Original Article

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Invitro antioxidant capacity, phytochemical screening and Gas chromatography GC-MS analysis of Ethanol root extract of *Sphenocentrum jollyanum* (Pierre)

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Abstract

Background: *In-vivo* antioxidants potentials of *Sphenocentrum jollyanum* had been reported in disease conditions. However, there is a paucity of information on its *invitro* capacity compared to standard antioxidants. The objective of the study is to screen ethanol root extract of *Sphenocentrum jollyanum* (SJE) for its *invitro* antioxidants capacity and phytochemical components.

Methodology: Antioxidant capacity and phytochemical content were determined using ABTS, DPPH and *GC-MS* techniques.

Results: Free radicals were scavenged by *SJE* in all the *In vitro* methods used in a concentration dependent manner. Howbeit, the free radical scavenging ability and reducing power potentials of SJE were significantly lower ($p<0.05$) when compared to standard antioxidants such as Trolox and Vit.E The qualitative screening shows the presence of alkaloids, phenols, phlobatanins, saponins, tannins, amino acid and reducing sugar w*hile GC-MS revealed* thirty-four plant bioactive principles among which are members of Octadecadienoic acid family including 12-Octadecadienoic acid, ethyl ester also known as Linoleic acid.

Conclusion: *Sphenocentrum jollyanum* ethanol root extract possesses antioxidant capacity and important phytochemicals that can be useful in the design, synthesis, and advancement of new medications.

Keywords: *Sphenocentrum jollyanum*; free radicals; phytochemicals; phenols; Octadecadienoic acid; linoleic acids

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Capacité antioxydante in vitro, criblage phytochimique et analyse gc-ms par chromatographie en phase gazeuse d'extrait ethanoïque de racine de *sphenocentrumjollyanum* (pierre)

Oladokun, O.O.

Résumé

Contexte général de l'étude: Des potentiels antioxydants *in vivo de Sphenocentrum jollyanum* ont été rapportés dans des conditions pathologiques. Cependant, il existe peu d'informations sur sa capacité *in vitro* par rapport aux antioxydants standards. L'objectif de l'étude est de cribler l'extrait éthanoïque de racine de *Sphenocentrum jollyanum* (RSJ) pour sa capacité antioxydante *in vitro* et ses composants phytochimiques.

Méthode de l'étude: La capacité antioxydante et la teneur phytochimique ont été déterminées à l'aide des techniques ABTS, DPPH et *GC-MS.*

Résultats de l'étude: Les radicaux libres ont été piégés par *SJE* dans toutes les méthodes *Invitro* utilisées de manière dépendante de la concentration. Cependant, la capacité de piégeage des radicaux libres et les potentiels de puissance réductrice de SJE étaient significativement plus faibles ($p < 0,05$) par rapport aux antioxydants standard tels que Trolox et Vit.E Le dépistage qualitatif montre la présence d'alcaloïdes, de phénols, de phlobatanines , de saponines , de tanins, acide aminé et sucre réducteur tandis que la *GC-MS a révélé* trente-quatre principes bioactifs végétaux parmi lesquels des membres de la famille des acides octadécadiénoïques, y compris l'acide 12-octadécadiénoïque, ester éthylique également connu sous le nom d'acide linoléique.

Conclusion: L'extrait de racine d'éthanol de *Sphenocentrum jollyanum* possède une capacité antioxydante et des composés phytochimiques importants qui peuvent être utiles dans la conception, la synthèse et l'avancement de nouveaux médicaments.

Mots-clés: *Sphenocentrumjollyanum,* radicaux libres, phytochimiques, les phénols, acide octadécadiénoïque, acides linoléiques

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INTRODUCTION

Oxidants are essential for normal physiological function, cellular homeostasis and in aiding the immune system (1, 2). A serious disturbance in the balance between prooxidants and antioxidants in favor of prooxidants, can however lead to possible damage to cells and organs. This is known as oxidative stress (3). Many free radicals in biological systems are derived from oxygen (reactive oxygen species, ROS) but there are also derivatives of nitrogen-Reactive Nitrogen Species (RNS). ROS such as superoxide anion ($O_2^{\cdot-}$), hydroxyl ($^{\cdot}$ OH), peroxyl ($ROO^{\cdot}$) alkoxyl radicals ($RO^{\cdot}$), hydrogen peroxide (H_2O_2), and singlet oxygen $O_2(^1\Delta_g)$ may attack biological macromolecules, giving rise to protein, lipid and DNA damage, cell aging, oxidative stress originated diseases such as cardiovascular and neurodegenerative diseases, and cancer. Antioxidants scavenge or quench ROS and reactive nitrogen species products of respiration, including free radicals (4). There are two basic categories of antioxidants: synthetics and naturals (5). However, restrictions on the use of synthetic compounds are being imposed, because of their carcinogenicity and other toxic properties (6) resulting in increased demand for phytotherapy globally over the years (7). Phenolic compounds in natural plants such as polyphenols with either monomeric or complex structures like phenolic acids, phenylpropanoids and flavonoids have shown close correlations with antioxidant capacity (8).

In traditional folklore and traditional medicine, *Sphenocemtrum jollyanum* (SJ), is believed to possess positive hemostatic and stomachic properties. It occurs primarily in the undergrowth of the forest, often in deep shade and open forest, from sea level up to 400 m. It is therefore native to the tropical forest zones of West Africa, widely cultivated in Cameroun, Sierra Leone, Nigeria, Ghana, and Côte d'Ivoire. It has displayed wide spectra of biological and pharmacological activities (9, 10). Olorunnisola and Afolayan (11) reported *in-vivo* dose dependent protective capacity and antioxidant activity of the leaf extract in P. berghei infected mice. The root is used traditionally as an aphrodisiac for men. The juice from the root is believed to relieve abdominal pain, constipation and increase sexual drive, exploring this plant can therefore form the basis for investigation, validation, development and application of suitable biotechnologies for cost effective disease control strategies with correspondingly high efficiency and ecologically acceptable aphrodisiac and fertility drug as suggested by Yongabi (12) and Koparde (13) . This study was therefore conducted to evaluate invitro antioxidant properties and phytochemical constituent of Sphenocentrum jollyanum ethanol root extract.

MATERIALS AND METHODS

Plant material

Sphenocentrum jollyanum plant was collected and authenticated at Forestry Research Institute Nigeria (FHI No.: 106994). The plant root was air-dried at room temperature for four weeks, after which it was ground into uniform powder. Cold maceration was performed with 1 litre of ethanol to 1 kilogram of dry root for 72 hours. The resulting mixture was filtered with Whatman filter paper. Filtrate was concentrated to dryness using rotary evaporator to obtain *Sphenocentrum jollyanum* ethanol extract (SJE).

Invitro antioxidant capacity: 2, 2'- Azinobis-(3-ethylbenzothiazoline 6 sulfonic acids) (ABTS)

The antioxidant activity/capacity of ethanol extract of *SJE* was evaluated using 2,2'-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) assay. Radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS solution 5 ml of 7 mM with 5 ml of 2.45 mM potassium persulfate ($K_2S_2O_8$) at ratio 1:1 and the mixture was allowed to stand in dark at room temperature for 16 h before use. ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at a wavelength of 745 nm. 1ml of ABTS reagent was added to 10µl of different concentration of SJE and the absorbance was measured at 735 nm. Trolox was used as standard antioxidant. The percentage inhibition was calculated using the formular

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] * 100$$

A_0– absorbance in the absence of sample

A_1- absorbance of SJE or Trolox

The median inhibitory concentration (IC_{50}) of SJE was also compared with Trolox (14).

1, 1-Diphenyl-2 picryl-hydrazyl radical scavenging assay (DPPH)

DPPH is a stable alcoholic free radical with red color. It is reduced to yellow non-radical 2,2-diphenyl-1-picrylhydrazine (DPPH-H) in the presence of a hydrogen-donating antioxidant. This common principle was utilized in this assay (14).

Assay: Briefly 1.2 ml of test SJE or Vit. E in 0.1ml of 1M Tris-HCL buffer (pH 7.9) was mixed with 1.2 ml of 5 mM DPPH in methanol. The reaction

mixture will be incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 517 nm. The percentage of inhibition was calculated using the following equation

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] * 100$$

A_0– absorbance without sample
A_1-absorbance of SJE or Vit. E

Ferric reducing antioxidant power assay (FRAP)

FRAP assay was done according to method described by Tsai (15), with slight modification. FRAP uses antioxidant as reductants in a redox linked colorimetric method to test the total antioxidant power directly. Reagent containing 0.83 mmol L1 TPTZ and 1.67 mmol L1 ferric chloride at 0.1 mol L1 acetate buffer (pH 3.6). Sample was mixed with 0.9 ml of reagent and incubated at 25°C for 10 minutes. Formation of a blue colored ferrous tripyridyltriazine (Fe2+ - TPTZ) from a yellow ferric tripyridyltriazine (Fe3+ -TPTZ) was observed at absorbance of 593 nm. Values were expressed in µmole Fe^{2+}/mg dry weight of the test sample. Ascorbic acid was used as standard reference.

Cupric ions reducing assay (CUPRAC)

Cupric reducing assay was done according to method described by Koksal (16), with little modification. 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanol neocuproine solution (7.5 mM) and 0.25 mL CH_3COONH_4 buffer solution (1.0 M) were added to each test tube then, different concentrations (20, 40, 60, 80 and 100 µg/mL) of standard antioxidants (trolox) or extracts were added to each tube, separately. Total volume was adjusted to 2 mL with distilled water and mixed. Tubes were incubated at room temperature for 30 min. Absorbance was measured at 450 nm against a reagent blank.

Phytochemical Screening

Test for Alkaloids

Test for alkaloid was carried out by a method previously described by Siddiqui and Ali (17). The alkaloidal base of the SJE in acidified alcohol and ammonia was extracted with acetic acid to obtain the chloroform layer. This was divided into three portions. Mayer's reagent, Draggendorff's reagent and Wagner's reagent were added to each of the portions respectively. The formation of a cream-colored precipitate with Mayer's reagent, a mist or a reddish-brown precipitate with the Draggendorff reagent and a hazy brown color with the Wagner reagent was considered positive for the presence of alkaloids.

Cardenolides/Cardiac Glycosides test (Keller-Killiani test)

The Keller-Killiani test for glycosides was performed according to a method described by Sim (18). Briefly, 5 ml of water was used to prepare 0.5 g of plant. 2 ml of glacial acetic acid containing a drop of ferric chloride solution was added to form a precipitate. This was filtered and 1 ml of concentrated sulfuric acid was added. A brown ring at the interface indicated the presence of a characteristic deoxy sugar of the cardenolide acid layer.

Test for Anthraquinones

Test for total anthraquinones (bound and free) was carried out according to the method described by Harbone (19). Briefly, 0.5 g of the plant was boiled for 5 minutes with 10 ml of sulfuric acid (H_2SO_4). The solution was filtered hot, allowed to cool and 10 ml of chloroform was added and mixed vigorously. The chloroform layer was separated into a new test tube and mixed with 1 ml of diluted ammonia. The resulting solution was examined to determine the red color in the upper phase of ammonia, indicating the presence of anthraquinones.

Saponins Test (Frothing test)

One decigram (0.1g) of the plant extract was mixed with 5.0 ml distilled water, shaken for 20 min. Presence of foams indicates presence of saponins (20).

Tannins Test

Five milligram of plant extract was boiled with 5 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added. A brownish green or blue-black color indicated the presence of tannins (21).

Test for Flavonoid

Approximately 0.2 g of the plant extract was dissolved in 2 ml of methanol and heated. A piece of metallic magnesium was added, followed by few drops of concentrated H_2SO_4. The appearance of red or orange spots indicated flavonoids (22).

Test for Starch

The extract (0.2g) was dissolved in 5 ml of distilled water and filtered. The filtrate was treated with 2 drops of α-naphthol alcohol solution (Molisch test). The formation of a purple ring indicated the presence of starch.

Test for reducing sugar (Fehling's test)
Two milliliters (2.0 ml) of Fehling solution A and B was added to 1.0 ml (v/v) of the extract and boiled for 5 minutes. Reducing sugar forms red precipitate with Fehling solution (21).

Test for Phenols (Ferric Chloride test)
Phenols forms a blue-black colour with the addition of few drops Ferric chloride solution (23).

Test for amino acid (Ninhydrin test)
The crude extract (5mg) was boiled with 2 ml of 0.2% Ninhydrin solution. Amino acids and proteins give purple color.

Gas Chromatography – Mass Spectroscopy

The GC-MS analysis was carried out according to the method described by Ajayi (24) using a Hewlett Packard Gas Chromatograph (Model 6890 series) furnished with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 mins holding time and raised from 50 to 250°C at a rate of 2°C /min, using helium (99.99%) as carrier gas at a constant flow of 22 cm / s. One micron extract (1 mg dissolved in 1 ml of absolute alcohol) in a divided ratio of 1:30 was injected

The MS analysis was performed on an Agilent Technology Network mass model (model 5973) coupled to a Hewlett Packard gas chromatograph (model 6890) equipped with a NIST08 library software database. Mass spectra were recorded at 70 eV/200°C; Scan speed of 1 scan / s. Compounds were identified using the NIST library database. Mass spectrum of a single unknown compound was compared with the known compounds stored in the software database of NIST library.

Statistical analysis

Data were analyzed statistically using GraphPad prism Version 5.0 software. Differences between means were determined using the student t test at $P < 0.05$. The data are presented as mean ± SEM.

RESULTS

***Invitro antioxidant* properties of ethanol root extract of SJE**

Total antioxidant capacity (TAC) of SJE as measured by 2, 2'- Azinobis-(3-ethylbenzothiazoline 6 sulfonic acids) (ABTS), shows that SJE has radical scavenging properties. However, its inhibition capacity is significantly lower than values recorded when compared to standard antioxidant trolox in dose dependent manner (Figure 1 A). Trolox and SJE has median inhibition concentration values of IC_{50} 0.56 and 9.95 respectively (Figure 1B). Radical scavenging capacity of SJE as evaluated in 2, 2-di (4-tert-octylphenyl) -1 picrylhydrazyl (DPPH) assay, showed a dose dependent inhibition capacity of SJE in reducing DPPH to the yellow colored diphenylpicrylhydrazine DPPH-H (Figure 2A). The median inhibitory concentration (IC_{50}) of SJE towards DPPH as compared to standard antioxidant Vit E is however high as seen in Figure 2B. As shown in table 1, the antioxidant power of SJE as measured by Ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) gave mean values of 116.09 ± 0.178 and 24.44 ±0.740 mg/mL respectively when compared with standard antioxidant ascorbic acid and trolox.

Phytochemical Analysis

The phytochemical screening of ethanol root extract of SJE showed it contained alkaloids, phenols, phlobatanins, saponins, tannins, amino acid and reducing sugar (Table 2). Tannins were the most abundant phytochemicals present while alkaloids, phlobatanins and reducing sugar were trace substance.

Gas chromatograph of ethanol root extract of (SJE)

The extract contained Hexadecanoic acid, ethyl ester,n-Hexadecanoic acid ,9-Octadecenoic acid, methyl ester, (E) Heptadecanoic acid, ethyl ester, 11-Octadecenoic acid, methyl ester ,12-Octadecadienoic acid, ethyl ester, 9,12-Octadecadienoic acid, ethyl ester, 9,17-Octadecadienal, (Z)-, 9,17 Octadecadienal, (Z)- 9,12-Octadecadienoic acid (Z,Z)-, cis-Vaccenic acid, Octadecanoic acid, ethyl ester, Octadecanoic acid, ethyl ester, Cyclopropanedecanoic acid, 2-hexy-, methyl esterl, Nonadecanoic acid, ethyl ester, cis-11-Eicosenoic acid, 11-Eicosenoic acid, methyl ester, Methyl 19-methyl-eicosanoate Nonadecanenitrile, Octadecanoic acid, ethyl ester and 11-Oxadispiro[4.0.4.1]undecan-1-on (Table 3) among others.

GC-MS chromatogram of SJE peaks between numbers 19-26 with retention time 31.08-31.56. Compounds in this range were

family member of Octadecadienoic acid. The family made up 73.48 percent of the whole essential oil present in the ethanol root extract (Figure 3A; Table 3), and 12-Octadecadienoic acid, ethyl ester also known as linoleic acid (Figure 3B) alone contributed about 35.86 percent.

DISCUSSION

Among several mechanisms by which antioxidants act is scavenging reactive oxygen and nitrogen free radicals and its reducing power. It was observed that free radicals were scavenged by *SJE* in all the *invitro* methods used in a concentration dependent manner. The percentage scavenging and IC_{50} values calculated for all doses were also compared with standard antioxidants such as Vit E and Trolox. The scavenging activity of *SJE* was however significantly lower when compared with standard antioxidant such as Vit.E and Trolox. In drug discovery, medicinal herbs are regularly considered the leading source of pharmaceuticals employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality (25,26). Natural antioxidants have gained utmost importance in the treatment of "hard to treat diseases", such as inflammation, heart disease and cancer (7,27). Exploring the knowledge of medicinal plants and improving on it is therefore a useful tool to reduce health and health related challenges in our immediate locality (28).

Many studies have shown the content and species of phenolic compounds to antioxidant scavenging and reducing properties (29). However, emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low values of antioxidant indices in plants do not translate to poor medicinal properties (30). Mineral elements, other secondary plant metabolites not detected or evaluated, and vitamins may contribute to the synergy of phytochemicals that confer medicinal properties on plants (30).

The present qualitative study shows moderate presence of alkaloids, phlobatanins, reducing sugar, phenols, saponins, and amino acid, while there is high composition of tannins in SJE. This finding is similar to phytochemicals earlier reported in the stem bark of the plant (31). Several workers have reported on the analgesic properties of alkaloids (32, 33). Anticarcinogenic and antimutagenic as well as the anti-inflammatory and antibacterial properties had been attributed to tannins (34). Its anti-carcinogenic and antimutagenic potentials may be connected to its antioxidant properties which are important in protecting cellular oxidative damage including lipid peroxidation (35). These compounds have shown healing effect against various bacteria. It is not surprising afterwards that these plant extracts are traditionally used to cure bacterial diseases (36).

Isoquinoline alkaloids have vasodilation potentials and a variable progression of neurological effects from relaxation and euphoria to seizures (37, 38). Flavonoids have antibacterial, antiallergic, antiviral, antimutagenic, antineoplastic, anti-inflammatory, antithrombotic and vasodilatory potentials (39). The powerful ability of flavonoids in scavenging hydroxyl radicals, superoxide anions and peroxy-lipid radicals, may be their most important function (39). However, the result of the qualitative phytochemical analysis of *SJE* revealed the absence of flavonoid and flavonol derivatives in its root.

Saponin was found in the SJE analyzed. Studies have shown that saponins, although not toxic, can cause adverse physiological reactions such as growth impairment and reduction in food intake in animals that eat them (40). In addition, saponins reduce nutrient bioavailability and decrease enzyme activity (40). They exhibit cytotoxic and growth inhibitory effect against a variety of cell, making them anti-inflammatory and anti-cancer agents (40). In addition, they show tumor inhibitory activity (41), and possesses anti-hyperglycaemic and anti-diabetic potentials. The result of our findings is similar to an earlier report by Mbaka *et. al.,* (42) that *SJE* is rich in compounds responsible for anti-hyperglycaemic activities comparable to saponins.

Tannins were also detected in SJE. Presence of tannins has been implicated for free radical scavenging effects (43). Tannins are phenolic compounds reported to have primary free radical scavenging activities (44). The antioxidant properties earlier reported by Mbaka *et al*., (42) as well as Olorunnisola and Afolayan (11), may be due to the presence of tannins among other phytochemicals.

Phytochemicals in fruits and vegetables have been demonstrated to have complementary and overlapping mechanism(s) of action such as modulation of detoxifying enzymes, stimulating the immune system, scavenging of oxidative agents, hormone metabolism and regulation of gene expression in cell proliferation and

programmed cell death (45). Pharmacological studies have also shown that many phytochemicals have extensive naturapeutic activities as seen in *Clerodendrum* genus (46). This may account for the widespread use of SJE in ethnomedicine and its acclaimed pharmaceutical importance in modern research.

Taking the benefit offered by the maximum unmatched capacity of GC capillary columns (47), gas chromatographic technique was used to isolate the various compounds in SJE. Octadec-9-enolic acid is unsaturated fatty acid present in several plants. It has been found to lower level of cholesterol in the blood and so lowers the risk of heart problem, lowers blood pressure, atherosclerosis and helps with cancer deterrence (48). N-hexacadecanoicacid have antioxidants, hypochloresterolemic, nematiside, pestiside, lubricant, antiandrogenic flavor, hemolytic properties (49). Oleicacid, hexadecanoic acid and otadecanoic acid are known to have potential antibacterial and antifungal activity (50). Polyunsaturated fatty acids (PUFA) such as omega-3 (n-3) and omega-6 (n-6) must be ingested because they are indispensable for various biological processes including growth, brain development, reproduction, and reduction in the risk of heart disease. However, animals cannot synthesize them (51, 52). 12-Octadecadienoic acid, ethyl ester, the most abundant compound found in the SJE is a family member of Octadecadienoic acid comprising of linoleic acid. Linoleic acid (LA), an omega-6 polyunsaturated fatty acid, is a colorless liquid at room temperature. A carboxylic acid with 18 carbon atoms and two double cis bonds (53). Kumar *et al* (54) attributed antimicrobial, anti-inflammatory, antioxidant, anti-histimic, hypocholesterolemic, hepatoprotective, antiarthritic, antieczemic and cancer preventive activities of some of the phytochemical compounds of flavonoids to the presence of palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecanoic acid), unsaturated fatty acid and linoleic acid (docosatetraenoic acid and octadecatrienoic acid). This could be the rationale behind the use of the plant as antimalaria in folkloric medicine and the report by Aboaba and Ekundayo (55), on its essential oil antibacterial activity against *B. subtilis* and *P. eruginosa.*

Linoleic acid ethyl ester and its oxidized ester 9,12-Octadecadienoic acid are said to have antiarthritic, nematicide, hepatoprotective, antiandrogenic, antiacne, insectifuge, antihistaminic, hypocholesterolemic and 5-alpha reductase inhibitory activities (56). Linoleic acid can be converted to conjugated linoleic acids (CLA) when the double bonds are replaced by alternate double and single bond through a microbial or chemical reaction (57). *Conjugated linoleic acids (CLAs) can be produced commercially by alkaline isomerization of LA-rich oils (*58). Since the time CFAs have been reported as having antioxidant, antitumor, immunomodulatory and serum lipid lowering ability (59), *interest in its health benefits has increased tremendously (*60). A number of case–control studies suggested that dietary intake or/and moderate plasma or adipose concentrations of linoleic acid are associated with a reduced risk of coronary heart disease (52, 61), and therefore possess relevant pharmacological activities in the prevention and treatment of atherosclerosis, hypertension, obesity, and cancer (62).

CONCLUSION

SJE contains free radical scavenging components and numerous phytochemicals that can be evaluated for their biological importance and possible drug development. Therefore, knowledge of the pharmacology and safety of the species is of paramount importance to adequately exploit its therapeutic potential and enhance its possible improvement and inclusion into the national health care scheme.

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Table 1: Mean Ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) of ethanol extract of ethanol root extract of SJE

Antioxidant assay	**SJE mean reducing antioxidant power (mg/ml)**	**Reference antioxidant**
(FRAP)	116.09± 0.178	Ascorbic acid
(CUPRAC)	24.44±0.740	Trolox

Values are represented as mean± SEM, n=5 replicate

Table 2: Phytochemical Screening of ethanol root extract of SJE

Phytochemicals	**Result**
Alkaloids	+ve
Phenols	++ve
Phlobatanins	+ve
Cardenolides	-ve
Anthraquinones	-ve
Saponins	++ve
Tannins	+++ve
Flavoniods	-ve
Flavonol Derivatives	-ve
Amino acid	++ve
Reducing sugars	+ve
Starch	-ve

Key:
-ve: Negative test result (absence of expected colour change, turbidity, flocculation and precipitation)
+ve: Trace
++ve: Weak positive test (very weak colour change, turbidity, flocculation and precipitation)
+++ve: Positive test (clear and noticeable colour change, turbidity, flocculation and precipitation)

Table 3: Phytochemical components identified by Gas Chromatography – Mass Spectroscopy (GC-MS) in SJE.

PK no	Retention time	% total	%Quality	Weight g/mol	formula	Identified compound
1	7.982	0.11	72	146.14	$C_6H_{10}O_4$	Ethyl hydrogen succinate
2	16.51	0.09	96	278.5	$C_{17}H_{30}OSi$	Phenol, 2,4-bis(1,1-dimethylethyl)
3	18.32	0.02	95	224.43	$C_{16}H_{32}$	1-Hexadecene
4	20.21	0.12	97	182.17	$C_9H_{10}O_4$	Benzaldehyde, 4-hydroxy-3,5-dimeth oxy-
5	22.1	0.09	86	180.20	$C_{10}H_{12}O_3$	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
6	23.01	0.03	98	252.5	$C_{18}H_{36}$	1-Octadecene
7	23.04	0.04	93	256.43	$C_{16}H_{32}O_2$	Tetradecanoic acid, ethyl ester
8	24.61	0.04	58	282.5	$C_{18}H_{34}O_2$	E-11-Hexadecenoic acid, ethyl ester
9	25.22	0.16	95	270.46	$C_{17}H_{34}O_2$	Pentadecanoic acid, ethyl ester
10	25.89	0.06	98	270.45	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
11	26.62	0.02	91	236.39	$C_{16}H_{28}O$	E,E-10,12-Hexadecadienal
12	26.78	0.56	99	282.46	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate
13	26.87	0.17	99	282.46	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate
14	27.51	14.28	98	284.5	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
15	27.73	0.05	95	256.43	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
16	28.83	0.65	93	296.49	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
17	29.32	2.03	95	298.51	$C_{19}H_{38}O_2$	Heptadecanoic acid, ethyl ester
18	29.45	0.41	99	296.48	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester
19	31.08	35.86	99	308.5	$C_{20}H_{36}O_2$	12-Octadecadienoic acid, ethyl ester
20	31.11	3.35	97	308.5	$C_{20}H_{36}O_2$	9,12-Octadecadienoic acid, ethyl ester
21	31.15	5.38	90	264.4	$C_{18}H_{32}O$	9,17-Octadecadienal, (Z)-
22	31.24	10.54	90	264.4	$C_{18}H_{32}O$	9,17-Octadecadienal, (Z)-
23	31.29	9.71	95	280.45	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
24	31.48	10.88	99	282.46	$C_{18}H_{34}O_2$	cis-Vaccenic acid
25	31.51	0.91	99	312.53	$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester
26	31.56	2.23	90	312.53	$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester
27	32.62	0.09	72	270.5	$C_{17}H_{34}O_2$	Cyclopropanedecanoic acid, 2-hexy-, methyl esterl
28	33.09	0.1	94	326.6	$C_{21}H_{42}O_2$	Nonadecanoic acid, ethyl ester
29	34.26	0.28	96	310.51	$C_{20}H_{38}O_2$	cis-11-Eicosenoic acid
30	34.64	1.05	60	324.5	$C_{21}H_{40}O_2$	11-Eicosenoic acid, methyl ester
31	35.14	0.24	87	340.6	$C_{22}H_{44}O_2$	Methyl 19-methyl-eicosanoate
32	35.85	0.1	49	279.5	$C_{19}H_{37}N$	Nonadecanenitrile
33	37.63	0.1	78	312.53	$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester
34	38.25	0.08	46	166.22	$C_{10}H_{14}O2$	11-Oxadispiro[4.0.4.1]undecan-1-on
	TOTAL	**99.83**				

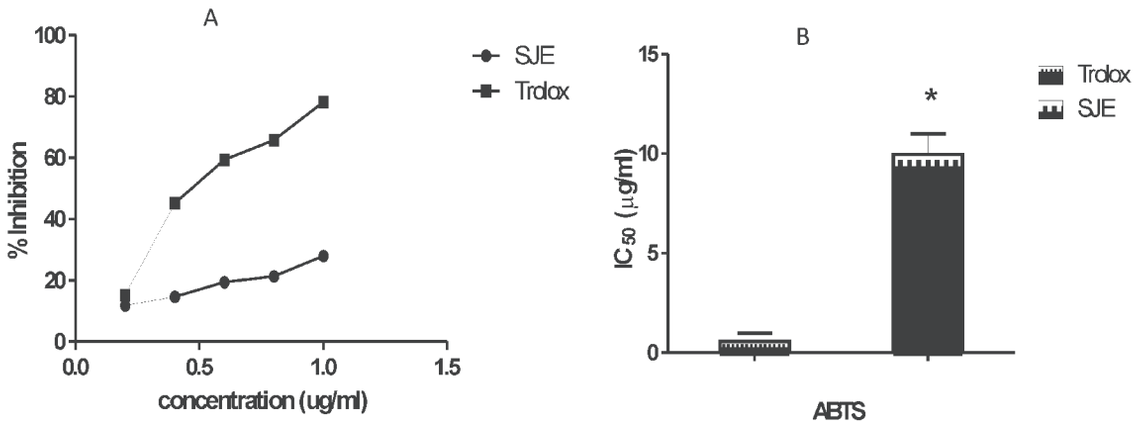


Figure 1: 2, 2'- Azinobis-(3-ethylbenzothiazoline 6 sulfonic acids) (ABTS)/Trolox equivalent antioxidant capacity (TEAC). *P<0.05 significant difference from Trolox.

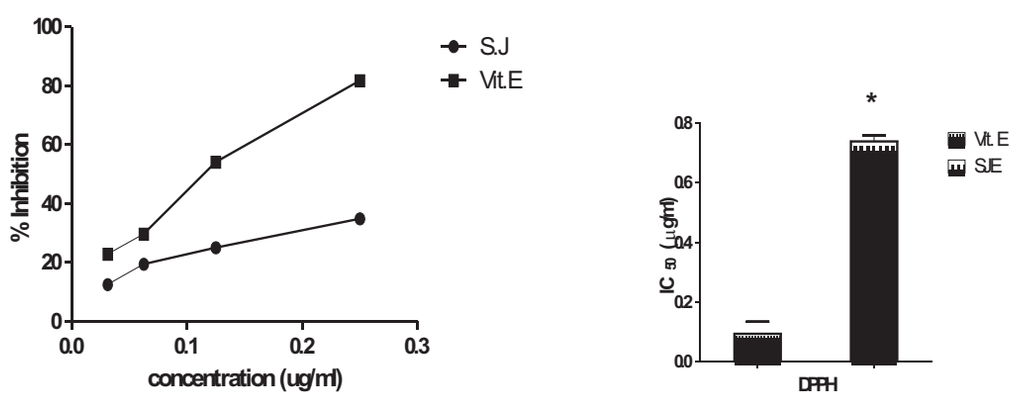
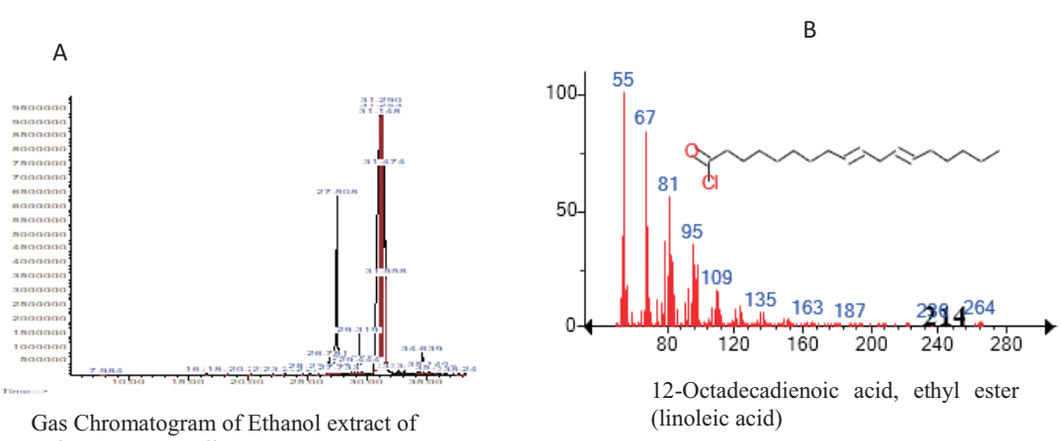


Figure 2: DPPH scavenging activity of SJE and Vit. E, Median Inhibitory concentration (IC_{50}) of SJE as compared to Vit. E. *P<0.05 significant difference from Vit. E



Gas Chromatogram of Ethanol extract of *Sphenocentrum jollyanum* root

12-Octadecadienoic acid, ethyl ester (linoleic acid)

Figure 3: Gas chromatogram A: SJE; B: linoleic acid