Phytochemical Examination, Compound Identification, and *In-vitro* Antioxidant Activity of Ethanolic Extract of *Shuteria involucrata*

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Abstract

The aim of the present work is to evaluate the phytochemical investigation, identification of chemical compounds and biological action of leaf extracts of *Shuteria involucrata*. The dry plant material was successively extracted with ethanol in soxhlet apparatus. The extracts were concentrated by rotary vacuum evaporator and were screened for major phytochemical compounds using established procedures. The GC-MS analysis of *S. involucrata* leaf was performed using Agilent 6890-JEOL GC-Mate-II Mass Spectrometer. The result of the study showed the presence of twenty seven bioactive compounds in the ethanolic extract. The radical scavenging potencies of the ethanolic extract was explored by employing DPPH assays, in which exhibited highest inhibitory effect on the radicals (IC50 = 59.92 µM).

Keywords: Phytochemical investigation, Soxhlet apparatus, *Shuteria involucrata*, GC-MS analysis, DPPH assays.

1. Introduction

Medicinal plants are believed to be with healing powers, and people have used them for many centuries to maintain health, as well as to prevent, diagnose, improve or treat physical sufferings all over the world. Herbal medicines are becoming popular in both developing and developed countries because of their natural origin and less side effects. In present time medicinal plants and their products are employed as home remedies as well as playing important role in the development of novel and useful drugs used in modern medicine.[1-4] Natural products have played a vital role in health care and prevention of life killing diseases. Now a days about 80% of the developed countries use traditional medicine, which has compounds derived from medicinal plants.[5]

Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological applications. Medicinal plants are expensive gift from nature to human. For thousands of years, natural products have played a vital role in health care and prevention of life killing diseases. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the phytochemical pathway.[6]

The present study revealed the qualitative phytochemical analysis, chemical compound identification and antioxidant activity of *S. involucrata* medicinal plant *Fabiaceae* family used by the peoples of Kolli hills, Namakkal district, Tamilnadu, India. It is well known for its ayurvedic medicine. All parts of the plant have medicinal properties.

2. Materials and Methods

2.1 Plant material

The plant material (leaves) was collected from the natural habits of Kolli hills, Namakkal District, Tamilnadu, India in February 2015. The plant was identified and authenticated by Dr.S. Susairaj, Associate Professor, Department of Botany, St Joseph's college, Tiruchirappalli, Tamilnadu, India.

2.2 Preparation of Plant Extracts

Collected plant material were washed under running tap water, then with distilled water and chopped into small pieces and air dried under shade at room temperature for fifteen to twenty days. The dried plant materials were pulverized into the powder form. An ethanol extract of the plant was prepared by hot continuous extracion method using soxhlet extractor. It was concentrated by using a rotary vaccum evaporator and subjected to dryness to yield crude residue. This residue was used for the investigation. The powdered materials were stored in air tight polythene bags until use.[7]

Fig. 2.1 Plant Powder

Fig. 2.2 Soxhlet Apparatus

2.3 Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of the crude ethanolic extract of *S. involucrata* was carried out according to the method described by J.B. Harborne.[8]

2.4 GC-MS analysis

GC-MS analysis was carried out in Bishop Heber College (HAIF), Tiruchirappalli. GC-MS analysis was performed by using JEOL GC MATE II (GC Model), quadruple double focusing detector. One microliter of extract was injected in split mode in injection port of GC column. The inlet temperature was set at 220 ^{0}C and oven temperature was programmed as 50 to 250 ^{0}C for 1min then 10 ^{0}C min^{-1}. Total run time was 60 min. Helium gas was used as the carrier gas at constant flow rate of 1.0 mL/min. The interface temperature (GC to MS) was set at 250 ^{0}C.

MS was set in scan mode. MS quad temperature was 250 ^{0}C, MS source temperature was 250 ^{0}C. Ions were obtained by electron ionization mode. Molecular ions (mass range) were monitored for identification which was set 50-600 m/z. Peak area denoted the relative percentage of constituents.[9]

2.5 Antioxidant Activity

The free radical scavenging activity of *Shuteria involucrata* leaves extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Diphenylpicrylhydrazyl) by the method of Brand-Williams *et al.*, 1995[10] at different concentrations (500, 400, 300, 200, 100 µg/ml). 2 ml of ethanolic solution of sample (extract/standard) was mixed with 3.0 ml of a DPPH methanolic solution (20 µg/ml). The mixture was kept in a dark place at room temperature for 30 min and later absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[11] The capability to scavenge the DPPH radical was calculated using the following equation:

% scavenging activity = (1- A test sample/A control) × 100.

Here, A stands for Absorbance.

The graph plotted with inhibition percentage against extract/standard concentration; extract concentration providing 50% inhibition (IC50) was calculated.

3. Result and Discussion

3.1 Phytochemical Screening Test

The phytochemical characteristics of *S. involucrata* tested are summarized in the table 4.1 The results revealed the presence of medically active compounds in the selected plant. From the table, it could be seen that, alkaloids, carbohydrates, coumerin, flavonoids, glycosides, lipids and fat, phenols, proteins, quinines, resin, saponin, starch, steroids, tannin, terpenoids, and vitamin C & ascorbic acid were present in ethanol extracts.

Table 4.1 Phytochemical Screening of Ethanolic Extract of *Shuteria involucrata*

S.No	Phytoconstituents	Ethanol
1	Alkaloids	+
2	Carbohydrates	+
3	Carotenoids	-
4	Coumerin	+
5	Essential oil	-
6	Flavonoids	+
7	Glycosides	+
8	Gum & Mucilage	-
9	Lipids & Fat	+
10	Phenols	+
11	Proteins	+
12	Quinines	+
13	Resin	+
14	Saponin	+
15	Starch	+
16	Steroids	+
17	Tannin	+
18	Terpenoids	+
19	Vitamin C & Asc. acid	+
20	Phytosterols	-

Note: '+' indicates presence and '–' indicates absence

3.2 Identification of Chemical Compounds (GCMS)

GC-MS analysis of ethanolic extract of *S. involucrata* identified twenty four different compounds. Which were listed in Table 4.2. GC-MS chromatograph ethanol extract showed different peaks, each peaks were indicating the different chemical compounds. The major compounds detected in ethanol extract were methyl phenyl(2-piperidinyl)acetate, 3-cyclohexene-1-methanol,alpha,alpha,4-trimethyl-acetate, coumarin, eugenol, 9H-Pyrido[3,4-b]indole, 1-methyl, Di-n-octyl phthalate, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), behenic alcohol, 4,4'-((p-Phenylene)diisopropylidene)diphenol and stigmast-5-en-3-ol respectively and others are minor compounds.[12]

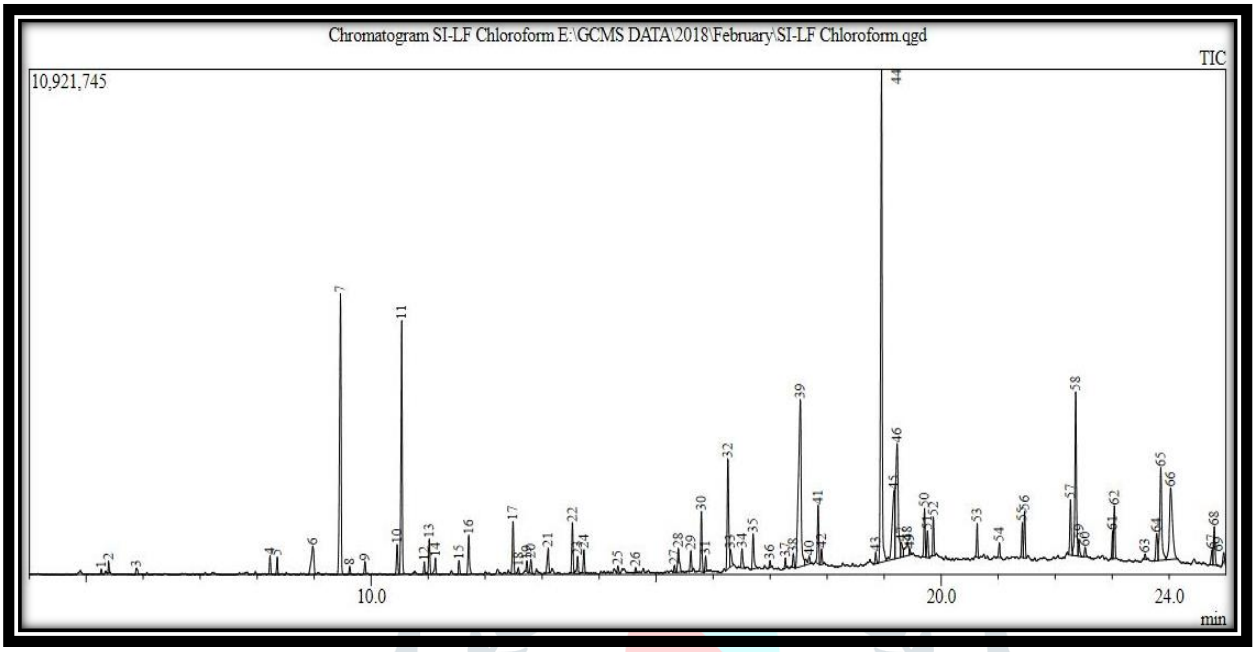
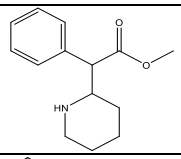
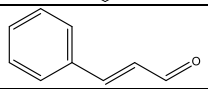
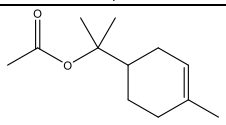
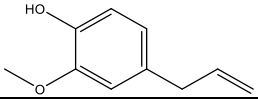
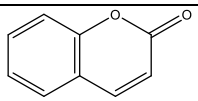
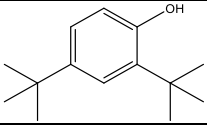
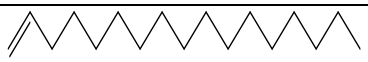
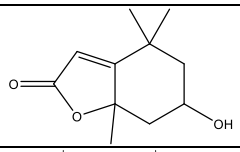
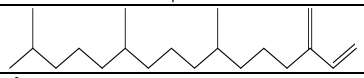
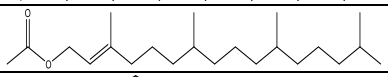
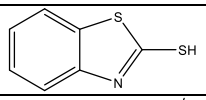
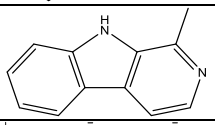
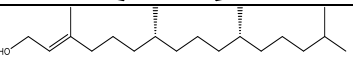
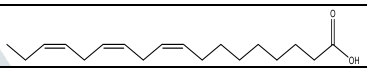
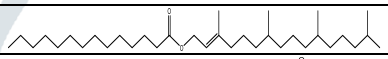
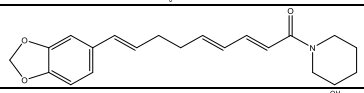
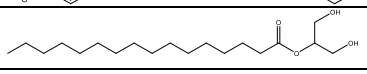
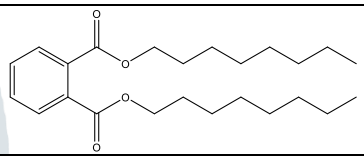
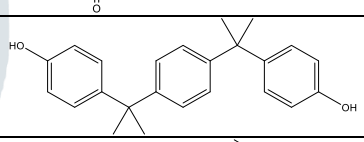
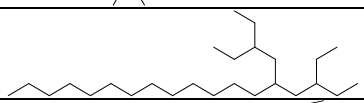
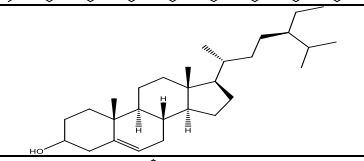
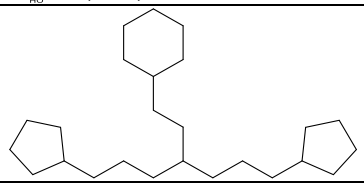


Fig 4.1 GCMS Spectrum of Ethanol extract of *S. involucrta*

Table 4.2 GCMS Analysis of Ethanol extract of *S. involucrta*

S.No	R.T	M.F	M.W	Name	Structure
1	8.975	$C_{14}H_{19}NO_2$	233	methyl phenyl(2-piperidinyl)acetate	
2	9.460	C_9H_8O	132	2-Propenal, 3-phenyl	
3	10.455	$C_{12}H_{20}O_2$	196	3-cyclohexene-1-methanol,alpha,alpha,4-trimethyl-acetate	
4	10.543	$C_{10}H_{12}O_2$	164	Eugenol	
5	11.020	$C_{14}H_{28}$	196	1-Tetradecene	
6	11.710	$C_9H_6O_2$	146	Coumarin	
7	12.485	$C_{14}H_{22}O$	206	2,4-ditert-butylphenol	

8	13.530	$C_{17}H_{34}$	238	1-Heptadecene	
9	15.610	$C_{11}H_{16}O_3$		6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	
10	16.255	$C_{20}H_{38}$	278	Neophytadiene	
11	16.705	$C_{22}H_{42}O_2$	338	Phytol acetate	
12	17.405	$C_7H_5NS_2$	167	2-Mercaptobenzothiazole	
13	17.690	$C_{12}H_{10}N_2$	182	9H-Pyrido[3,4-b]indole, 1-methyl	
14	18.955	$C_{20}H_{40}O$	296	2-hexadecen-1-OL, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	
15	19.225	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	
16	19.710	$C_{22}H_{46}O$	326	Behenic alcohol	
17	19.865	$C_{34}H_{66}O_2$	506	Phytyl tetradecanoate	
18	21.020	$C_{21}H_{25}NO_3$	339	(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)nona-2,4,8-trien-1-one	
19	22.360	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	
20	22.530	$C_{24}H_{38}O_4$	390	Di-n-octyl phthalate	
21	23.580	$C_{24}H_{26}O_2$	346	4,4'-((p-Phenylene)diisopropylidene)diphenol	
22	23.855	$C_{26}H_{54}$	366	octadecane, 3-ethyl-5-(2-ethylbutyl)	
23	24.030	$C_{29}H_{50}O$	414	stigmast-5-en-3-ol	
24	24.750	$C_{25}H_{46}$	346	Cyclohexane, [6-cyclopentyl-3-(3-cyclopentylpropyl)hexyl]	

3.3 Free Radical Scavenging Activity by DPPH Method

In this investigation, the crude ethanolic extract of *S. involucrta* leaves showed the free radical scavenging activity with IC50 (50% inhibition Concentration) value of 59.92 µg/ml respectively. The maximum inhibition was found as 82.49% and 79.64% and the minimum inhibition was found to as 59.61% & 69.18% respectively.

Table 4.2 Trends in the radical scavenging potential of the ethanol extracts at various concentrations.

Concentration	Test 1	Test 2	Mean	IC50
100	59.02	60.20	59.61	59.92
200	69.95	68.41	69.18	
300	76.15	77.85	77.00	
400	79.25	80.02	79.64	
500	81.69	83.29	82.49	

Since the present crude extract an exhibit good DNA binding ability, it was further considered to study the radical scavenging potencies of the ethanolic extract by their ability to stabilize the DPPH radicals. Fig. 4.2 shows the antioxidant activity of the test compounds against the selected free radicals.[13]

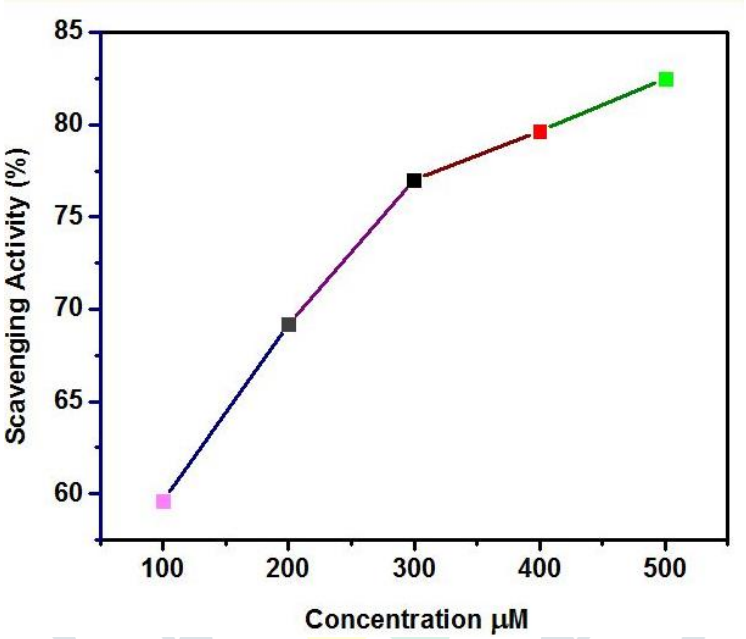


Fig 4.2 DPPH Radical Scavenging Activity of Ethanol Extract

4. Conclusion

The plant extracts were screened for major phytochemical compounds using established procedures. Around sixteen phytoconstituents like alkaloids, carbohydrates, coumerin, flavonoids, glycosides, lipids & fat, phenols, proteins, quinines, resin, saponin, starch, steroids, tannin, terpenoids, and vitamin C & ascorbic acid were present in an ethanol extracts. GC-MS analysis of *S. involucrata* identified twenty four different chemical (including major and minor) compounds to be presented. The ethanolic extract of *S. involucrta* leaves showed the free radical scavenging activity with IC50 value of 59.92 µg/ml respectively. The maximum inhibition was found as 82.49% and 79.64% respectively.

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