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Phytochemical Screening, GC-MS Analysis and Antioxidant Activity of *Curcurbita pepo* L. using Its Leaf Sample

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EAS and NCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAS, WGP and GBO managed the analyses of the study. Authors EAS, NCN and GBO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis and antioxidant activity of *Curcurbita pepo* L. using its leaf sample with standard methods. The sample used for the study was procured from Imo State University school farm and was properly identified. Result of phytochemical screening revealed the presence of saponins, flavonoids, alkaloids, steroids, phlobactannins, proteins, and anthraquinnones, while the GC-MS analysis revealed a total of 78 compounds, out which Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$) had the highest molecular weight, 2,4,6-Octatriene, 2,6-dimethyl- ($C_{10}H_{16}$) had the highest peak area of 10.21% while Morphinan-6-ol, 4,5-epoxy-N-methyl-, (5α 6α- ($C_{17}H_{21}NO_2$) had the highest retention time. The antioxidant activity of the studied sample was enhanced against the control. Some of the compounds as revealed by GC-MS analysis could be of healthcare or industrial importance. There

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is need for further studies on the leaf sample to ascertain further the observations of the present study. This study has evaluated the phytochemical screening, GC-MS analysis and antioxidant activity of *C. pepo* L. using its leaf sample.

Keywords: Antioxidants activity; C. pepo; medicinal plants; phytochemical screening; "ugboguru".

1. INTRODUCTION

The benefits of plants to man and his environment have long been recognized [1-9]. The use of products from plants transversed different human endeavors [10-18]. They contribute to the survival of man through provision of food substances [19-29], raw materials for industries [7,30], manures for agriculture and salvage the environment for man [3,7,9,30]. The use of plants in complementary and natural healthcare in recent years, has opened the door for numerous research studies on plants in relation to their efficacy over diseases and disease causing pathogens. Studies on plants have revealed many biologically active substances and compounds that are physiologically active against disease causing microorganisms [31-39]. Plants with such constituents and with disease salvaging potency are collective known as medicinal plants. Different authors have defined medicinal plants in acceptable terms within the research community [40-51].

Some medicinal plants also have potency against excessive production of reactive oxygen species (ROS) [52] and are said to have antioxidant capacity [53-55]. Various stresses associated with the excessive production of reactive oxygen species have been recognized, Some medicinal plants have also been with the capacity to boost a group of complex antioxidative system comprising ascorbate (AsA) and glutathione (γ-glutamyl-cysteinyl-glycine, GSH) as well as tocopherol, carotenoids, phenolic compounds, superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [56-59]. These group of complex antioxidants scavenge and as well combat the activities of ROS and prevent them from causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells [60-61].

Curcurbita pepo from *Cucurbitaceae* family, popularly known as "ugboguru" among the Igbos of Southeastern Nigeria, could be amongst the plants defined as medicinal plants. It is a herbaceous vine that grows to about 3-9 long and branches occasionally [62]. The vine can sprawl across the ground, but can as well climb adjacent vegetation and objects with the help of its tendrils [62]. It bears a light green stem with short-hairy stout or bluntly angular-grooved. The plant has been cultivated for its edible fruits for thousands of years. It remains a crop plants with great economic importance till date. The matured and immature flowers, fruits, and young leaves are used as vegetables [62]. The matured fruits are used as animal fodder while the large seeds, also known as pumpkin nuts are edible [62]. The seeds are rich in zinc [62-63]. The sap and pulp of *C. pepo* have long been used as a medicinal plant in the North and Central American [62]. The sap and pulp are applied to burns while the seeds are used as a diuretic and as a de-worming agent [62-64]. The plant is also recognised in Ayuvedic medicine where its fruit is considered as cooling and astringent agents. It is believed to cure the thirst for water and fatigue on consumption. It is also believed to purify blood fluid [62-65]. The leaves are used in the treatment of nausea, as a painkiller, and act as boost to haemoglobin content of the blood [62-66]. The seeds of *C.pepo* are affective against bronchitis and fever, and are considered very nutritious [62-67].

Much is not known on the possible bioactive constituents of *C. pepo* that could be physiologically active, and with the recent need to discover more medicinal plants within the context acceptable by the research community. There is urgent need for a detailed scientific study on *C. pepo*. This study evaluated the phytochemistry and antioxidant activity of *C. pepo*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The *C. pepo* leaves used in this study were collected from Imo State University school farm

and got identified in the Department of Plant Science and Biotechnology of the same institution by a Botanist in the Department. The leaves of interest were collected, properly cleaned, shade dried and coarsely powdered for further usage.

2.2 Aqueous Extract Preparation

The extraction was carried out as described by Ezekwe et al. [68]. Ten grams (10 g) of each sample was extracted by maceration in 50 mL of water for 3 days with frequent agitation at a speed of 280 rpm at 28°C in dark. Between extractions, the samples were centrifuged for 10 min with 2000 rpm. The combined supernatants were collected, filtered through Whatman No. 1 filter paper and concentrated in vacuum. They were kept in a vacuum desiccator for complete removal of solvent. The yield extract was thus used for some of phytochemical screening, GC-MS analysis and assessment of antioxidant activity.

2.3 Qualitative Phytochemical Determinations

2.3.1 Test for tannins

To 1 mL of the extract, equal volume of bromine water was added. The formation of a greenish to red precipitate was taken as the presence of tannins.

2.3.2 Test for saponins

One mL of the extract was boiled with 5 mL of distilled water for 5 min. and decanted while hot. 4 mL of distilled water was added to 1 mL of the filtrate before it was shaken vigorously for observation of stable froth on standing.

2.3.3 Test for flavonoids

0.5 g of the extract was added, in a test tube and 10 ml of distilled water, 5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of the extract followed by addition of 1 mL concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract..

2.3.4 Test for alkaloids

One (1) mL each of the extract was shaken with 5 mL of 2% HCl on a steam bath and then filtered. To I mL of the filtrate, Wagner`s reagent (iodine in potassium –iodide solution) was added and reddish brown precipitates was observed for positive result.

2.3.5 Test for steroids

Half (0.5 g) gram of the extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

2.3.6 Test for terpenoids

One gram of seed sample was shaken in a test tube with 10 mL of methanol, and then filtered. 5 mL extract was then mixed with 2 mL of chloroform and 3 mL of sulphuric acid was added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

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2.3.7 Cardiac glycosides

One mL of the seed extract was dissolved in 2 mL of chloroform in a test tube. 1 mL conc. H_2SO_4 was carefully added to the test tubes through the side and was observed for a red or reddish brown colouration at the interphase, which indicates positive result.

2.3.8 Test for phlobatannins

One percent aqueous hydrochloric acid was added to the seed extract in a test tube (about 2 mL), and then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

2.3.9 Test for phenolic compounds

To 2 mL of the seed extract, 1% $FeCl_3$ was added and observation was made for blue, violet, purple, green or red-brown colour.

2.3.10 Test for proteins

Five drops of 1% hydrated copper sulphate was added to 2 mL the seed extract in a test tubes. Two mL of 40% NaOH was also added, and the test tube was shaken vigorously to mix the content and presence of purple colouration indicated the presence of proteins.

2.3.11 Test for reducing sugars

One mL of ethanol was mixed with 2 mL each of the plant extract, after which 1 mL each of Fehling solution A and B were added to the test tubes. The test tubes were heated to boiling while observation was made for presence of reddish brown colouration which indicates positive results.

2.3.12 Test for anthroquinones

One gram of the seed extract was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which for the presence of anthraquinones. This was repeated with all the plant samples.

2.4 GC-MS Analysis of the Extracts

GC-MS analysis of the aqueous extracts was carried out using AOC-20i auto sampler and gas chromatograph interface to a mass spectrometer (GC-MS) instrument. Employing the following conditions; column Elite-1 fused silica capillary column (30 mm×0.25 mm ID×1µM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/ min, and an injection volume of 0.5µl, Split ratio of 10:1), with injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 mins isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 mins. The plant extract was dissolved in aqueous and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test were ascertained.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH (1, 1-Dipheny l-2-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity was measured by DPPH assay method. Four mg of DPPH (0.1 mM) was dissolved in 100 mL of distill water to obtain working solution. One mL of each extract was mixed separately with 2.0 mL of 0.1 mM DPPH followed by 30 min incubation in dark. The reduction of the DPPH free radical was measured by taking the absorbance at 517 nm [61]. Colour of DPPH was reduced from purple to yellow. The antioxidant activity of each extracts was evaluated by calculating the inhibition % of free radical formation using the formula:

$$\% \text{ inhibition} = [(A-A_1)/A] \times 100$$; A= absorbance of the blank (DPPH); A1= absorbance of the extract (DPPH+ extract).

2.6 Results and Discussion

Result of phytochemical screening of *C.pepo* as presented in Table 1 shows that tannins, saponins, flavonoids, flavonoids, alkaloids, steroids, terpenoids, cardiac glycosides, phlobactannins, phenolic compounds, proteins, reducing sugars, and anthraquinnones were screened. However, only saponins, flavonoids, alkaloids, steroids, phlobactannins, proteins, and anthraquinnones were found present at different concentrations. Flavonoids, phlobactannins and proteins were present in high concentrations. The strategic roles of saponins [68-70], flavonoids [68], alkaloids [68,72], steroids [68, 73], phobactannins [68, 74-75], proteins [68,76], and anthraquinnones [68,76] in plants, on pathogenic organisms, and humans have been long been reported.

Result of GC-MS analysis of *C.pepo* showing retention time, molecular formula, molecular weight and peak area as presented on Table 2, revealed the presence of 78 constituents, which include Benzene, 1,1'-(oxydi-2,1-ethanediyl)bis [3-ethyl-, Piperidinone, Divinyl sulfate, Benzofuran, 2,3-dihydro-, 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-, Methyl cis-2-bromo-3-chloropropenate, 3-Hexyne, Benzenepropanoic acid, methyl ester, indole, 4-Hdroxy-3-methylacetophenone, Benzaldehyde, 3-hydroxy-oxime, 2-Cyclopenten-1-one, 2-methyl-, Butanoic acid, 3-methyl-2-methylpropyl ester, 1,4-Hexadiene, 2,3,4,5-tetramethyl, 2,5,10-Undecatrienoic acid, methyl ester, 2-Hydroxy-4-

hydroxylaminopirimidine, Benzeneacetamide, α-ethyl-, 3,5-Octadiene, 4,5-diethyl-, (E,Z)-, Allyl undecylenate, (Cyclopropyl)trivinylsilane, Silane, ethenyldiethylmethyl-, 3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro1-1-benzofuran, Benzene, 1-ethynyl-4-fluoro-, 1-Methoxy-1,4-cyclohexadiene, Propanedinitrile, (1,2,2-trimethylpropylidene)-, Ethanol, 2-bromo-, 3-Hydroxy-.beta.-damascone, 3-Hydroxy-7,8-dihydro-β-ionol, 1,6-Octadien-3-ol, 3,7-dimethyl-,4-Picoline, 3-(tert-butylthio)-, 2,4,6-Octatriene, 2,6-dimethyl-, 7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-, 3H-Pyrazol-3-one, 1,2-dihydro-1,2,5-trimethyl-, 2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-,2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-, 2H-Pyran-2-one, 4-hydroxy-6-(2-oxopropyl)-, 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-, 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2,4-pentadienyl)-, (Z)-(+)-, Cyclotridecane, 5-Ethyl-2-furaldehyde, 2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-, 2,4,6-Octatriene, 2,6-dimethyl-,2H-Inden-2-one, octahydro-3a-methyl-, cis-,7,8-Epoxy-α-ionone, 2-Heptenal, 2-propyl-,7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-, 6-Octenal, 3,7-dimethyl-, Solavetivone, 5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol, Hexadecanoic acid, methyl ester, 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-,2(1H)-Azulenone, 4,5,6,7,8,8a-hexahydro-8a-methyl-, (S)-, 2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-, 1,3-Oxathiane, 2-ethyl-2,6-dimethyl-, cis-,1,6-Dimethyl-9-(1-methylethylidene)-5,12-dioxatricyclo[9.1.0.0(4,6)]dodecan-8-one, 2-Dodecen-1-yl(-)succinic anhydride, Ethanone, 1-(3,3-dimethylbicyclo[2.2.1]hept-2-yl)-, endo-,9-Octadecenoic acid (Z)-, methyl ester, Phytol, Octadecanoic acid, methyl ester, 2-Allyl-2-methyl-1,3-cyclopentanedione, β-l-Arabinopyranoside, methyl, p-Menth-8(10)-en-9-ol, cis-,(R)-(-)-14-Methyl-8-hexadecyn-1-ol, 1H-Indene, 5-butyl-6-hexyloctahydro-, Eicosanoic acid, methyl ester, 1,2-Dioxolan-3-ol, 4-bromo-3,5,5-trimethyl-, Nonadecanoic acid, methyl ester, Bis(2-ethylhexyl) phthalate, 5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol, Heneicosanoic acid, methyl ester, 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-,3-(3,4-Dimethoxyphenyl)propylamine, PFP, Benzenamine, 3-methoxy-2,4,6-trimethyl-, Temazepam, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, and Morphinan-6-ol, 4,5-epoxy-N-methyl-, (5α 6α-.

Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$) had the highest molecular weight of 390 $gmol^{-1}$ with a retention time of 10.301 secs. 2,4,6-Octatriene, 2,6-dimethyl- ($C_{10}H_{16}$) had the highest peak area of 10.21% while Morphinan-6-ol, 4,5-epoxy-N-methyl-, (5α 6α- ($C_{17}H_{21}NO_2$) had the highest retention time of 16.816 secs. These constituents in totally could be contributing to the few known medicinal efficacy of *C.pepo* in traditional healthcare system. Ezekwe et al. [68] noted that the compounds revealed by GC-MS in the plants and those of phytochemical screening become important when their functions and contributions in nature are considered.

Table 1. Phytochemical Screening of *C. pepo*

Phytochemical	*C. pepo*
Tannins	-
Saponins	+
Flavonoids	++
Alkaloids	+
Steroids	+
Terpenoids	-
Cardiac glycosides	-
Phlobactannins	++
Phenolic compounds	-
Proteins	++
Reducing sugars	-
Anthraquinnones	+

++: present in high concentration; +: present in moderate concentration; -: absent

C. pepo leaf tends to have a better antioxidant activity against that of ascorbic acid as observed in the present study (Fig. 1). Some of the observed GC-MS constituents could no doubt aid such activity. The antioxidant activities of plants such as *Gongronema latifolium* [54-55]; *Gongronema latifolium* Benth [68], *Petrocarpus mildbraedii* Harms [68] and *Piper guineense* [68] have been reported by different authors.

Table 2. Result of GC-MS analysis of *C. pepo* showing retention time, molecular formula, molecular weight and peak area

SN	Retention time	Name of compound	Formula	Molecular weight	Peak Area %
1	3.688	Benzene, 1,1'-(oxydi-2,1-ethanediyl)bis[3-ethyl-	$C_20H_{26}O$	282	1.85
2	3.827	Piperidinone	C_5H_9NO	99	1.91
3	3.894	Divinyl sulfide	C_4H_6S	86	0.57
4	3.995	Benzofuran, 2,3-dihydro-	C_8H_8O	120	8.96
5	4.082	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_7NO_2$	137	2.02
6	4.145	Methyl cis-2-bromo-3-chloropropenate	$C_4H_4BrClO_2$	198	0.55
7	4.217	3-Hexyne	C_6H_{10}	82	0.87
8	4.337	Benzenepropanoic acid, methyl ester	$C_{10}H_{12}O_2$	164	0.94
9	4.483	Indole	C_8H_7N	117	1.07
10	4.584	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	150	2.35
11	4.659	Benzaldehyde, 3-hydroxy-, oxime	$C_7H_7NO_2$	137	0.29
12	4.723	2-Cyclopenten-1-one, 2-methyl-	C_6H_8O	96	0.54
13	4.813	Butanoic acid, 3-methyl-, 2-methylpropyl ester	$C_9H_{18}O_2$	158	1.54
14	5.023	1,4-Hexadiene, 2,3,4,5-tetramethyl	$C_{10}H_{18}$	138	0.38
15	5.075	2,5,10-Undecatrienoic acid, methyl ester	$C_{12}H_{18}O_2$	194	0.28
16	5.150	2-Hydroxy-4-hydroxylaminopirimidine	$C_4H_5N_3O_2$	127	0.41
17	5.315	Benzeneacetamide, α-ethyl-	$C_{10}H_{13}NO$	163	0.25
18	5.427	3,5-Octadiene, 4,5-diethyl-, (E,Z)-	$C_{12}H_{22}$	166	0.37
19	5.521	Allyl undecylenate	$C_{14}H_{24}O_2$	224	0.29
20	5.566	(Cyclopropyl)trivinylsilane	$C_9H_{14}Si$	150	0.89
21	5.604	Silane, ethenyldiethylmethyl-	$C_7H_{16}Si$	128	1.26
22	5.832	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro1-1-benzofuran	$C_{10}H_{16}O$	152	0.62
23	6.023	Benzene, 1-ethynyl-4-fluoro-	C_8H_5F	120	0.42
24	6.072	1-Methoxy-1,4-cyclohexadiene	$C_7H_{10}O$	110	0.68
25	6.128	Propanedinitrile, (1,2,2-trimethylpropylidene)-	$C_9H_{12}N_2$	148	0.36
26	6.181	Ethanol, 2-bromo-	C_2H_5BrO	124	1.11
27	6.241	3-Hydroxy-.beta.-damascone	$C_{13}H_{20}O_2$	208	1.54
28	6.297	3-Hydroxy-7,8-dihydro-β-ionol	$C_{13}H_{20}O_2$	208	2.27
29	6.342	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H18O$	154	0.36
30	6.391	4-Picoline, 3-(tert-butylthio)-	$C_{10}H_{15}NS$	181	0.98
31	6.462	2,4,6-Octatriene, 2,6-dimethyl-	$C_{10}H_{16}$	136	10.21
32	6.514	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	168	3.31

SN	Retention time	Name of compound	Formula	Molecular weight	Peak Area %
33	6.593	3H-Pyrazol-3-one, 1,2-dihydro-1,2,5-trimethyl-	$C_6H_{10}N_2O$	126	1.36
34	6.649	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	$C_{11}H_{18}O_2$	182	0.28
35	6.698	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$	208	0.71
36	6.784	2H-Pyran-2-one, 4-hydroxy-6-(2-oxopropyl)-	$C_8H_8O_4$	168	0.74
37	6.822	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	$C_{13}H_{22}O_3$	226	1.44
38	6.904	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2,4-pentadienyl)-, (Z)-(+)-	$C_{11}H_{14}O_2$	178	1.02
39	6.964	Cyclotridecane	$C_{13}H_{26}$	182	1.76
40	7.028	5-Ethyl-2-furaldehyde	$C_7H_8O_2$	124	3.9
41	7.111	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	1.32
42	7.178	2,4,6-Octatriene, 2,6-dimethyl-	$C_{10}H_{16}$	136	4.56
43	7.242	2H-Inden-2-one, octahydro-3a-methyl-, cis-	$C_{10}H_{16}O$	152	0.39
44	7.275	7,8-Epoxy-α-ionone	$C_{13}H_{20}O_2$	208	0.79
45	7.324	2-Heptenal, 2-propyl-	$C_{10}H_{18}O$	154	1.42
46	7.373	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	168	2.14
47	7.399	6-Octenal, 3,7-dimethyl-	$C_{10}H_{18}O$	154	5.84
48	7.542	Solavetivone	$C_{15}H_{22}O$	218	0.29
49	7.579	5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol	$C_{15}H_{28}O$	224	0.31
50	7.617	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.25
51	7.677	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	$C_{10}H_{16}O$	152	1.05
52	7.703	2(1H)-Azulenone, 4,5,6,7,8,8a-hexahydro-8a-methyl-, (S)-	$C_{11}H_{16}O$	164	1.08
53	7.774	2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}O_2$	168	1.93
54	7.905	1,3-Oxathiane, 2-ethyl-2,6-dimethyl-, cis-	$C_8H_{16}OS$	160	0.29
55	7.995	1,6-Dimethyl-9-(1-methylethylidene)-5,12-dioxatricyclo[9.1.0.0(4,6)]dodecan-8-one	$C_{15}H_{22}O_3$	250	0.30
56	8.201	2-Dodecen-1-yl(-)succinic anhydrid	$C_{16}H_{26}O_3$	266	0.50
57	8.314	Ethanone, 1-(3,3-dimethylbicyclo[2.2.1]hept-2-yl)-, endo-	$C_{11}H_{18}O$	166	0.83
58	8.363	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	2.93
59	8.419	Phytol	$C_{20}H_{40}O$	296	1.43
60	8.460	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	1.03
61	8.535	2-Allyl-2-methyl-1,3-cyclopentanedione	$C_9H_{12}O_2$	152	0.46

SN	Retention time	Name of compound	Formula	Molecular weight	Peak Area %
62	8.587	β-l-Arabinopyranoside, methyl	$C_6H_{12}O_5$	164	0.33
63	8.655	p-Menth-8(10)-en-9-ol, cis-	$C_{10}H_{18}O$	154	0.26
64	8.715	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252	0.55
65	9.221	1H-Indene, 5-butyl-6-hexyloctahydro-	$C_{19}H_{36}$	264	0.21
66	9.300	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.37
67	9.465	1,2-Dioxolan-3-ol, 4-bromo-3,5,5-trimethyl-	$C_6H_{11}BrO_3$	211	0.46
68	10.166	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312	0.55
69	10.301	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.61
70	10.994	5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol	$C_{15}H_{28}O$	224	0.28
71	11.189	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	0.23
72	11.841	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	0.44
73	13.536	3-(3,4-Dimethoxyphenyl)propylamine, PFP	$C_{14}H_{16}F_5NO_3$	341	0.37
74	14.237	Benzenamine, 3-methoxy-2,4,6-trimethyl-	$C_{10}H_{15}NO$	165	0.86
75	16.029	Temazepam	$C_{16}H_{13}ClN_2O_2$	300	1.21
76	16.445	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298	0.21
77	16.625	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298	0.55
78	16.816	Morphinan-6-ol, 4,5-epoxy-N-methyl-, (5α 6α-	$C_{17}H_{21}NO_2$	271	0.70

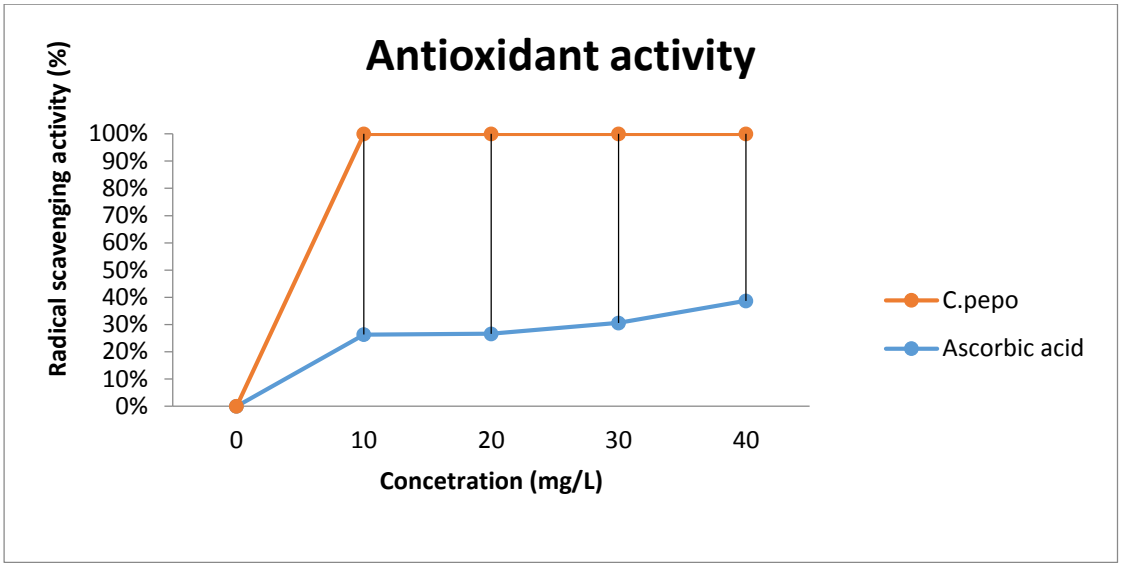

Fig. 1. Antioxidant activity of *C. pepo* leaf

3. CONCLUSION

This study has shown the phytochemical constituents of *C. pepo* leaf. The GC-MS analysis further revealed detailed compounds, majority of which could be very useful in healthcare and industries. The leaf also had an enhanced antioxidant activity than ascorbic acid used as the control. However, there is need for further studies on the leaf sample to ascertain further the observations of the present study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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