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# Antioxidant activity, acetylcholinesterase inhibitory potential and phytochemical analysis of *Sarcocephalus latifolius* Sm. bark used in traditional medicine in Sudan

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## Abstract

**Background:** *Sarcocephalus latifolius* is used as a traditional medicine for curing many diseases in Sudan. The main objective of the current study was to determine the antioxidant activity and acetylcholinesterase inhibition (AChEI) of *S. latifolius*, and to estimate its total phenolic and flavonoid contents.

**Methods:** Antioxidant activity of the tested plant extracts was carried out by determining their ability to scavenge the 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical. On the other hand, AChE inhibitory activity was determined spectrophotometrically using the Ellman's colorimetric method. The levels of total phenols and flavonoids were determined quantitatively using spectrophotometric methods. MTT assay was consumed to assess the cytotoxic effect of the most active fractions. These fractions were subjected to phytochemical analysis using GC-MS techniques to determine their chemical composition.

**Results:** Hexane and chloroform fractions exhibited the highest antioxidant activity with IC<sub>50</sub> values of (0.098 ± 0.08 and 0.099 ± 0.029 mg/ml) respectively. Standard propyl gallate had the lowest IC<sub>50</sub> value of 0.0414 ± 0.11 mg/ml. The ethanolic crude extract showed low AChEI activity with 40.2 ± 0.10%. High concentrations of phenolic and flavonoid contents were observed. GCMS revealed the presence of well-known antioxidants compounds e.g. Vitamin E and caffeic acid.

**Conclusion:** The ethanolic extract of bark of *S. latifolius* showed potent antioxidant effects and low AChEI activity, high phenolic and flavonoid contents and presence of pharmacologically active compounds. These findings explain its wide usages in traditional medicine.

**Keywords:** *S. latifolius*, Antioxidant, Acetylcholinesterase, Phenolics, Flavonoids, Gcms

## Background

Due to its high reactivity, Oxygen is capable of becoming part of potentially damaging molecules called reactive oxygen species (ROS) [1]. Humans have evolved a highly sophisticated antioxidant protective system, both endogenous and exogenous in origin [2]. Whenever the balance between ROS production and antioxidant effect is disturbed, 'oxidative stress' results leading to various pathological conditions [3]. Many researchers have

focused on antioxidant activity of phenolic compounds especially flavonoids and a positive correlation was observed [4–6]. ROS are playing a dangerous role in the pathogenesis of various diseases, including neurodegenerative disorders, cancer and atherosclerosis [7, 8]. Oxidative processes are the pathogens associated with the central nervous system in Alzheimer's disease (AD). The brain in particular is highly vulnerable to oxidative damage as it consumes about 20% of the body's total oxygen, with a high content of polyunsaturated fatty acids and lower levels of endogenous antioxidants [9, 10]. The brain of patients suffering from AD is said to be under oxidative stress. [11, 12]. AD is the

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most common neurodegenerative disorder, characterized clinically by progressive memory deficits and impaired cognitive function [13]. It is a major public health concern due to the increasing number of sufferers, placing strains on caregivers as well as on financial resources [14]. A deficiency in levels of the neurotransmitter acetylcholine (ACh) has been observed in the brains of AD patients, and inhibition of acetylcholinesterase (AChE), the key enzyme which hydrolyses ACh, is a major treatment option for AD [15]. Traditionally plants have been shown to be good options in the search for AChE inhibitors. Recently, several plants have been identified as containing AChEI activity [7]. In this respect, medicinal plants provide a rich source of biologically active constituents with multiple activities. *Sarcocephalus latifolius* Sm. (family: *Rubiaceae*), locally known as “Karmadoda”, have many uses in traditional medicine including malaria, dysentery, fever, hypertension and health promotion (antioxidant) [16–18].

## Methods

### Plant materials

Bark samples of *S. latifolius* were collected from South Kordfan state in February 2015. Identified and authenticated by Prof. Hatel H. Alkamali, Faculty of Science and Technology, Omdurman Islamic University, and confirmed by plant taxonomists at the herbarium of Medicinal and Aromatic Plants Research Institute, National Center for Research. Khartoum, Sudan.

### Extraction

The fresh samples were dried in shade for seven days, pulverized then used for extraction. Cold maceration methodology was carried out according to published method of Osama and Awdelkarim, 2015 [19].

### Fractionation

The crude extract was fractionated using liquid-liquid extraction methodology, which were carried by dissolving the samples in dist. H<sub>2</sub>O then partitioned between n-hexane, chloroform, ethyl acetate and n-butanol respectively using separating funnel apparatus.

### Qualitative phytochemical evaluation

Phytochemical screening was conducted to determine the presence of natural products in the fractions of selected plants using standard methods [20, 21].

### Determination of total phenolic content

Total phenolic content was determined by Folin Ciocalteu method [22]. Calibration curve was constructed using gallic acid standards Fig. 1 and the total phenolic

**Table 1** Preliminary screening of secondary metabolites in the fractions of *S. latifolius*

Family of compound	Type of test	interference				
		n-hexane	CHCl <sub>3</sub>	EtOAc	n-BuOH	H <sub>2</sub> O
Phenols	FeCl <sub>3</sub>	+v	+v	+v	+v	+v
Tannins	FeCl <sub>3</sub>	-v	+v	+v	+v	+v
Flavonoids	KOH	+v	+v	+v	+v	+v
	Alkaline	+v	+v	+v	+v	+v
	Lead acetate	+v	+v	+v	+v	+v
Quinones	H <sub>2</sub> SO <sub>4</sub>	-v	+v	+v	+v	+v
Alkaloids	Dragendorff's	+v	+v	+v	+v	+v
	Wagner's	+v	+v	+v	+v	+v
Triterpenes	Salkowski	+v	+v	+v	+v	+v
Diterpenes	Copper acetate	-v	-v	-v	-v	-v
Steroids	Salkowski	+v	+v	+v	+v	+v
Saponins	Forth	-v	+v	+v	-v	+v

+ve positive -ve negative

content was expressed as mg gallic acid equivalents (GAE)/g dry weight (DW).

### Determination of flavonoid content

The flavonoid content was measured by the aluminium chloride colorimetric assay [23]. Calibration curve was plotted using quercetin standards and flavonoid content was expressed as mg quercetin equivalents (QE)/g DW.

### GC-MS analysis

GC-MS analysis was carried out using GCMS instrument (Model GCMS-QP2010 Ultra, Shimadzu Co., Japan) equipped with a capillary column Rtx-5 (0.25 μm film × 0.25 mm i.d. × 30 m length). The instrument was operated in electron impact mode at ionization voltage (70 eV), injector temperature (250 °C), and detector temperature (280 °C). The carrier gas used was helium (99.9% purity) at a flow rate of 1.2 mL/min and about one μL of the sample was injected. The oven temperature was initially programmed at 110 °C (7 min) to 200 °C at 10 °C/min and from 200 to 280 °C at 5 °C/min with hold time 0 and

**Table 2** Yield percentages, Total phenolic and flavonoid contents of ethanolic extract and solvent fractions of *S. latifolius*

Sample	Extraction yield (w/w% of dry weight)	Phenolic content (mg /g GAE)	Total flavonoid (mg/g QE)
Crude	47.33	78.21 ± 2.4	91.36 ± 0.84
Hexane	6.18	98.78 ± 2.1	81.01 ± 0.012
CHCl <sub>3</sub>	1.64	71.49 ± 0.5	118.29 ± 0.21
EtOAc	3.09	86.12 ± 0.7	80.23 ± 0.03
BuOH	19.09	56.20 ± 1.23	94.32 ± 0.71
H <sub>2</sub> O	70.91	83.20 ± 3.7	40.22 ± 0.28

**Table 3** Antioxidant activity (%RSA and IC<sub>50</sub>) of solvent fractions of *S. latifolius*

Sample	% RSA	IC <sub>50</sub> (mg/ml)
Hexane	76 ± 0.02	0.098 ± 0.08
CHCl <sub>3</sub>	86 ± 0.03	0.099 ± 0.029
EtOAc	84 ± 0.03	0.148 ± 0.33
BuOH	83 ± 0.02	0.104 ± 0.19
H <sub>2</sub> O	79 ± 0.10	2.015 ± 0.3

9 min respectively. The identification of compounds from the spectral data was based on the available mass spectral records (NIST and WILEY libraries).

#### Antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The test samples were prepared in DMSO as 10× stocks from each test concentration (between 0 and 100 µg/ml) and briefly sonicated when necessary in an ultrasonic water bath. Solvents fractions producing radical scavenging activities equal to or higher than 50% at 100 µg/ml in a preliminary screen were further tested and IC<sub>50</sub> (concentration of the sample producing 50% scavenging of DPPH radicals) determined using EZ-Fit Enzyme Kinetic Program. propyl gallate was tested in the assay as positive control. The assay method used in the present study was based on Shimada et al., 1992 [24] method. The samples stock solutions (20 µL/well) were dispensed in triplicate onto 96-well plates. The assay was started with the addition of DPPH reagent (300 µM in ethanol, 180 µL/well). Appropriate blanks were prepared using the solvent only in addition to the same amount of DPPH reagent to get rid of any inherent solvent activity. Negative controls were also run in parallel to correct for any non-DPPH absorbance by coloured extracts at the test wavelength. The plate was immediately shaken for 30 s and incubated in the dark for 30 min at room temperature. The remaining DPPH was measured in the microplate reader at 517 nm. Percentage of radical scavenging activity (RSA) was calculated as following:

$$\%RSA = 100 - \{(Ac - At) / Ac\} \times 100$$

Where, At = Absorbance value of test compound;  
Ac = Absorbance value of control.

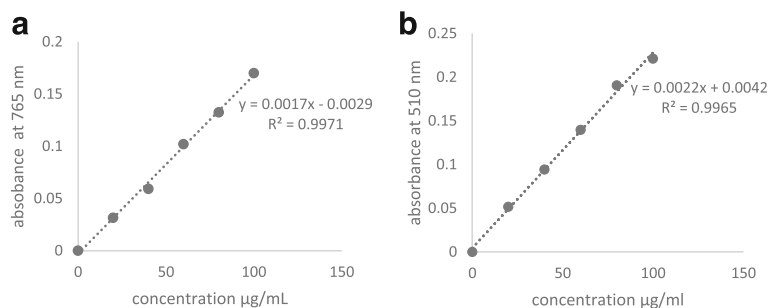
#### The multi-well plate AChE inhibition assay

The AChE inhibitory activity was tested using 96 well micro-plate assay based on Ellman et al., 1961 [25] method with minor modifications. Each extract (10 µL of 5 mg/mL in ethanol) was dispensed in triplicate onto 96 well microplate and mixed with 190 µL of Ellman's mixture containing 20 µL of enzyme, 140 µL to phosphate buffer, pH 8, containing 10 µL of 0.5 mM of 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, Germany) and 20 µL acetylthiocholine iodide (ATCI, Sigma-Aldrich, Germany). The control wells contained ethanol instead of the extract. The enzymatic activity was monitored at 412 nm every 30 s intervals for 3 min (linear reaction). The enzyme rate was calculated from the slope of the curve of absorbance vs time. As screening strategy, final concentration of 1000 µg/mL from each extract was examined and the average % inhibition was calculated relative to the enzyme rate at the control wells according to the following equation:

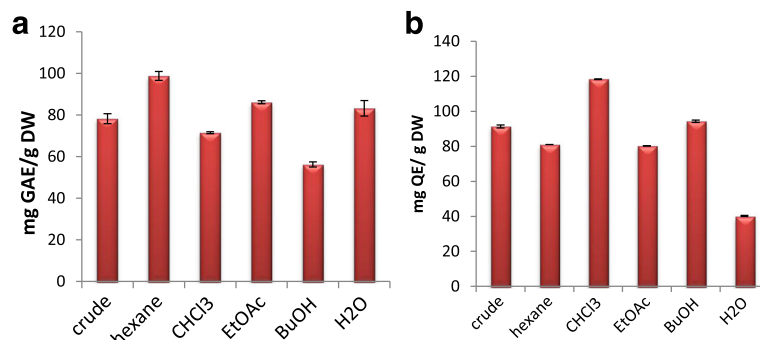
$$\%Inhibition = 100 - \{(Ac - At) / Ac\} \times 100$$

#### MTT (3-(4, 5-dimethylthazol-2-yl)-2, 5-diphenyl tetrazolium bromide) cytotoxicity assay

In the present investigation, Vero (normal, African green monkey kidney) cell line was used and cytotoxicity on these cells was assessed as described previously [26]. For each experiment, cultures were seeded from frozen stocks. Vero cells were maintained complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM). The cells were incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere and were in the logarithmic phase of growth at the time of the neutral red (NR) and tetrazolium (MTT) assays. Cells were harvested and seeded into 96-well tissue culture plates at a density of 1 × 10<sup>4</sup> cells per well of aliquots of medium (200 µL).



**Fig. 1** Standard curves of (a) Gallic acid and (b) Quercetin shows the absorbance against concentration



**Fig. 2** Comparison charts of (a) Total phenolic content. b Total flavonoid content of ethanolic extract and solvent fractions of *S. latifolius*

The cells were allowed to adhere to the wells for 24 h at 37 °C in a humid atmosphere optimized with 5% CO<sub>2</sub> in air. The next day, the plant fractions were added at the desired final concentrations and incubated for 72 h. All experiments were performed at least four times. Phosphate-buffered saline (PBS) was used as a negative. After the 72 h exposure period, the toxic endpoints were determined at 570 nm. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells that served as negative control.

#### Statistical analysis

All data were expressed as means  $\pm$  SD for three experiments. *P* values <0.05 were considered statistically significant. Statistical analyses were performed using Excel software (Microsoft 2010).

## Results and discussion

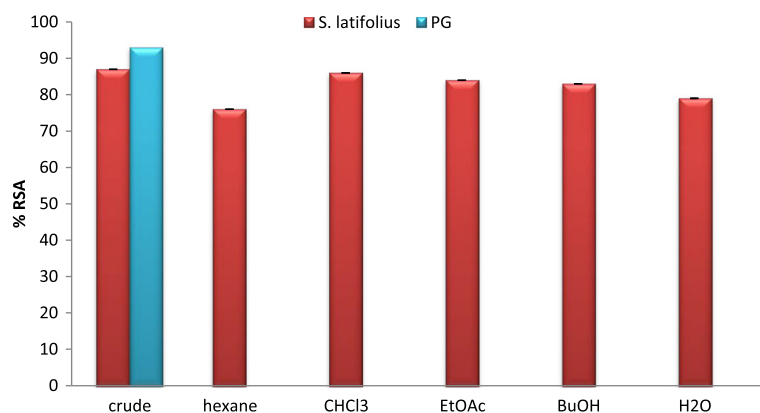
### Extraction yield and phytochemical screening

The percentage yield of *S. latifolius* ethanolic extract was 47.33% of dry weight. The polar fractions (water and butanol) showed the highest percentage yield (Table 2), this could be attributed to the polar nature of the crude extract obtained with polar solvent (ethanol).

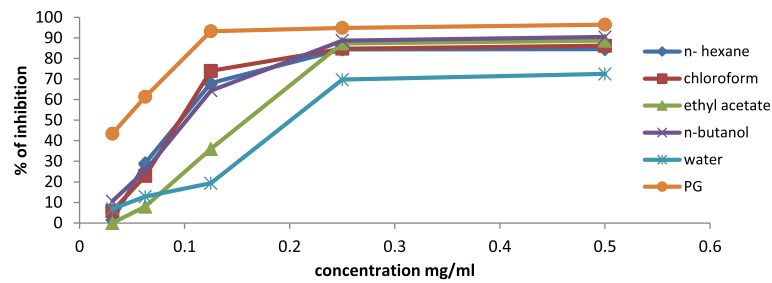
The results of preliminary investigation on the phytochemicals present in different solvent fractions are presented in Table 1. Different phytoconstituents such as phenolics, flavonoids, tannins, alkaloids, saponins, quinones, steroids, and terpenoids were detected in the tested fractions. The phytochemicals investigated in the present study are known to be beneficial in industrial and medicinal sciences [27]. Also, this preliminary knowledge can be looked at as a decipher in the search of a new source of economically valued chemical compounds [28, 29].

### Total phenolic and flavonoid contents

Crude natural extracts and compounds purified from these extracts can serve as better herbal drug sources owing to their fewer side effects and nutritional value [30]. As presented in Table 2, the ethanolic crude extract of *S. latifolius* bark exhibited total phenolic content of  $78.21 \pm 2.4$  mg GAE/g DW. Fig. 2 a similar study carried out using the methanolic leaf and root extracts of *S. latifolius*, has shown that the total phenolic content present was ( $0.016 \pm 0.03$  and  $0.036 \pm 0.05$  mg GAE/g DW) respectively, which is very low compared to the present study [31]. This



**Fig. 3** Percentage of RSA of *S. latifolius* ethanolic extract and solvent fractions



**Fig. 4** Comparison Chart of Antioxidant potentials of *S. latifolius* fractions with the decrease of concentration (IC50 evaluation)

high variation could be due to many reasons including, the part of plant under study, which contains different chemical composition with different percentage, the method, solvent used for extraction, the origin of plant samples, and the environmental factors. The

hexane fraction showed the highest phenolic content ( $98.78 \pm 2.1$  mg GAE/g DW), this result indicates the presence of high lipid soluble phenolic compounds such as vitamin E, whose existence was confirmed by GCMS analysis.

**Table 4** GC-MS spectral analysis of hexane fraction of *S. latifolius* bark

Peak no.	R. Time	Area %	Compound name	Molecular Formula	Mass
1	13.736	1.68	5,5-Dimethyl-1,5-oxasilonan-9-one	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub> Si	186
2	15.467	5.67	Quinic acid	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	192
3	19.188	0.29	Pentadecanoic acid, methyl ester	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
4	19.425	0.67	Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222
5	19.805	6.89	Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242
6	20.147	2.20	Palmitic acid ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
7	20.761	0.53	2-Cyclopropylideneadamantane	C <sub>13</sub> H <sub>18</sub>	174
8	22.455	16.49	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
9	22.694	3.23	Methyl linoleate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
10	22.765	1.64	Ethyl octadec-9-enoate	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310
11	22.814	0.48	Linolenic acid methyl ester	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292
12	23.122	3.91	Heptadecanoic acid, ethyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
13	25.920	14.21	Oleic acid amide	C <sub>18</sub> H <sub>35</sub> N O	281
14	25.994	0.23	Oleic acid amide	C <sub>18</sub> H <sub>35</sub> N O	281
15	26.187	0.44	Palmitic acid ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
16	26.265	0.60	Stearic acid amide	C <sub>18</sub> H <sub>37</sub> NO	283
17	26.509	0.43	2-Pentyl-2-nonenal	C <sub>14</sub> H <sub>26</sub> O	210
18	26.761	0.43	Oxirane, hexadecyl-	C <sub>18</sub> H <sub>36</sub> O	268
19	28.299	0.48	Octadecanal	C <sub>18</sub> H <sub>36</sub> O	268
20	28.665	9.23	Phthalic acid, mono-(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
21	29.192	0.97	Ethyl palmitate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
22	29.881	19.50	Cinchol	C <sub>29</sub> H <sub>50</sub> O	414
23	32.086	0.57	Ethyl docosanoate	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	368
24	32.642	1.64	Spinacene	C <sub>30</sub> H <sub>50</sub>	410
25	33.176	0.59	3-[[Trimethylsilyloxy]lanosta-8,24-diene	C <sub>33</sub> H <sub>56</sub> OSi	498
26	33.343	1.44	Lupenyl acetate	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468
27	35.907	3.46	Stigmast-4-en-3-one	C <sub>29</sub> H <sub>48</sub> O	412
28	38.248	1.09	Cholesteryl bromide	C <sub>27</sub> H <sub>45</sub> Br	448
29	38.974	1.01	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Higher concentrations of more bioactive flavonoid compounds were detected with 80% ethanol [32]. Therefore, ethanol was chosen for extraction. Plant phenolic compounds especially flavonoids are currently receiving greater interest due to their antioxidants potential [27, 33]. Aluminium chloride colorimetric assay yielded total flavonoid content of (91.36 ± 0.84 mg QE/g DW) for the crude extract. Highest flavonoid contents were observed in chloroform fraction (118.29 ± 0.21 mg QE/g DW), which indicate the high amount of less polar flavonoids (aglycones) such as isoflavones, flavanones, highly

methoxylated flavones, and flavonols [34]. These results could give a clue interpreting the observed high bioactivities of this plant.

#### Antioxidant and AChE inhibitory activities

Recently, interest has increased in naturally occurring antioxidants that can be used to protect human beings from oxidative stress damages [35, 36]. In the current study, the ethanolic crude extract exhibited high antioxidant activity with 87 ± 0.03%. The order of the activity (IC<sub>50</sub> mg/ml) was as follow: hexane (0.098 ± 0.08) > chloroform (0.099 ± 0.029) > butanol (0.104 ± 0.19) > ethyl acetate (0.148 ± 0.33) and eventually water fraction (2.015 ± 0.3), Table 3 and

**Table 5** GC-MS spectral analysis of chloroform fraction of *S. latifolius* bark

Number	R. Time	Area %	Compound name	Molecular Formula	Mass
1	13.735	2.97	5,5-Dimethyl-1-oxa-5-silacyclononanone-9	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub> Si	186
2	14.635	0.29	9-Eicosene, (E)-	C <sub>20</sub> H <sub>40</sub>	280
3	15.989	1.99	2H-Pyran-2-one, 5-ethylidenetetrahydro-4-(2-hydroxyethyl)-	C <sub>9</sub> H <sub>14</sub> O <sub>3</sub>	170
4	17.029	0.85	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	180
5	17.334	0.35	1-Heptadecene	C <sub>17</sub> H <sub>34</sub>	238
6	18.302	0.84	p-Hydroxycinnamic acid, ethyl ester	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>	192
7	19.187	0.35	9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]-	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354
8	19.361	0.18	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	276
9	19.422	0.15	alpha-D-Xylofuranose, 1,2-O-isopropylidene-5-(t-butyl dimethylsilyl)-	C <sub>14</sub> H <sub>28</sub> O <sub>5</sub> Si	304
10	19.800	5.89	Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242
11	20.115	0.30	9-Tricosene, (Z)-	C <sub>23</sub> H <sub>46</sub>	322
12	20.360	7.84	2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy-	C <sub>10</sub> H <sub>8</sub> O <sub>4</sub>	192
13	20.761	0.62	Bicyclo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene-	C <sub>14</sub> H <sub>20</sub>	188
14	21.139	0.22	Trimethylsilyl 3-methoxy-4-(trimethylsilyloxy)cinnamate	C <sub>16</sub> H <sub>26</sub> O <sub>4</sub> Si <sub>2</sub>	338
15	21.769	0.31	9-Octadecenoic acid (Z)-, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
16	21.996	25.46	2-Propenoic acid, 3-(3,4-dihydroxyphenyl)-	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180
17	22.450	9.78	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
18	22.762	3.28	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
19	23.104	1.19	1-Hexadecene	C <sub>16</sub> H <sub>32</sub>	224
20	23.740	0.61	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280
21	25.816	0.39	O O'-BIPHENOL, 4,4',6,6'-TETRA-T-BUTYL-	C <sub>28</sub> H <sub>42</sub> O <sub>2</sub>	410
22	25.909	1.08	9-Octadecenamamide, (Z)-	C <sub>18</sub> H <sub>35</sub> N O	281
23	26.155	0.93	1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266
24	28.301	0.19	Oxirane, heptadecyl-	C <sub>19</sub> H <sub>38</sub> O	282
25	28.662	1.17	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
26	29.163	1.28	1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266
27	29.732	1.15	gamma-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	414
28	32.053	1.83	1-Triacontanol	C <sub>24</sub> H <sub>50</sub> O	354
29	35.350	1.02	17-Pentatriacontene	C <sub>35</sub> H <sub>70</sub>	490
30	35.848	5.09	Methyl commate C	C <sub>31</sub> H <sub>50</sub> O <sub>4</sub>	486
31	38.123	22.40	Lup-20 (29)-en-3-ol, acetate, (3.beta.)-	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468



Figs. 3, and 4 illustrate these results. The activity of hexane and chloroform fractions ( $0.098 \pm 0.08$  and  $0.099 \pm 0.029$  mg/ml) respectively, is comparable values to the standard antioxidant PG ( $0.0414 \pm 0.11$  mg/ml); they also showed high amount of phenolic and flavonoid contents, it's possible that could be the reason of this high antioxidant potential.

Inhibition of AChE has been considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis, in which a deficit in cholinergic neurotransmission is involved [37, 38]. The side effects of anti-AChE drugs such as toxicity, tolerability, and loss of efficiency stimulates the researchers to screen alternative natural anti-AD drugs for medication switch [39]. Ethanolic extract displayed low AChE inhibitory activity with ( $40.2 \pm 0.10$ ). GCMS analysis of chloroform fraction indicates the presence of caffeic acid which has been reported to be a potent inhibitor of both AChE and BChE [40]. It is possible that the solvent used for extraction was not able to isolate the active ingredients with a proper amount.

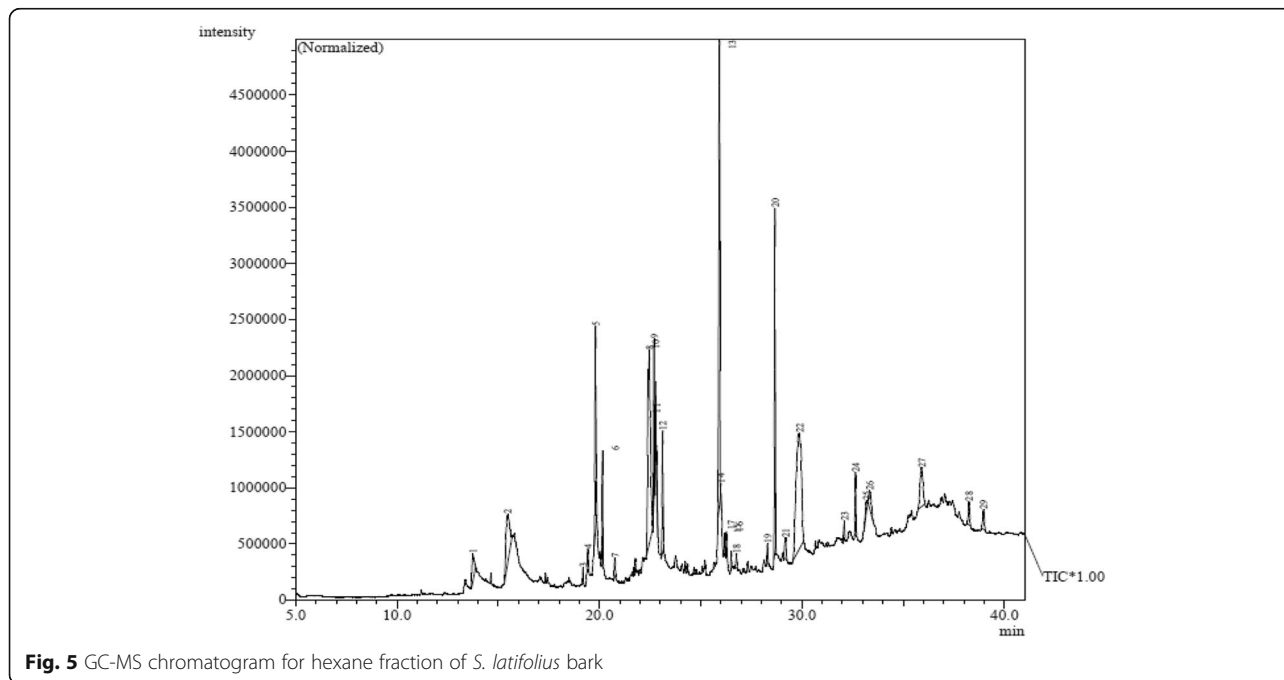
#### GCMS analysis

Due to their superior antioxidant activities hexane and chloroform fractions were analyzed with GC-MS, to identify their chemical composition which may be responsible of the measured activities. The GC-MS analysis lead to the identification of a number of compounds. These compounds were identified through

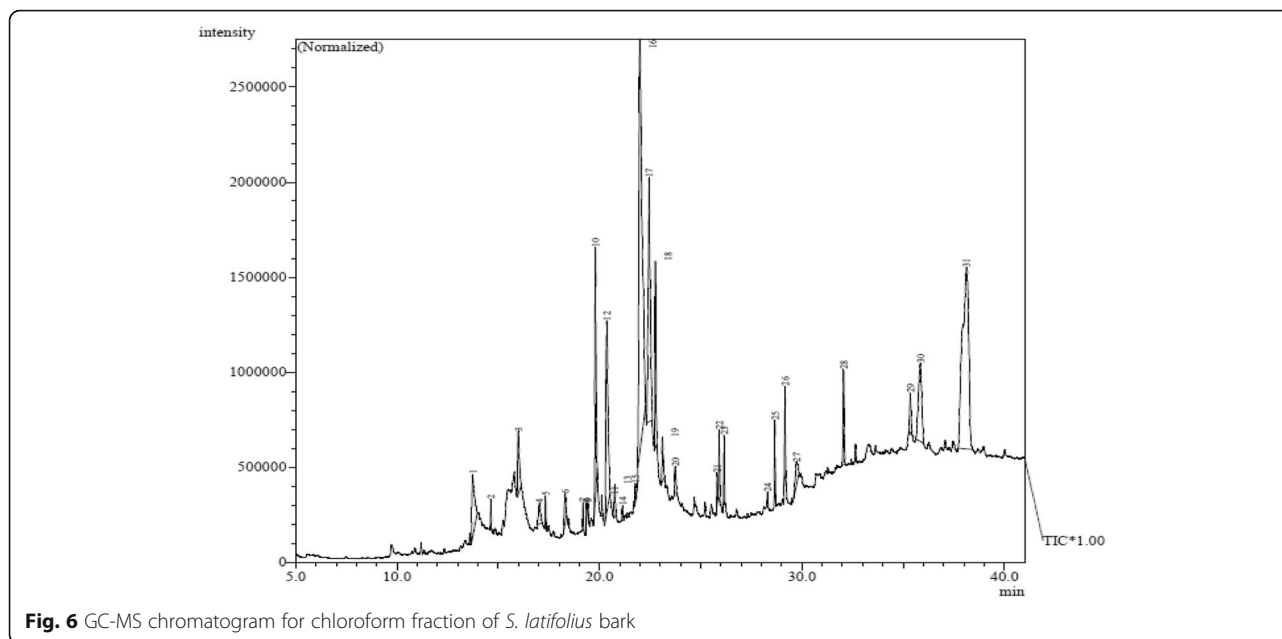
mass spectrometry attached with GC. Interpretation of mass spectrum was conducted using the database of National Institute Standard and Technology (NIST). The name, molecular weight and structure of the components of the test materials were ascertained, illustrated in Tables 4, and 5 and Figs. 5, and 6.

GC-MS spectrum of the hexane and chloroform fractions revealed the presence of 29 and 31 compounds respectively. Two phenolic compounds (Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate and Vitamin E) were observed in hexane fraction. Fat soluble vitamin E is one of the most active natural antioxidants, it is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. The supplemental intakes of this powerful antioxidant have been documented to be useful against cancer [41]. Vitamin E also acts in the prevention of free radical formation.

chloroform fraction declared the presence of five phenolic substances (4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; p-Hydroxycinnamic acid, ethyl ester; 2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy; Caffeic acid, O O'-biphenol, 4,4',6,6'-tetra-T-butyl) these phenolic compounds are known antioxidants, therefore the antioxidant potentials of this plant could be justified. However, further studies on the isolation, characterization, and biological evaluation of these identified compounds are necessary to confirm their potential benefits.



**Fig. 5** GC-MS chromatogram for hexane fraction of *S. latifolius* bark



**Fig. 6** GC-MS chromatogram for chloroform fraction of *S. latifolius* bark

### Cytotoxicity study

MTT assay revealed that, all tested fractions have no toxic effects on Vero cells with  $IC_{50}$  more than 200  $\mu\text{g/ml}$ , these results are shown in (Table 6).

The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance relying on the conversion of yellow MTT to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells [42]. Increasing concentrations of the tested fractions did not affect mitochondrial respiration as measured by the MTT cytotoxicity assay. However, the results of this assay measuring cell integrity showed that these solvent fractions are not toxic over this concentration range tested.

### Conclusion

*S. latifolius* is a Sudanese medicinal plants commonly used as herbal medicine for several purposes. In the present study, the selected plant was investigated in vitro for AChEI and antioxidant properties. In addition, the total phenolic and flavonoids contents were measured.

**Table 6** The cytotoxic effect (expressed as % inhibition and  $IC_{50}$  values) of hexane and chloroform fractions of *S. latifolius* tested at 125, 250 and 500  $\mu\text{g/ml}$  against Vero cells

Code of extract	Concentration ( $\mu\text{g/ml}$ )			$IC_{50}$ ( $\mu\text{g/ml}$ )	$IC_{50}$
	Inhibition % $\pm$ SD				
	500	250	125		
Hexane	55.3 $\pm$ 0.04	50.4 $\pm$ 0.02	40.9 $\pm$ 0.03	277.8	>100
Chloroform	67.6 $\pm$ 0.08	55.0 $\pm$ 0.01	35.9 $\pm$ 0.05	224.9	>100
Control	95.3 $\pm$ 0.00				<30

$IC_{50} < 30 \mu\text{g/ml}$ : High toxic. Control = triton was used as positive control at 0.2  $\mu\text{g/ml}$ . the maximum concentration used was 500  $\mu\text{g/ml}$

GC-MS analysis of *S. latifolius* (hexane and chloroform) fractions revealed the presence of well known antioxidant compound such as Vitamin E and Caffeic acid. According to these findings it could be suggested that *S. latifolius* (hexane and chloroform) fractions might be potent and safe antioxidant materials in medicine or food industry.

### Abbreviations

Abs: Absorbance; Ac: Absorbance value of Control; Ach: Acetylcholine; AChE: Acetylcholinesterase; AChEI: Acetylcholinesterase Inhibitory; AD: Alzheimer's Disease; At: Absorbance value of Test compound; BuOH: Butanol; DMSO: Dimethyl sulfoxide; DPPH: 2,2-diphenyl-1-picryl hydrazyl; DTNB: 5,5'-Dithiobis (2-nitro benzoic acid); EI: Electron Ionization; EtOAc: Ethyl Acetate; FBS: Fetal Bovine Serum; GAE: Gallic Acid Equivalent; GC: Gas Chromatography; GC-MS: Gas Chromatography- Mass Spectrometer; MEM: Minimal Essential Medium; MTT: Microculture tetrazolium; NIST: National Institute of Standards and Technology; PG: Propyl Gallate; QE: Quercetin Equivalent; RSA: Radical Scavenging Activity

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### Availability of data and materials

All data and materials used in this research are available from the corresponding author on reasonable request.

### Authors' contributions

AO Conduct the extraction, fractionation, phytochemical screening, total phenolic and total flavonoid content as well as the GCMS analysis and wrote the first draft. SA Supervised all the experimental work and data interpretation and also corrects the first draft. AA Conduct the antioxidant and anti- AChE inhibitory activities and the cytotoxicity experiment. All authors read and approved the final manuscript.



**Competing interests**

The authors declare that they have no competing interests.

**Ethics approval and consent to participate**

Not applicable.

**Declaration**

The experimental work described in this article was conducted in the Chemical Laboratories Complex, Department of Chemistry, Faculty of Sciences and Technology, Omdurman Islamic University, and Research Lab, Medical Biochemistry Department, Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan, from December 2014 to September 2015. These studies are the result of our own investigations, except where the work of others is acknowledged and have not been submitted in any other form to another journal.

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