Antioxidant activities and GC-MS analysis of essential oil extracted from *Salvia officinalis* L.

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**Abstract**

*Salvia officinalis* L., the common sage, is considered to be the most representative species among the Salvia species. The aim of the present study was to evaluate the antioxidant activity and identify the bioactive compounds by performing GC-MS analysis of essential oil extracted from *Salvia officinalis*. The oil was extracted by employing chloroform as solvent in Soxhlet apparatus. The phytochemical screening was carried out by standard methods which revealed the presence of important phyto constituents such as alkaloids, terpenoids, steroids, and phenolic compounds. The antioxidant assays such as DPPH· radical, ABTS·+ radical cation, OH· radical scavenging assays, Phoshomolybdenum reduction and Fe3+ reducing power assays were studied for the essential oil of *Salvia officinalis*. GC-MS analysis showed the presence of volatile compounds. The IC50 values DPPH· radical, ABTS·+ radical cation, OH· radical scavenging assays were found to be 10.47, 10.32 and 68.27 µg/mL concentration respectively. The results of this study portray the effective antioxidant activity of *S. officinalis*.

**Keywords:** *Salvia officinalis*, phytochemical screening, Antioxidant, GC-MS, Radical, DPPH

**Introduction**

Essential oils could be extracted from leaves, stems, flowers, roots, herbs, brushes, and trees via distillation. For many years, essential oil has been used for medicinal and healing purposes all over the world. The popularity of aromatherapy has lead to interest in essential oils that has increased in recent decades, which proves the beneficial effects of essential oil and other aromatic compounds. They are widely used in perfumes, cosmetics, soaps, cleaning products and other products and also for flavouring of foods and drinks[1-4].

The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal genera of the Lamiaceae family which is present throughout Mediterranean region, South-East Asia and Central America[5-7]. Some members of this genus are used as food spices to flavour meats and as flavouring agents in perfumery and cosmetics. The most common uses of sage are antioxidant, anti-inflammatory, antihydrotic, spasmyloytic, anticholinesterase, antiseptic, insecticide and also in the treatment of mental and nervous conditions. Salvia is a rich source of phytochemicals including phenolic acids, polyphenols, flavonoid glycosides, anthocyanins, sesquiterpenoids, diterpenoids, sesenterpenes and triterpenes[8-12]. *Salvia officinalis* is one considered to be one of the essential plant herb with an unique savoury and slightly peppery flavour. Sage leaf contains various phyto constituents such as flavones, flavonoid glycosides, cornsile, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic substances[13]. Solvent extraction is commonly used an alternative method to steam distillation but it involves issues leading to the destruction of thermally liable constituents due to application of high temperatures[14]. In order to overcome the disadvantages, Soxhlet Apparatus was utilized to extract the essential oil from the selected species which includes *Salvia officinalis* L. The antioxidant and anticancer activities have been studied to find out the potential of the active compounds for their medicinal use.
Materials and Methods
Collection of plant material
The plant species of *Salvia officinalis* was collected from the local store in Chennai, Tamil Nadu, India. The collected raw material was stored in a container for further use.

Preparation of essential oil
The essential oil was extracted from the plant material by utilising Soxhlet apparatus and employing suitable solvent system, (here), chloroform. The freshly prepared essential oil was stored in a container for later use.

Qualitative phytochemical screening procedure
The chloroform extract of *S. officinalis* was subjected to preliminary phytochemical screening using standard methods [15]. The extract was screened for different classes of phytoconstituents such as alkaloids, steroids, terpenoids, flavonoids and phenolic compounds using specific standard reagents [16, 17].

**In vitro antioxidant assays**

**DPPH** \(^{-}\) radical scavenging assay
The antioxidant activity of chloroform extract of *Salvia officinalis* was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical [18]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of essential oils. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

\[
\% \text{ of DPPH}^{-}\text{ radical inhibition} = \frac{\text{(Control} - \text{Sample)/ Control}} *100
\]

**ABTS** \(^{+}\) radical cation scavenging assay
The ABTS\(^{+}\) radical cation scavenging activity was carried out by the procedure described by Delgado-Andrade et al [19]. ABTS\(^{+}\) was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left undisturbed in the dark at room temperature for 12-16 hrs. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of methanol extract of varying concentrations (20-120 µg/mL) to 1 mL of diluted ABTS\(^{+}\) solution, the absorbance was measured after 10 min. The ABTS\(^{+}\) radical-scavenging activity of the samples was expressed as

\[
\% \text{ of ABTS}^{+}\text{ radical cation inhibition} = \frac{\text{(Control} - \text{Sample)/ Control}} *100
\]

**Hydroxyl (OH)\(^{-}\) scavenging activity**
The hydroxyl radical scavenging capacity of chloroform extract of *Salvia officinalis* was evaluated by the method described by Olabinri et al [20]. Various concentrations (20-120 µg/mL) of extract were added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). Initially, 0.5 mL of ascorbic acid (0.22%) was added and incubated at 80-90°C for 15 min in a water bath. After incubation, 1.0 mL of ice-cold TCA (17.5% w/v) was added in the end. Three mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone was added and made up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The absorbance was measured spectrophotically at 412 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated using the following formula:

\[
\% \text{ of OH}^{-} \text{ radical inhibition} = \frac{\text{(Control} – \text{Sample)/ Control}} *100
\]

**Phosphomolybdenum reduction assay**
The antioxidant capacity of the chloroform extract of *Salvia officinalis* was assessed as described by Prieto et al [21]. The essential oils with concentrations ranging from 20 to 120 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 90°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference.

**Ferric (Fe \(^{3+}\)) reducing power assay**
The reducing power of chloroform extract of *Salvia officinalis* was determined by slightly modified method of Chen, 1995 [22]. One mL of plant extract of different concentrations (10 - 60 µg/mL) was combined with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe (CN)₆] (1 mL, 1 %). The mixtures were then incubated at 50°C for 20 min. One mL of trichloracetic acid (10 %) was added to each mixture. Then to the mixture 1 mL of FeCl₃ (0.1 %) was added and the absorbance was measured at 700 nm using Spectrophotometer. Ascorbic acid was used as the standard reference.

**Thin layer chromatography**
Thin layer chromatography (TLC) was carried out for the chloroform extract of *Salvia officinalis* on Merck TLC aluminium sheets, silica gel 60 F254 (20 x 20 cm), preloaded plates [23, 24]. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. Development of a chromatogram was seen in a mixture of suitable solvent system. The spots were visualized with UV light at 356 nm. The Rₐ values of the coloured spots were recorded. The ratio in which distinct bands appeared was optimized and R十九届 values were calculated.

**Calculation of R十九届 value**

\[
\text{R十九届 value} = \frac{\text{Distance travelled by the solute}} {\text{Distance travelled by the solvent}}
\]

**Gas chromatography–Mass Spectrometry (GC–MS)**
The samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

**Identification of components**
The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the
interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Result and Discussion
Phytochemical screening
The phytochemical analysis was carried out according to the standard procedure. The results showed the presence of alkaloids, terpenoids, phenolic compounds and flavonoids.

Table 1: Qualitative analysis of chloroform extract of *Salvia officinalis*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical Constituents</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>1. Mayer’s test 2. Salkowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Terpenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Phenolic Compounds</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>NaOH test</td>
<td>+</td>
</tr>
</tbody>
</table>

DPPH\(^{-1}\) radical scavenging assay
The ability of chloroform extract of *Salvia officinalis* to scavenge free radicals formed was assessed using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH\(^{-1}\) radical scavenging activity was 75.37% at 30 μg/mL. Chloroform extract of *Salvia officinalis* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH\(^{-1}\) (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC\(_{50}\) value was found to be 10.47 μg/mL (Figure 2) and was compared with standard (Ascorbic acid, IC\(_{50}\) = 11.98 μg/mL concentration).

ABTS\(^{+}\) radical cation scavenging assay
ABTS\(^{+}\) is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum ABTS\(^{+}\) radical cation scavenging activity was 79.37% at 30 μg/mL concentration. The experiment demonstrated high antioxidant activity the IC\(_{50}\) of 10.32 μg/mL concentration (Figure 2) and was compared with standard Ascorbic acid (IC\(_{50}\) = 4.21 μg/mL concentration).

Hydroxyl (OH\(^{-}\)) radical scavenging activity
The hydroxyl radical is a highly reactive free radical formed in biological systems and reacts with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Scavenging of hydroxyl radical is an important antioxidant activity. The maximum hydroxyl radical scavenging activity was 73.82% at 30 μg/mL concentration. This experiment also showed reliable antioxidant activity with IC\(_{50}\) of 68.27 μg/mL concentration (Figure 2). The chloroform extract of *Salvia officinalis* significantly inhibited generation of OH\(^{-}\) radicals in a dose-dependent manner.

Phosphomolybdenum reduction assay activity
The total antioxidant activity of chloroform extract of *Salvia officinalis* was measured spectrophotometrically by phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum absorbance was 0.975 at 120 μg/mL concentration (Figure 3). It was compared with the standard (0.359) Ascorbic acid.

Ferric (Fe\(^{3+}\)) reducing power activity
The reducing power of Fe\(^{3+}\) to Fe\(^{2+}\) by the chloroform extract of *Salvia officinalis* was studied and showed reduction ability in a dose dependent manner (Figure 3). The maximum absorbance was 0.777 at 120 μg/mL and was compared with the standard (0.289) Ascorbic acid.

Table 2: DPPH\(^{-1}\) radical, ABTS\(^{+}\) radical cation and OH\(^{-}\) radical scavenging activities of chloroform extract of *Salvia officinalis*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/mL)</th>
<th>DPPH(^{-1}) Reduction Assay Absorbance @ 695 nm</th>
<th>ABTS(^{+}) Reducing Assay Absorbance @ 700 nm</th>
<th>Fe(^{3+}) Reducing Power Assay Absorbance @ 700 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.148±0.010</td>
<td>0.031±0.002</td>
<td>0.669±0.046</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.367±0.025</td>
<td>0.215±0.015</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>0.542±0.037</td>
<td>0.387±0.027</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.630±0.044</td>
<td>0.387±0.027</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>0.868±0.060</td>
<td>0.584±0.040</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0.949±0.066</td>
<td>0.584±0.040</td>
<td>0.031±0.002</td>
</tr>
</tbody>
</table>

Table 3: Phosphomolybdenum reduction and Fe\(^{3+}\) reducing power assays of chloroform extract of *Salvia officinalis*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/mL)</th>
<th>Phosphomolybdenum Reduction Assay Absorbance @ 695 nm</th>
<th>Fe(^{3+}) Reducing Power Assay Absorbance @ 700 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>34.6±2.4</td>
<td>44.3±3.12</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>47.7±3.3</td>
<td>48.4±3.39</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>58.9±4.12</td>
<td>53.6±3.75</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>65.3±4.57</td>
<td>70.3±4.92</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>71.4±2.99</td>
<td>78.8±5.52</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>75.3±5.27</td>
<td>79.3±5.55</td>
</tr>
</tbody>
</table>

Fig 2: Radical scavenging assay

Fig 3: Reduction Assay

Thin layer chromatography
Thin layer chromatography analysis was carried out in the
solvent system of Toluene: Ethyl Acetate with the ratio of 1:1. The separated compounds in TLC were showed in Figure 5.

**Fig 4:** Compounds separated by Thin Layer Chromatography

<table>
<thead>
<tr>
<th>Spot</th>
<th>( R_f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
</tr>
<tr>
<td>4</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>0.42</td>
</tr>
</tbody>
</table>

### GC-MS analysis

GC-MS analysis was carried out for the chloroform extract of *S. officinalis* and the eluted compounds were showed in Table 5. Phytol compound (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol) was eluted by GC-MS which is a potential antioxidant compound and could be one of the reasons for the antioxidant property of the extract.

**Table 5:** Active compounds identified in chloroform extract of *Salvia officinalis* by GC-MS analysis

<table>
<thead>
<tr>
<th>Structure</th>
<th>IUPAC name</th>
<th>Molecular weight G/mol</th>
<th>Molecular formula</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-</td>
<td>154.2493</td>
<td>C₁₀H₁₆O</td>
<td>14.17</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>8H-Azecino(5,4-b)indol-8-one, 5-ethylidene-1,2,3,4,5,6,7,9-octahydro-6-(2-hydroxyethyl)-3-methyl-(S-(E))</td>
<td>326.4374</td>
<td>C₂₀H₂₆N₂O₂</td>
<td>20.83</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>4H-Benz[de][1,3]benzodioxolo[5,6-g]quinoline,5,6,6a,7-tetrahydro-1,2,3-trimethoxy-6-methyl-[S]</td>
<td>369.16</td>
<td>C₂₁H₂₁NO₅</td>
<td>25.77</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Bicyclo[3.3.1]non-6-ene-2-carboxylic acid, ethyl ester</td>
<td>194.13</td>
<td>C₁₂H₁₆O₂</td>
<td>16.12</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Tricosan-2-ol</td>
<td>340.63</td>
<td>C₂₃H₄₀O</td>
<td>27.57</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>3,7,11,15-tetramethyl-2-hexadecen-1-ol</td>
<td>296.53</td>
<td>C₂₀H₃₄O</td>
<td>18.8</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>4H-1-Benzopyran-4-one, 5,7-dihydroxy-3-phenyl-</td>
<td>254.06</td>
<td>C₁₄H₁₀O₄</td>
<td>17.78</td>
</tr>
</tbody>
</table>
Reference