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Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of *n*-hexane and methylene chloride extracts of *Senna italica*

Hassan M.F. Madkour[1], Mosad A. Ghareeb[2*], Mohamed S. Abdel-Aziz[3], Omar M. Khalaf[1], Amal M. Saad[2], Ahmed K. El-Ziaty[1], Mamdouh Abdel-Mogib[4]

[1]Chemistry Department, Faculty of Science, Ain Shams University, El-Khalifa El-Mamoun, 11566 Abbassia, Cairo, Egypt. [2]Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornaish El-Nile, 12411 Warrak El-Hadar, Imbaba (P.O. 30), Giza, Egypt. [3]Microbial Chemistry Department, Genetic Engineering and Biotechnology Division, National Research Center, El Behoos Street 33, Dokki-Giza, Egypt. [4]Chemistry Department, Faculty of Science, Mansoura University, Mansoura-35516, Mansoura, Egypt.

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ABSTRACT

The current study was aimed to investigate the antimicrobial, anticancer, and antioxidant activities of the *n*-hexane and methylene chloride extracts from *Senna italica* as well as the characterization of their chemical constituents via gas chromatography-mass spectrometry analysis (GC-MS). The results revealed that the *n*-hexane extract showed weak antimicrobial activity with inhibition zones were ranged from 3.8 to 7mm, and the methylene chloride extract showed a potent antimicrobial activity with inhibition zones were ranged from 4.2 to 18mm compared to five standard antibiotics. Also, according to the criteria of the American National Cancer Institute (USNCI), the *n*-hexane showed weak anticancer activity with IC_{50} equal to 81.6±4.1, 73.0±3.90, 66.8±3.12, and 92.8±4.82 µg/ml, while the methylene chloride exhibited a potent anticancer activity with IC_{50} of 16.9±1.30, 17.4±1.36, 18.3±1.59, and 14.2±1.18µg/ml, compared to 5-fluorouracil with IC_{50} of 7.9±0.28, 4.8±0.21, 8.3±0.35, and 5.4±0.20, all respectively for HePG-2, Hela, PC3, and MCF-7 tumor cell lines. The activity against ABTS radical was recorded with the methylene chloride (% inhibition= 86.3%), compared to ascorbic acid with 89.2%. Moreover, GC-MS analysis revealed that the major constituents of the *n*-hexane were *n*-hexadecanoic acid (30%), (Z,Z,Z)9,12,15-octadecadienoic acid (21%), vitamin E (7.32%), and for methylene chloride were 3-methyl-4-oxopentanoic acid (16.36%), (E)-stilbene (11.86%), and 2,6-di-tert-butylphenol (10.70%).

INTRODUCTION

Senna L. (Fabaceae) is a widespread genus of flowering plants growing in the tropical and subtropical regions around the world *i.e.*, Latin & North America, Africa, Southeastern Asia, and Australia (Yagi *et al.*, 2013). *Senna* comprised nearly 350 species (Randell and Barlow, 1998; Yagi *et al.*, 2013). For a long time different species belonging to this genus are used traditionally to treat many ailments such as intestinal disorders, anti-tick, rheumatic, sexually transmitted diseases, laxative, and purgative (Al-Said 1993;Tshikalange *et al.*, 2005;Magano *et al.*, 2008;Hennebelle *et al.*, 2009;Masoko *et al.*, 2010). *Senna italica* is native to many regions in Africa. The plant has a great history in the folk medicine for the treatment of many diseases and pains (Al-Said, 1993; Maleho, 2015). The literature survey on the plant revealed that the previous chemical investigations on the different partsof *S. italica* led to the separation and characterization of certain phytochemicals *viz.*, physcion, chrysophanol, 10,10′-chrysophanol bianthrone, 3,4',5-trihydroxystilbene, 1,8-dihydroxy-3-methylanthraquinone and 1,2-benzenedicarboxylic acid (Magano *et al.*, 2008; Mokgotho *et al.*, 2013; Yagi *et al.*, 2013).

* *Corresponding Author*
Mosad A. Ghareeb, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornaish El-Nile, 12411 Warrak El-Hadar, Imbaba (P.O. 30), Giza, Egypt. Email: m.ghareeb @ tbri.gov.eg

From the biological activity point of view, different parts of the plants were reported for their vital biological activities *i.e.*, antioxidant (Jothi *et al.*, 2015), cytotoxicity (Kuete *et al.*, 2013), antibacterial (Masoko *et al.*, 2010; Dabai *et al.*, 2012), and and antiproliferative (Masoko *et al.*, 2010). Since, the microbial resistances against antibiotics is a great challenge and rapidly increase, so scientists search for a new trend aiming to identify naturally occurring antimicrobial agents from medicinal plants as alternative to the current antibiotics (Cowan, 1999; Ghareeb *et al.*, 2015). Cancer is the rapid and uncontrolled cells growth leading to death, and must be fixed by chemotherapy (Ghareeb *et al.*, 2013). The plant derived naturally occurring compounds are considered as good chemotherapeutic anticancer agents (Schwartsmann *et al.*, 2002). Therefore, the current study aims to investigate the chemical constituents of Senna italica aerial parts as well as the evaluation of their *in vitro* antimicrobial, anticancer, and antioxidant activities.

MATERIALS AND METHODS

Plant material

The aerial parts of *Senna italica* were collected from Alkharga Oasis, Alwady Algaded, Egyptduring March, 2015. The plant was identified by Prof. Dr. Ibrahim A. Mashaly, Professor of Plant Ecology and Flora, Botany Department, Faculty of Science, Mansoura University.

Extraction

The air dried powdered aerial parts of *S. italica* (500 g) were soaked in a mixture of organic solvents composed CH_2Cl_2/MeOH (1:1) for 72 hat room temperature and after filtration, the solvent was evaporated using rotatory evaporator, then the resulting crude extract was undergo further fractionation processusing different organic solvents *i.e.*,*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol.

Antimicrobial activity

The antimicrobial activity was evaluated by filter paper disc methods**(**Murray *et al.*, 1998; Sardari *et al.*, 1998**)**.Stock cultures of the test organisms were obtained from the microbiological Laboratory, Faculty of Medicine, Mansoura University. Bacteria test microbes used were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *E. coli*, *Bacillus subtilis* and *Erwenia carotovora*. Whereas the fungus used was; *Candida albicans.*

Anticancer activity (MTT assay)

The anticancer activity was evaluated according to the reported procedure (Mauceri *et al.*, 1998), using four human tumor cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), Human prostate cancer (PC3), and Epitheliod Carcinoma (Hela). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Briefly, MTT assay is a colorimetric technique is based on the change of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cells were seeds in a 96-well plate at a density of 1.0x104 cells/wellat 37 °C for 48 hrs under 5%CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 hr. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 hr. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

Antioxidant activity (ABTS assay)

The antioxidant activity was evaluated via ABTS method **(**El-Gazzar *et al.*, 2009**)**.Briefly,2 ml of ABTS solution (60 mM) was added to 3 M MnO_2 solution (25 mg/ml) all prepared in phosphate buffer (pH 7, 0.1 M). Then, the mixturewasn, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at 734 nm. Then, 50 ml of (2 mM) solution of the test sample in highly grade spectroscopic methanol/phosphate buffer (1:1) was added. The absorbance (A_{test})was measured and the decrease in color strength was expressed as % inhibition. The % inhibition for each sample is calculated according to the following equation:% Inhibition = $A_{control}$-A_{test}/$A_{control}$ x 100.Ascorbic acid was used as standard, and the blank sample contain methanol/phosphate buffer (1:1) instead of sample in absence of ABTS. While the negative control sample containmethanol/phosphate buffer (1:1).

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial potentials of the n-hexane and methylene chloride extracts of *Senna italica* were examined via disc diffusion assay, using twelve pathogenic microbial species. The results in Table 1 revealed that *n*-hexane extract showed weak to moderate antimicrobial effect against all the tested organisms with inhibition zones ranged from 3.8 to 7 mm. While the methylene chloride extract showed a remarkable effect against eight organisms compared to standard antibiotics *i.e.*, *Escherichia coli* (18mm/ ampicillin24mm), *Staphylococcus aureus* (15mm/ ampicillin 24mm), *Streptococcus pyogenes* (12mm/ ampicillin 20mm), *Candida albicans* (10mm/ clotrimazole 20mm), *Klebsiella pneumoniae* (9.2mm/ ampicillin 25), *Erwinia spp.* (9mm/ streptomycin 35mm), *Staphylococcus epidermis* (9mm/ ampicillin 24mm), and*Bacillus subtilis* (9mm/ kanamycin 20mm). At concentration 30 mg/ml the *n*-hexane extract of *Senna italica* leaf growing in Nigeria was tested as antibacterial agent, which showed a strong activity against five bacterial microorganisms namely;

Table 1: The inhibition zone (mm) and activity index% of *n*-hexane and methylene chloride extracts of *S. italica* compared to standard antibiotics.

Microorganism	Standard Antibiotic/ Inhibition zone (mm)	*n*-hexane		Methylene chloride	
		Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%
Klebsiella pneumoniae	Ampicillin/ 25	0	0	9.2	36.8
Shigella spp.	Streptomycin/ 14	3.8	27.1	4.2	30.0
Erwinia spp.	Streptomycin/ 35	0	0	9	25.7
Escherichia coli	Ampicillin/ 24	5.2	21.6	18	75
Enterobacter aerogenes	Kanamycin/ 20	0	0	7	35.0
Pseudomonas aeruginosa	Tobramycin/ 15	4.3	28.6	8.2	54.6
Proteus vulgaris	Ampicillin/ 18	0	0	5.4	30.0
Staphylococcus epidermis	Ampicillin/ 24	7	29.1	9	37.5
Streptococcus pyogenes	Ampicillin/ 20	0	0	12	60
Staphylococcus aureus	Ampicillin/ 24	5	20.8	15	62.5
Bacillus subtilis	Kanamycin/ 20	0	0	9	45.0
Candida albicans	Clotrimazole/ 20	7	35.0	10	50.0

Staphylococcus aureus, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, with inhibition zones of11.60, 11.60, 16.0, 12.60, and 14.0 mm respectively (Dabai *et al.*, 2012).Masoko et al (2010) reported that acetone extract of the roots of *Senna italica* growing in South Africa showed antibacterial activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus* with MICs ranging from 0.08 to 0.16 mg/ml(Masoko *et al.*, 2010).Also, another study revealed that the methanolic extract of *Cassia italica* leaves growing in Iraq showed a significant inhibition in growth of three pathogenic microbial starins, *E.coli*, *Staphylococcus aureus* and *Candida albicans*, exhibited inhibition zones of 15.35, 21.35, and 14.35 mm respectively at concentration 50 mg/ml (Al-Naimy *et al.*, 2010).Moreover, As four et al (2015) investigated the *n*-hexane, ethyl acetate, aqueous and total methanol extracts from *Cassia italica* aerial parts growing in Saudi Arabia exhibited antimicrobial activity. The *n*-hexane exhibited weak effect against two organisms *S. aureus* (6mm) at 500 mg/ml compared to Gentamycin (27mm/ 50 mg/ml) and *C. albicans* (5mm)compared to Clotrimazole (22mm50 mg/ml)(Asfour *et al.*, 2015).

Anticancer activity

The *n*-hexane and methylene chloride extracts from the aerial parts of *Senna italica* were evaluated as anticancer agents against four human tumor cell lines compared to 5-fluorouracil as standard. The results in Table 2 and Figures1 (a-c) revealed that the *n*-hexane showed a weak anticancer activity against HePG-2(IC_{50} 81.6 µg/ml),MCF-7 (IC_{50}, 92.8 µg/ml), PC3 ($IC_{50,}$ 66.8 µg/ml), and Hela (IC_{50}, 73.0 µg/ml) compared with 5-FU with IC_{50}values of 7.9, 5.4, 8.3, and 4.8 µg/ml respectively. On the other hand, the methylene chloride extract showed strong activity against HePG-2(IC_{50}, 16.9 µg/ml),MCF-7 ($IC_{50,}$ 14.2 µg/ml), PC3 ($IC_{50,}$ 18.3 µg/ml), and Hela ($IC_{50,}$ 17.4 µg/ml.5-FU exhibited IC_{50} of 7.9, 4.8, 8.3 and 5.4 µg/mlagainst HePG-2,MCF-7, PC3, and Hela respectively. According to the American Cancer Institute (USNCI), the curd extracts are considered to be potent anticancer agents when their IC_{50} values are less than 20 µg/ml (IC_{50}< 20 µg/ml) (Suffness and Pezzuto, 1990; Boik, 2001; Ghareeb *et al.*, 2014; Shoeb *et al.*, 2014).

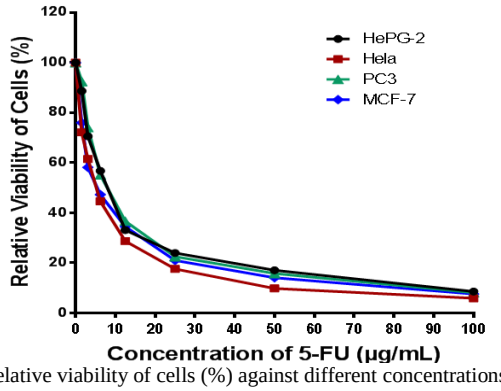


Fig. 1a: Relative viability of cells (%) against different concentrations 5-fluorouracil as standard.

Therefore, the*n*-hexane extract is considered to be weak anticancer, while the methylene chloride showed a potent anticancer activity against four tested tumor cell lines according to USNCI criteria. The acetone extract of *Senna italica* root inhibited proliferation and viability of Jurkat T cells (Masoko *et al.*, 2010). Kuete *et al* (2013) found that the 80% methanol extract of the aerial part from *Senna italica* growing in Saudi Arabia inhibited the growth of leukemia cancer cell lines CCRF-CEM by 53.57% and IC_{50} of 37.13 µg/ml, also inhibited the growth of HL60 cancer cell lines by 48.0% (Kuete *et al.*, 2013).Accordingly, the current findings confirmed the ability to use the plant as a source of naturally occurring anticancer agents.

Table 2: Anticancer activity of n-hexane and methylene chloride extracts of *S. italica* against human tumor cells compared to 5-fluorouracil as standard.

Samples	*In vitro* Cytotoxicity IC_{50} (µg/ml)[1]			
	HePG2	Hela	PC3	MCF-7
5-FU[2]	7.9±0.28	4.8±0.21	8.3±0.35	5.4±0.20
n-hexane-	81.6±4.15	73.0±3.90	66.8±3.12	92.8±4.82
Methylene chloride	16.9±1.30	17.4±1.36	18.3±1.59	14.2±1.18

[1]IC_{50} (µg/ml): 1-10 (very strong). 11-20 (strong). 21-50 (moderate). 51-100 (weak) and above 100 (non-cytotoxic). [2]5-FU = 5-fluorouracil.

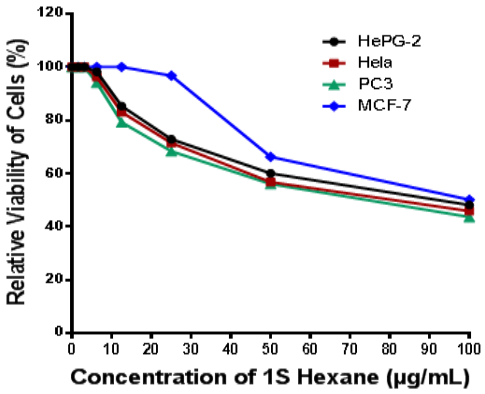


Fig. 1b: Relative viability of cells (%) against different concentrations of *n*-hexane extract.

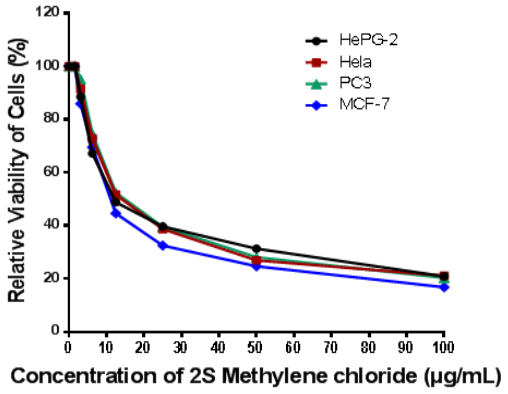


Fig. 1c: Relative viability of cells (%) against different concentrations of methylene chloride extract.

ABTS free radical scavenging antioxidant activity

The *in vitro* free radical antioxidant activity of the *n*-hexane and methylene chloride extracts was evaluated using ABTS assay. The results in Table 3 showed that the antioxidant activity (% inhibition) against ABTS radical was 36.5% and 86.3% respectively for the *n*-hexane and methylene chloride extracts, compared to ascorbic acid as standard with % inhibition of 89.2%.The results showed that methylene chloride extract had a potent antioxidant activity to scavenge ABTS radicals with nearly the same effect of ascorbic acid. There are limited previously published data about the ABTS radical scavenging activity of plant. For instance, Amutha *et al* (2014) studied the radical scavenging activity of ABTS of six solvent extracts of *Cassia senna* leaf growing in India. The inhibition percentages (%) were 92%, 90.6%, 93%, 92%, 90.8%, and 92% respectively for petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and aqueous extracts (Amutha *et al.*, 2014).Also, Jothi et al (2015) reported that the ABTS radical scavenging activity of different solvent extracts of the aerial part of *Senna italica i.e.*, (methanol, ethanol, petroleum ether, benzene, and ethyl acetate), with inhibition percentages 49.84%, 43.18%, 36.84%, 29.92%, and 40.18% at 100 µg/ml respectively for methanol, ethanol, petroleum ether, benzene, and ethyl acetate extracts(Jothi *et al.*, 2015).

Table 3: Antioxidant activity of *n*-hexane and methylene chloride extracts of *S. italica* using ABTS assay.

Sample	Absorbance of samples	% inhibition
n-hexane	0.324	36.5%
Methylene chloride	0.070	86.3%
Ascorbic acid	0.055	89.2%
Control of ABTS	0.510	0%

GC-MS investigations of n-hexane and methylene chloride extracts

The chemical constituents were identified by comparing their mass spectra with those of their analogous reported by NIST, Wiley9, Mainlib, Replib libraries and/or authentic spectra (Adams, 1995). The GC-MS analysis of the *n*-hexane extract of *S. italica* revealed the presence of forty-six compounds **(**Figure 2and Table 4), representing (93.69%) of the total composition. *n*-hexadecanoic acid (30%), (Z,Z,Z)9,12,15-octadecadienoic acid (21%), vitamin E (7.32%), turmerone (7.28%), phytol (4.66%), curlone (2.77%), squalene (2.57%), and hexadecanoic acid, methyl ester (1.94%)were identified as a major compounds in *n*-hexane extract. On the other hand, the GC-MS analysis of the methylene chloride extract revealed the presence of twenty-seven compounds (Figure 3 and Table 5), representing (95.44%) of the total composition. 3-methyl-4-oxopentanoic acid(16.36%), (E)-stilbene (11.86%), 2,6-di-tert-butylphenol (10.70%), 2-methoxy-4-vinylphenol (8.77%), N-ethylaniline (6.06%), N,N-dimethyl-1-phenylmethanamine(5.57%), 3-(1-methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one (5.13%), trans-sinapyl alcohol(4.68%), 1-nonadecene (4.18%), (E)5-eicosene (4.04%), 3-buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- (2.66%), and hexadecanoic acid methyl ester (2.30%)were identified as a major compounds in methylene chloride extract. Moreover, the chemical structures of major compounds identified in the *n*-hexane extract and some selected fragmentation pattern are shown in (Figure 6), *i.e.*, turmerone showed a base peak at m/z 83.20 (100%) corresponding to the fragment with molecular formula (C_5H_7O), hexadecanoic acid, methyl ester showed a base peak at m/z 74.20 (100%) corresponding to the fragment (dimethyl ester) with molecular formula ($C_3H_6O_2$), and hexadecanoic acid showed a base peak at m/z 73.20 (100%) corresponding to the fragment (propionic acid) with molecular formula ($C_3H_6O_2$). On the other hand, the chemical structures of major compounds identified in the methylene chloride extract and some selected fragmentation pattern are shown in (Figure 7),*i.e.*, N,N-dimethyl-1-phenylmethanamine showed a base peak at m/z 58.20 (100%) corresponding to the fragment (tri-methylamine) with molecular formula (C_3H_8N), 2-methoxy-4-vinylphenol showed a base peak (M.wt.) at m/z 150.10 (100%), (E)-stilbene showed a base peak (M.wt.) at m/z 180.20 (100%), and *trans*-sinapyl alcohol showed a base peak (M.wt.) at m/z 210.20 (100%) (Adams, 1995).

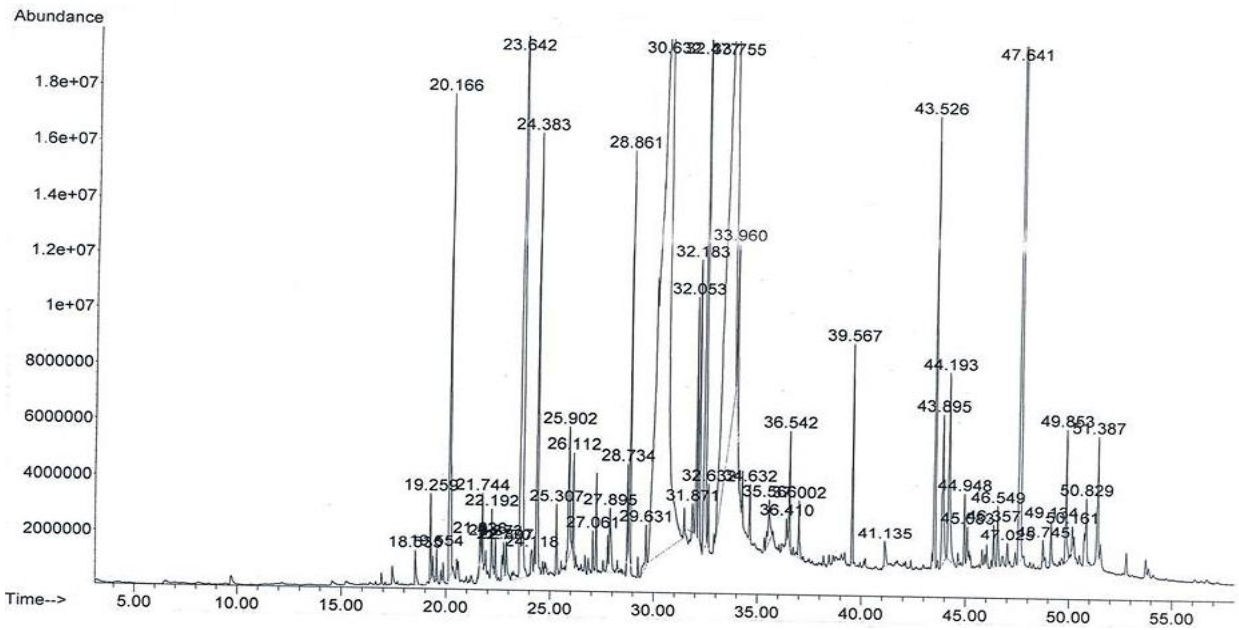


Fig. 2: Gas ion chromatogram of the of the *n*-hexane extract of *S. italica* aerial part.

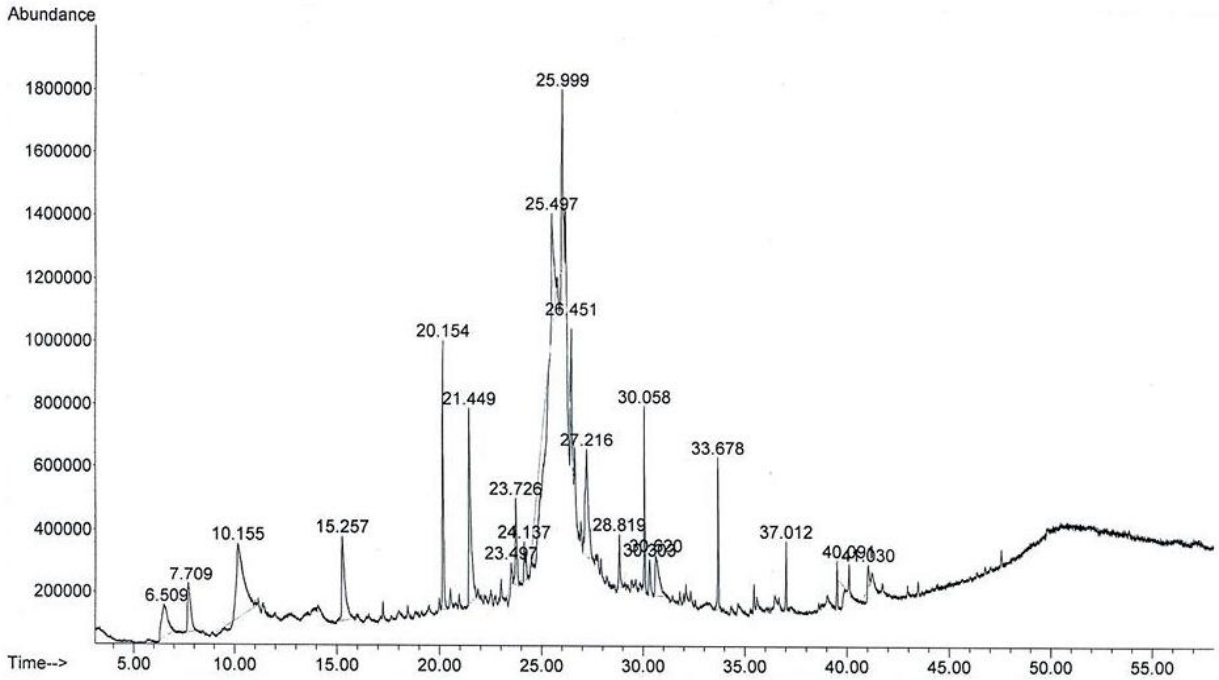
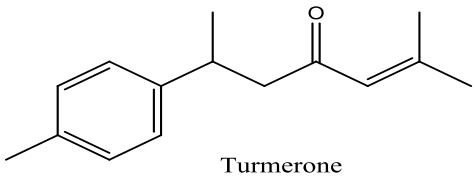


Fig. 3: Gas ion chromatogram of the methylene chloride extract of *S. italica* aerial part.

Reviewing the literature showed that, the GC-MS investigation of the *n*-hexane extract from *Senna italica* growing in Sudan led to identification of major components *viz.*, 2,6,-di-*sec*-butylphenol (36.69 %), di-*n*-octylphthalate (12.06%), eicosane (5.46%), tetratriacontane (4.87%), and 2,2'-methylenebis[6-(1,1-dimethyl)-4-methylphenol (4.18%)(Yagi *et al.*, 2013). Also, the GC-MS analysis of the methanol extract of the Indian species revealed the presence of Seventeen components, and the major constituents were identified as; 3-*O*-methyl-D-glucose (48.37%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (16.30%), *n*-hexadecanoic acid (11.70%), 1-butanol, 3-methyl, formate (4.38%), squalene (2.92%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (2.37%), ricinoleic acid (2.26%), phytol (1.64%), 11-dodecenoic acid, 10-hydroxy-, methyl ester (1.46%), and 9,12-octadecadienoic acid (Z,Z)- (1.47%) (Sermakkani and Thangapandian, 2012).



Turmerone

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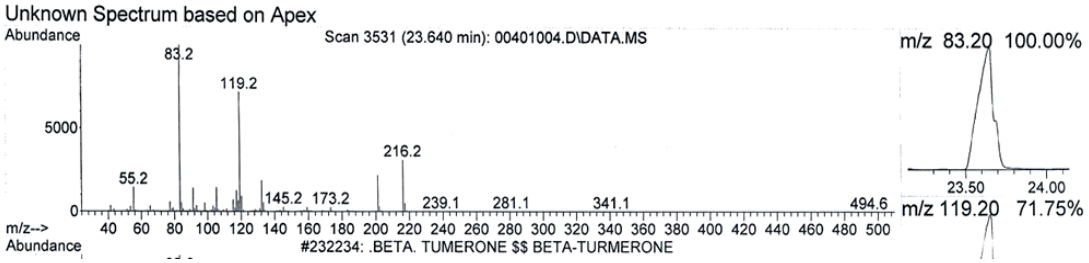

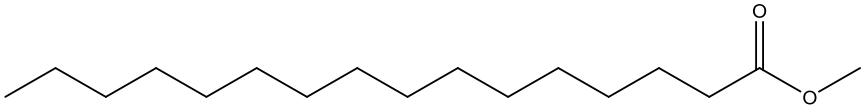

Hexadecanoic acid, methyl ester

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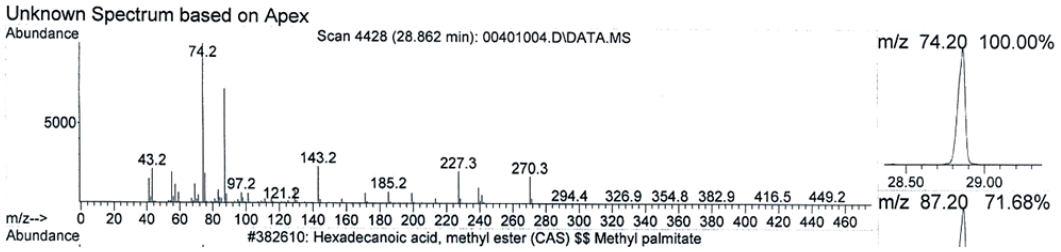

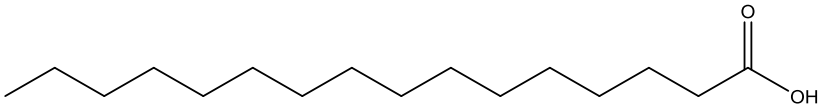

n-Hexadecanoic acid

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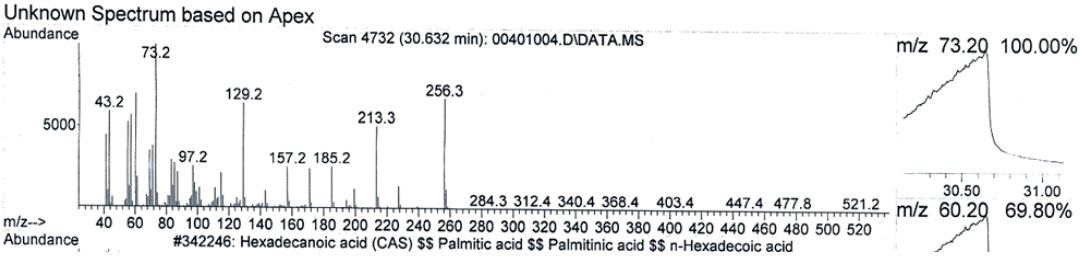
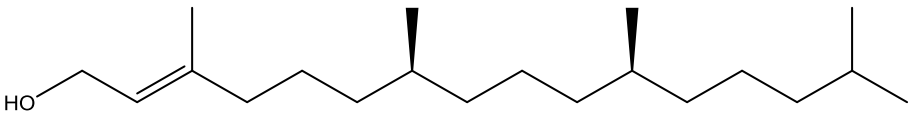




Phytol

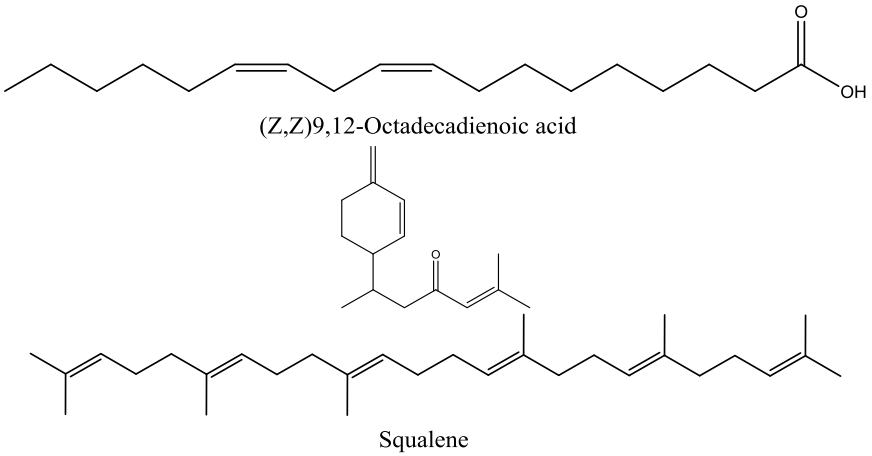
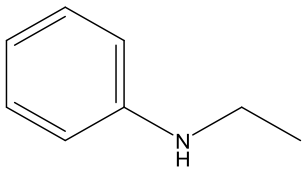


Fig. 6: Major compounds identified in the *n*-hexane extract

Table 4: The compounds identified in the n-hexane extract of S. italica aerial part by GC/MS analyses.

R.T	No.Compound	Name	Area%	M.F.	m/z
18.534	1	(E)-6,10-dimethylundeca-5,9-dien-2-one	0.23	$C_{13}H_{22}O$	194
19.261	2	curcumene	0.36	$C_{15}H_{22}$	202
19.552	3	(-)-zingiberene	0.09	$C_{15}H_{24}$	204
21.637	4	2,4-dimethylbenzyl 2,5-dimethylbenzoate	0.05	$C_{18}H_{20}O_2$	268
21.742	5	cetene	0.25	$C_{16}H_{32}$	224
22.190	6	pyridine, 5-ethenyl-2-methyl	0.37	C_8H_9N	119
22.370	7	α-cedrene	0.17	$C_{15}H_{24}$	204
22.472	8	β-cedrene	0.19	$C_{15}H_{24}$	204
22.772	9	zingiberenol	0.29	$C_{15}H_{26}O$	222
22.894	10	N,N-dimethylaniline	0.23	$C_8H_{11}N$	121
23.640	11	turmerone	7.28	$C_{15}H_{20}O$	216
24.117	12	1,4-dimethyl-2-(3,7-dimethyloctyl)benzene	0.22	$C_{18}H_{30}$	246
24.385	13	curlone	2.77	$C_{15}H_{20}O$	218
25.305	14	(6R,7R)-bisabolone	0.25	$C_{15}H_{24}O$	220
25.905	15	verbenone	0.97	$C_{10}H_{14}O$	150
26.114	16	1-octadecene	0.43	$C_{18}H_{36}$	252
27.063	17	neophytadiene	0.17	$C_{20}H_{38}$	278
27.896	18	benzoic acid, 2-hydroxy-, phenylmethyl ester	0.78	$C_{14}H_{12}O$	228
28.734	19	farnesyl acetone	0.32	$C_{18}H_{30}O$	262
28.862	20	hexadecanoic acid, methyl ester	1.94	$C_{17}H_{34}O_2$	270
30.632	21	*n*-hexadecanoic acid	30.0	$C_{16}H_{32}O_2$	256
31.872	22	heptadecanoic acid	0.12	$C_{17}H_{34}O_2$	270
32.053	23	(Z,Z)8,11-octadecadienoic acid methyl ester	1.20	$C_{19}H_{34}O_2$	294
32.181	24	(Z,Z,Z)9,12,15-Octadecatrienoic acid methyl ester	1.66	$C_{19}H_{34}O_2$	294
32.478	25	phytol	4.66	$C_{19}H_{32}O_2$	292
32.629	26	methyl stearate	0.25	$C_{20}H_{40}O$	296
33.753	27	(Z,Z)9,12-octadecadienoic acid	21.00	$C_{18}H_{32}O_2$	280
33.963	28	octadecanoic acid	0.77	$C_{18}H_{36}O_2$	284
34.632	29	methyl (2E,11E,13E)2,11,13-octadecatrienoate	1.26	$C_{19}H_{32}O_2$	292
35.564	30	(Z,Z,Z)9,12,15-octadecatrienoic acid	0.48	$C_{18}H_{30}O_2$	278
36.408	31	chrysophanic acid	0.14	$C_{15}H_{10}O_4$	254
36.452	32	4,8,12,16-tetramethylheptadecan-4-olide	0.54	$C_{21}H_{40}O_2$	324
37.002	33	geranylgeraniol	0.42	$C_{20}H_{34}O$	290
41.136	34	physcion	0.21	$C_{16}H_{12}O_5$	284
43.528	35	squalene	2.57	$C_{30}H_{50}$	410
44.949	36	solanesol	0.32	$C_{45}H_{74}O$	630
46.358	37	beta-Tocopherol	0.28	$C_{28}H_{48}O_2$	416
46.550	38	gamma-Tocopherol	0.37	$C_{28}H_{48}O_2$	416
47.028	39	fluorenone oxime	0.14	$C_{13}H_9N$	195
47.639	40	vitamin E	7.32	$C_{29}H_{50}O_2$	430
48.745	41	campesterol	0.04	$C_{28}H_{48}O$	400
49.135	42	stigmasterol	0.18	$C_{29}H_{48}O$	412
49.851	43	beta-Sitosterol	0.84	$C_{29}H_{50}O$	414
50.160	44	lupeol	0.11	$C_{30}H_{50}O$	426
50.830	45	dammaradienol	0.69	$C_{30}H_{50}O$	426
51.388	46	alpha-Tocopherol	0.76	$C_{29}H_{50}O_2$	430
		Total %	93.69		

R.T: Retention time. m/z[identity] (rel. abound. %). *M.F.: Molecular formula.*



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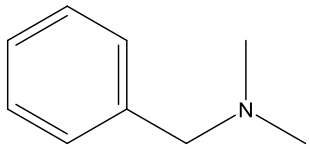

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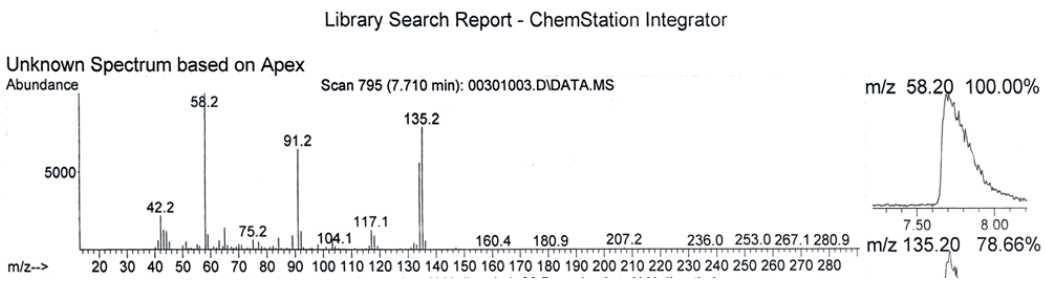

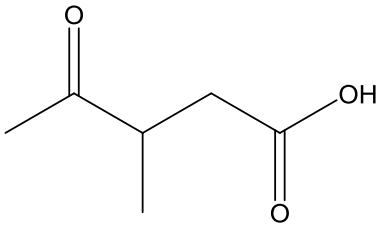

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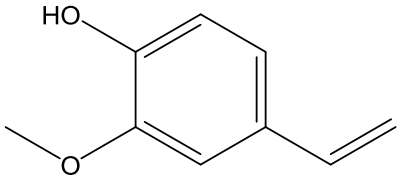
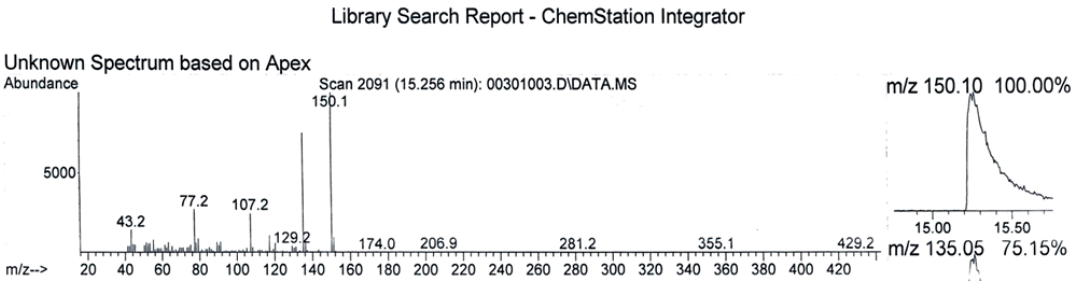
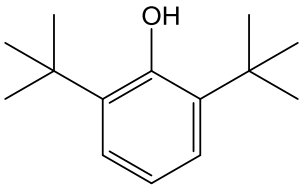


2-Methoxy-4-vinylphenol





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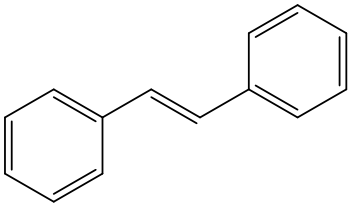
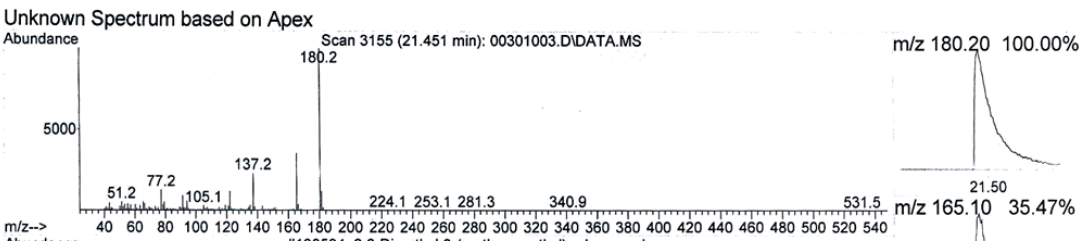


(E)-Stilbene



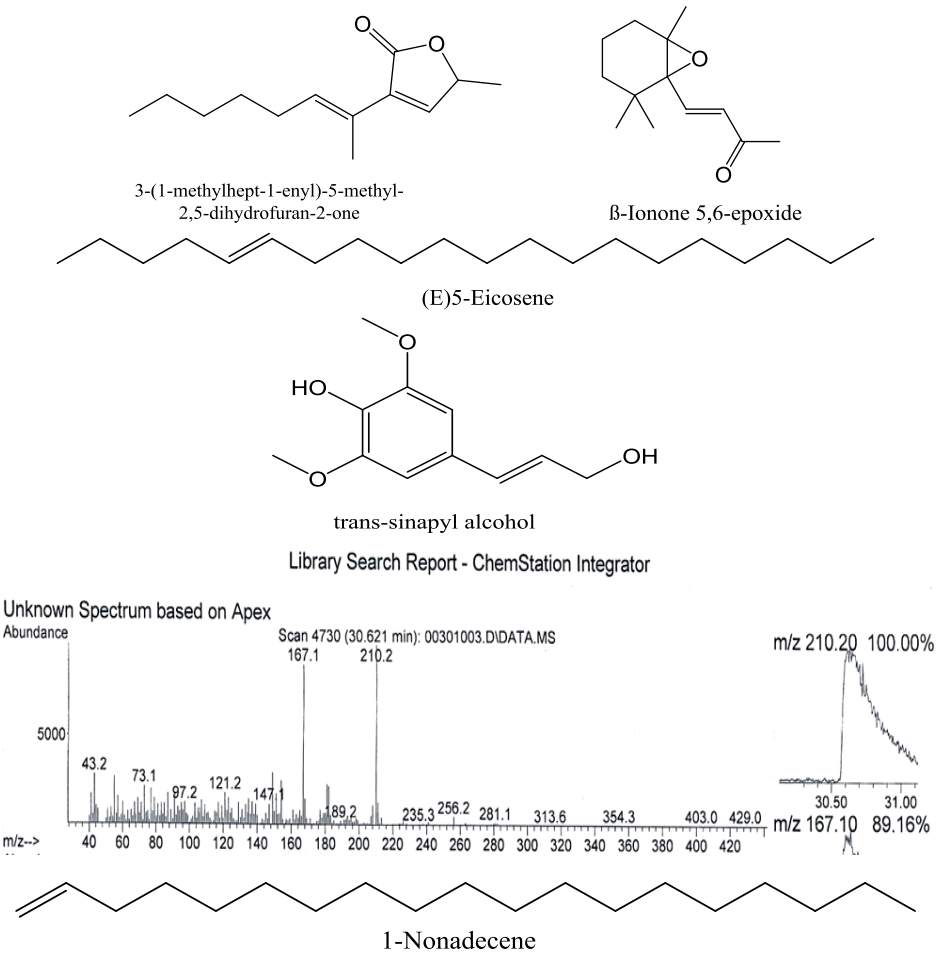


Fig. 7: Major compounds identified in the methylene chloride extract.

Table 5: The compounds identified in the methylene chloride extract of *S. italica* aerial part by GC/MS analyses.

R.T	Compound		Area%	M.F.	m/z
	No.	Name			
6.511	1	N-ethylaniline	6.06	$C_8H_{11}N$	121
7.710	2	N,N-dimethyl-1-phenylmethanamine	5.57	$C_9H_{13}N$	135
10.156	3	3-methyl-4-oxopentanoic acid	16.36	$C_6H_{10}O_3$	130
15.256	4	2-methoxy-4-vinylphenol	8.77	$C_9H_{10}O$	150
20.152	5	2,6-di-tert-butylphenol	10.70	$C_{14}H_{22}O$	206
21.451	6	(E)-stilbene	11.86	$C_{14}H_{12}$	180
23.500	7	anisyl propionate	0.32	$C_{11}H_{14}O_3$	194
23.550	8	1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.23	$C_{10}H_{16}$	136
23.727	9	3-(1-methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one	5.13	$C_{13}H_{20}O_2$	208
23.920	10	ß-Ionone 5,6-epoxide	0.62	$C_{13}H_{20}O_2$	208
24.135	11	3-buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	2.66	$C_{13}H_{20}O_3$	224
25.497	12	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	1.36	$C_{10}H_{12}O_3$	180
26.452	13	orcinol	0.22	$C_7H_8O_2$	124
28.816	14	hexadecanoic acid methyl ester	2.30	$C_{17}H_{34}O_2$	270
29.916	15	pentadecanoic acid 14-methyl-methyl ester	1.35	$C_{17}H_{34}O_2$	270
30.056	16	(E)5-eicosene	4.04	$C_{20}H_{40}$	280
30.106	17	1-octadecene	0.65	$C_{18}H_{36}$	252
30.621	18	trans-sinapyl alcohol	4.68	$C_{11}H_{14}O_4$	210
31.633	19	desaspidinol	1.38	$C_{11}H_{14}O_4$	210
33.677	20	1-nonadecene	4.18	$C_{19}H_{38}$	266
34.674	21	1-docosanol	1.34	$C_{22}H_{46}O$	326
36.670	22	1-tetracosanol	0.94	$C_{24}H_{50}O$	354
37.014	23	1-docosene	1.86	$C_{22}H_{44}$	308
40.093	24	9-nonadecene	0.23	$C_{19}H_{38}$	266
40.396	25	13-tetradecen-1-ol acetate	1.90	$C_{16}H_{30}O_2$	254
41.363	26	cycloeicosane	0.49	$C_{20}H_{40}$	280
42.031	27	8-methoxy-7-methyldibenzo[b,f]oxepine-1,6-diol	0.24	$C_{16}H_{14}O_4$	270
		Total %	95.44		

CONCLUSION

In the current study, the *n*-hexane and methylene chloride extracts of *Senna italic* aerial parts growing in Egypt were investigated for their antimicrobial, ABTS antioxidant and anticancer activities. The methylene chloride extract showed a noticeable activity than the *n*-hexane in the above mentioned assays. Furthermore, the two extracts were subjected to GC-MS analyses. *n*-hexadecanoic acid (30%), (Z,Z,Z)9,12,15-octadecadienoic acid (21%), vitamin E (7.32%), turmerone (7.28%), phytol (4.66%), curlone (2.77%) were recorded as a major compounds in *n*-hexane.Also,3-methyl-4-oxopentanoic acid(16.36%), (E)-stilbene (11.86%), 2,6-di-tert-butylphenol (10.70%), 2-methoxy-4-vinylphenol (8.77%), N-ethylaniline (6.06%), N,N-dimethyl-1-phenylmethanamine (5.57%), 3-(1-methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one (5.13%), trans-sinapyl alcohol(4.68%), 1-nonadecene (4.18%)were recognized as major compounds in methylene chloride extract. The identified compounds may be responsible for the antimicrobial, antioxidant and anticancer activities.

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Conflict of Interests: There are no conflicts of interest.

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