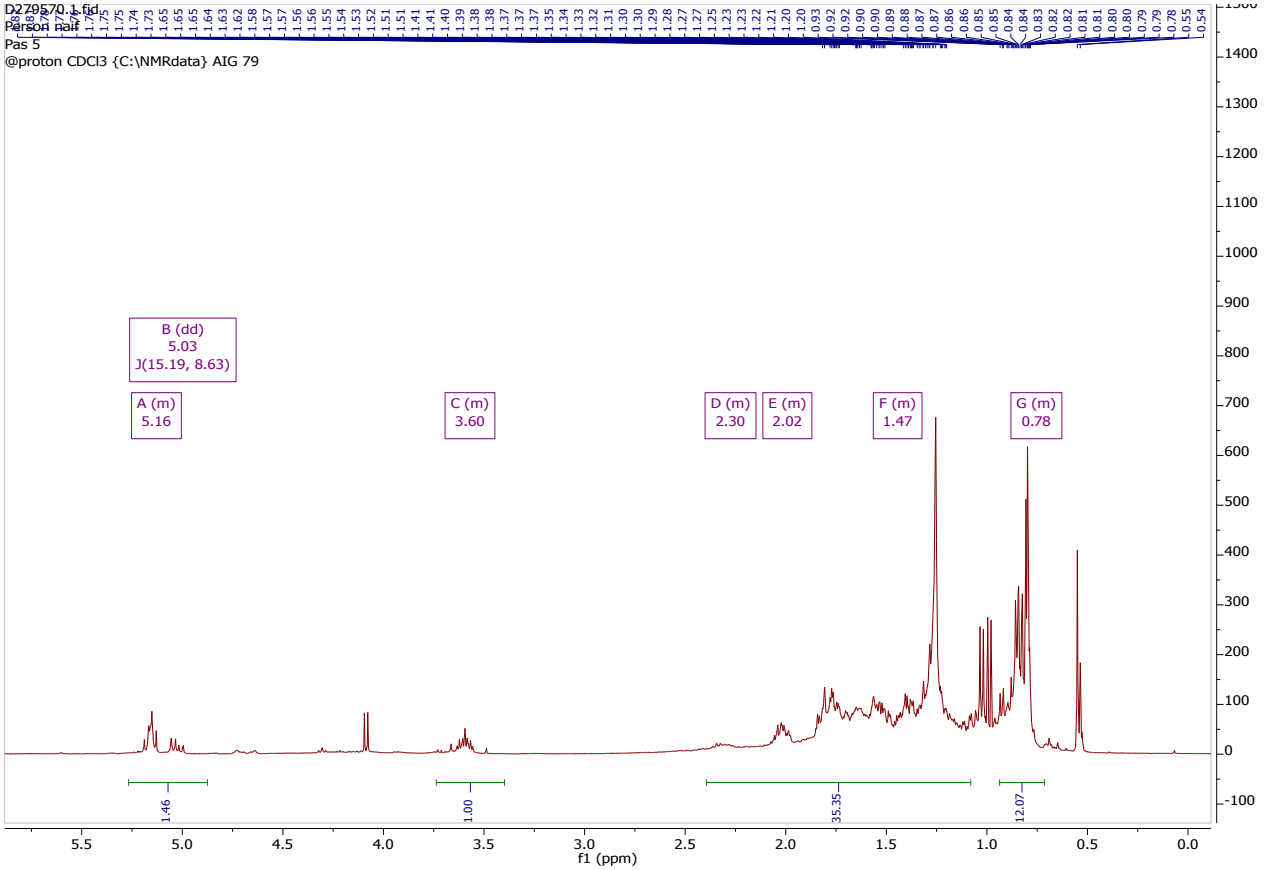

Fig. 3: NMR spectrum showing the presence of α-spinasterol based on evaluation of its integral areas

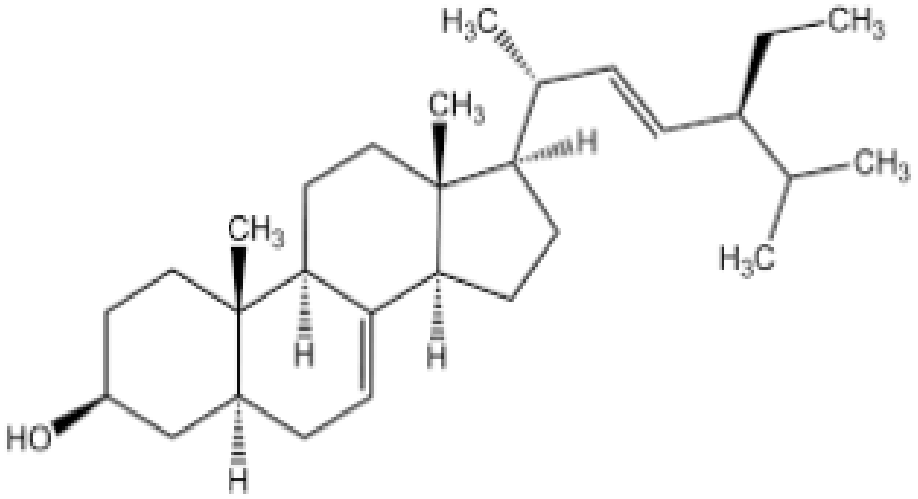

Fig. 4: Structure of spinasterol

7), 26.69 (C-8), 39.75 (C-9), 135.01 (C-10) ,124.31 (C-11), 28.28 (C-12), 17.68 (C-2), 16.03 (C-6), 16.01 (C-10) (Table 3).

Extracts of root of *Cucurbita pepo* showed the presence of Alpha (α)-spinasterol which has been reported to have therapeutic effects. Studies conducted by Socała *et al.*[29,30] revealed significant anticonvulsant effect of α-spinasterol and reduced depressive-like behaviour in mice while the study carried out by Jeong *et al.*[31] on α-spinasterol isolated from the roots of *Phytolacca americana* suggest that α-spinasterol has a significant therapeutic potential to modulate the development and progression of diabetic nephropathy. Apart from reducing cell infiltration in the injured tissue, oral administration of α-spinasterol also reduces postoperative pain when administered before incision or after incision[32]. It also has anti-inflammatory activity *in vivo* and inhibits benign prostatic hyperplasia in rats[33,34].

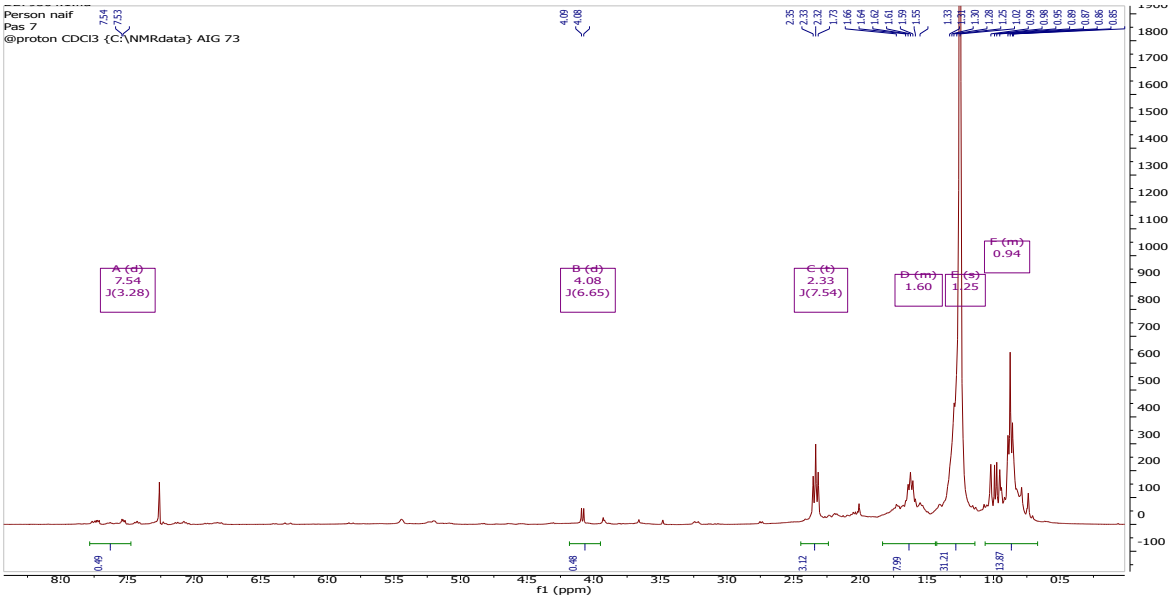

Fig. 5: Proton NMR spectrum of squalene

TABLE 2: ^{13}C NMR CHEMICAL SHIFT VALUES OF A-SPINASTEROL (100 MHz, $CDCL_3$)

Position	Chemical shift (δ)	Type of Carbon
1	37.2	CH2
2	31. 5	
3	71.5	
4	38	
5	40.3	
6	29.7	
7	117.5	CH (unsaturated)
8	139.6	
9	49.5	
10	34.2	
11	21.6	
12	39.6	
13	43.3	
14	55.1	
15	23	
16	28.5	
17	55.9	
18	12	C (unsaturated)
19	13	
20	40.8	
21	21.4	
22	138.1	CH (unsaturated)
23	129.5	
24	51.2	CH (unsaturated)
25	31.9	
26	21.1	
27	19	
28	25.4	
29	12.2	

TABLE 3: ^{13}C NMR CHEMICAL SHIFTS VALUES OF SQUALENE

C	^{13}C (δ)
1	25.70
2	131.28
3	124.32
4	26.73
5	39.76
6	134.88
7	124.28
8	26.69
9	39.75
10	135.01
11	124.31
12	28.28
13	17.68
14	16.03
15	16.01

Extracts of root of *Cucurbita pepo* also showed the presence of squalene, a terpene produced in plants which have been reported to have positive effects in the treatment of certain kinds of cancer. Examination of the seeds of *Cucurbita pepo* showed that it has high concentration of squalene[35]. The seeds oil of *Cucurbita pepo* is rich in phytosterols, fatty acids, squalene, tocopherols and other healthy components. These components make it as alternative medicine for management of men's health problem and have beneficial effects on benign prostatic hyperplasia, overactive bladder and androgenic alopecia[36].

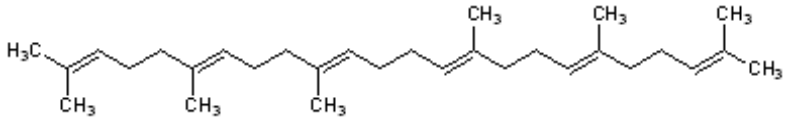

Fig. 6: Structure of squalene

Extracts of root of *Cucurbita pepo* showed the presence of palmitic acid. Palmitic acid is a fatty acid that is found naturally in animals and plants which is widely used in a variety of applications including personal care products and cosmetics. The *Cucurbita pepo* seed oil has been shown to contain palmitic acid in addition to other fatty acids such as oleic, linoleic and stearic acids[37]. Studies carried out by Mulugeta *et al.*[38] and Bulama *et al.*[39] on palmitic acid isolated from root wood of *Moringa stenopetala* and *Terminalia glaucescens* respectively revealed that palmitic acid has antibacterial activity which justify the use of the plants in the treatment of different bacterial infections traditionally[40,41].

The present phytochemical investigation of root of *Cucurbita pepo* revealed the presence of hexadecanoic acid, α-spinasterol and squalene. This is an initial report of these compounds from the root of this plant material.

Conflict of interest:

The authors declare no conflict of interest.

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