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ANTIOXIDANT STUDY OF ISOLATED CHEMICAL CONSTITUENTS FROM METHANOL EXTRACT OF THE CLERODENDRUM PHLOMIDIS LEAF

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ABSTRACT

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The main aim of the study was to isolate active antioxidant chemical constituent/s from methanol extract of the *Clerodendrum phlomidis* leaf. Methanol extract of the *Clerodendrum phlomidis* was subjected to isolation by column chromatography. Nine compounds were isolated from the methanol extract of the *Clerodendrum phlomidis* leaf and were characterized by IR (KBr), ^{1}H-Nuclear Magnetic Resonance (NMR), ^{13}C-Nuclear Magnetic Resonance and Mass spectrometry. Isolated compounds were subjected to antioxidant activity by DPPH free radical scavenging assay. Compounds 6 (3,6,7-trihydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one) and compound 9 (Isopropyl linoleate) showed good antioxidant activity with an IC_{50} value of 63.16 µg/mL and 61.13 µg/mL respectively. The compounds 3-hexen-1-yl benzoate**,** 2,3-dihydroxypropanal**,** 1-(2,4,5-trihydroxyphenyl)-1-butanone**,** 3-cyclohexen-1-ol, isopropyl linoleate, oleic acid eicosyl ester are reported for the first time from *Clerodendrum phlomidis* leaves.

KEYWORDS: Extraction, column chromatography, TLC, DPPH.

INTRODUCTION

Natural products once served as the source of all drugs. Natural products and their derivatives and analogs, still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing 25% of the total. In the past two centuries, the chemical investigation and purification of extracts of plants purported to have medicinal properties.[1] Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[2] The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds.[3]

Clerodendrum phlomidis is a small plant common in India and Sri Lanka. *Clerodendrum phlomidis* leaf extract possess various biological activity such as antifungal,[4] antioxidant,[5] antibacterial,[5] analgesic,[6] anti-asthmatic,[7] anti-diarrhoeal,[8] anti-inflammatory,[9] antimicrobial,[10] antiplasmodial,[11] hypoglycemic,[12] nematicidal,[13] psychopharmacological activity.[14] From the literature survey, the chemical constituents isolated so far was found to be a crystalline non-glucoside bitter principle ($C_{17}H_{16}O_6$), ceryl alcohol, β-sitosterol, γ-sitosterol, palmitic acid, cerotic acid and an unidentified sterol ($C_{28}H_{48}O$),[15] scutellarein (5,6,7,4′-tetrahydroxy flavones), pectolinaringenin (6,4′-dimethoxy scutellarein) and a flavanone,[16] a chemotaxonomic marker of the genus, (24S)-ethylcholesta-5,22,25-triene-3β-ol ($C_{29}H_{46}O$) was isolated from the leaf,[17] chalcone glycoside (4,2′,4′-trihydroxy-6′-methoxychalcone-4,4′α-D-diglucoside($C_{28}H_{34}O_{15}$), pectolinarigenin, 7-hydroxy flavone and 7-hydroxy flavanone-7-*o*-glucoside,[18] pectolinaringenin-7-*o*-β-D-glucopyranoside, 24β-ethycholesta-5, 22,E,25-triene-3β-o-β-D-glucopyranoside, (2S,3S,4R,10E)-2- [(2'R)-2'-hydroxytetracosanoylamino]-10-octadecene-1,3,4-triol,. andrographolide, 3,4,5-trihydroxy-6-[5-hydroxy-3-methoxy-2-(4-methoxy-phenyl)-4-oxo-4H-chromen-7-yloxy]-tetrahydro-pyran-2-carboxylic acid, 3,4,5-trihydroxy-6-[5-hydroxy-3-methoxy-2-(4-methoxy-phenyl)-4-oxo-4H-chromen-7-yloxy]-tetrahydro-pyran-2-carboxylic acid methyl ester.[19] Although methanol extract of *C. phlomidis* leaf has been reported for antioxidant activity, there was no systematic study performed leading to an active antioxidant chemical constituent. Hence, the current study was planned to further investigate the methanol extract for active antioxidant chemical constituent/s.

MATERIALS AND METHODS

The IR spectrum was recorded using KBr pellets in the range of 4000-400cm-1 using RXI-Perkin Elmer FTIR spectrometer. ^{13}C NMR spectra and ^{1}H proton NMR spectra was recorded using $CDCl_3$ as solvent in Jeol GSX liquid state NMR spectrometer operating at 200 MHz. Tetra methyl silane (TMS) was used as reference. Chemical shifts are reported in parts per million downfield with reference to internal standard. Mass spectra were recorded on GC Clarus 500Perkin Elmer system comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. For thin layer chromatography (TLCP) separations, precoated aluminum sheets of silica gel Merck, Darmstadt, Germany were employed. Other chemicals and solvents of analytical grade were obtained from STAR Scientific Chemicals, Salem, Tamil Nadu.

Collection of Plant Material

Leaves of *C. phlomidis* were collected from the outskirts of Tirupati, Andhra Pradesh, India. Plant material was authenticated by Dr. K. Madhava Chetty, Taxonomist, Department of Botany, Srivenkateswara University, Tirupati, Andhra Pradesh. Voucher specimen number CP/L/2015/1160.

Extraction

The leaves were dried in shade and homogenized to coarse powder and stored in opaque screw tight jars until use. Powdered drug was charged into soxhlet apparatus and extraction was carried out with methanol till exhausted. Extract was then concentrated using rotary vacuum evaporator at 50^0C under vacuum and dried residue was stored in an opaque glass bottles for further studies.

Isolation of Compounds by Column Chromatography

Methanol extract was chromatographed on a silica gel column of dimension (200g, 100 cm x 3.5 cm. Gradient elution technique was followed using chloroform: methanol as the mobile phase in different ratios**.** Fractions (1-15) A_1 eluted with chloroform: methanol (9.5:0.5), showed two spots in TLC solvent system of chloroform: methanol (9:1 v/v). Fraction A_1 was re-chromatographed on silica gel column (25 g, 2cm x 100cm). In the mobile phase ratio of chloroform: methanol (9.5:0.5) the compound 1 was started to elute to yield 60 fractions (20 mL each). The solvent was evaporated and the fractions were TLC monitored. The R_f value was found to be 0.47. They were combined and re-crystallized from acetone to get the yellow colour compound 1. Fractions (16- 24) B1 eluted with chloroform: methanol (9:1) started to

yield the compound 2 and 3 which was collected to yield 50 fractions (20 mL each). They were combined and re-crystallized from acetone to yield the pale yellowish colour compound 2 and light green colour compound 3. The R_f value of the compound was found to be 0.54 and 0.57. Fractions (25-36) C^1 eluted with chloroform: methanol (8:2) was found to contain two spots. Fraction C_1 was re-chromatographed on silica gel column (25 g, 2cm x 50cm), elution was carried out beginning with chloroform and the polarity was increased with the successive addition of methanol. At 2% addition of methanol i.e, chloroform: methanol (8:2), the compound 4 to 6 was started to coming out to yield 80 fractions (20 mL each). The solvent was evaporated and the fractions were TLC monitored. The R_f value was found to be 0.65, 0.68 and 0.73. They were combined and re-crystallized from acetone to yield brownish yellow, light green and pale yellow coloured compounds (4, 5 & 6). Again, the solvent polarity was increased gradually to elute another spot. At 4% addition of methanol i,e chloroform: methanol (6:4), the compound 7 and 8 was collected to yield 50 fractions (20 mL each). The solvent was evaporated and the fractions were TLC monitored. The R_f value was found to be 0.77 and 0.82. They were combined and re-crystallized from acetone to obtain light yellowish white needles of compound 7 and pale yellow colour compound 8. Fractions (38-45) D_1 eluted with chloroform: methanol (5:5) were found to have three spots. Fraction D_1 was re-chromatographed on silica gel column (25 g, 2cm x 100cm). At 5% addition of methanol i.e, chloroform: methanol (5:5), the compound 9 to 11 was obtained with R_f value 0.86, 0.88 and 0.90 they were combined and re-crystallized from acetone to obtain yellowish white needles of compound 9, yellowish colour compound 10 and green colour compound 11.

Diphenyl Picryl Hydrazyl (DPPH) Free Radical Scavenging Activity

The isolated compounds were subjected to free radical scavenging activity by DPPH assay method. DPPH reacts with an antioxidant compound that can donate hydrogen & get reduced. The change in colour (from deep violet to light yellow) was measured. The intensity of the yellow colour depends on the amount and nature of free radical scavenger present. DPPH stock solution of 100.0 µM was prepared using methanol as the solvent To 1.0 mL of 100.0 µM DPPH solution in methanol, equal volume of the sample in methanol of different concentrations (50, 125, 500 & 1000 µg/mL) were added separately and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 mL of methanol instead of test sample was added to the

control tube.[20] Each concentration was tested in triplicate. Percentage inhibition was calculated from the equation;

[(Absorbance of control - Absorbance of test)/ Absorbance of control] × 100

Inhibitory concentration (IC_{50}) values were determined using statistical analysis.

Spectral Characterisation Studies of the Isolated Compounds

The isolated compounds were characterized by infrared spectra (IR), proton magnetic resonance spectra (^{1}H NMR), carbon nuclear magnetic resonance (^{13}C NMR) and Mass spectra. Spectral characterization study was carried out for the compounds 1, 2, 3, 4, 6, 7, 8, 9 and 11.

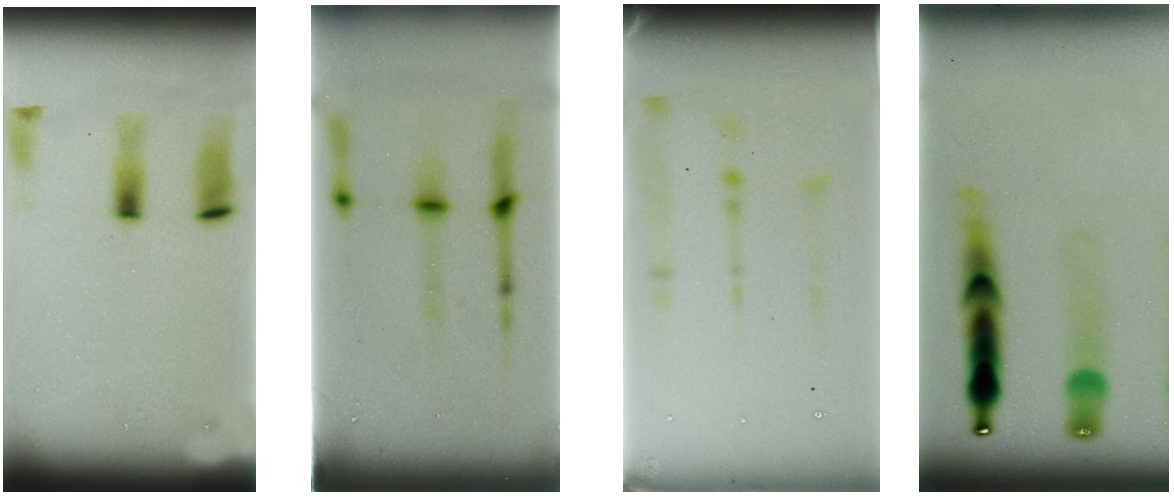
RESULTS AND DISCUSSION

Table 1: Data of the mobile phase system used in column chromatography elution system

Compound	Mobile Phase	Yield obtained	Colour obtained
Compound 1	chloroform: methanol (9.5:0.5)	10.5 mg	yellowish colour
Compound 2	chloroform: methanol (9:1)	10.9 mg	pale yellowish colour
Compound 3	chloroform: methanol (9:1)	8.5 mg	light green colour
Compound 4	chloroform: methanol (8:2)	8.6 mg	brownish yellow colour
Compound 5	chloroform: methanol (8:2)	4.1 mg	light green colour
Compound 6	chloroform: methanol (8:2)	10.1 mg	pale yellowish colour
Compound 7	chloroform: methanol (6:4)	8.3 mg	light yellowish white needles
Compound 8	chloroform: methanol (6:4)	8.7 mg	pale yellowish colour
Compound 9	chloroform: methanol (5:5)	8.5 mg	yellowish white needles
Compound 10	chloroform: methanol (5:5)	4.3 mg	yellowish colour
Compound 11	chloroform: methanol (5:5)	11.2 mg	green colour

Table 2: Data of the mobile phase system used in TLC and R_f value of compounds.

Compound	Mobile Phase (Chloroform: Methanol) Ratio	R_f Value
Compound 1	9:1	0.47
Compound 2	8:2	0.54
Compound 3	7:3	0.57
Compound 4	7:3	0.65
Compound 5	6:4	0.68
Compound 6	6:4	0.73
Compound 7	5:5	0.77
Compound 8	5:5	0.82
Compound 9	4:6	0.86
Compound 10	4:6	0.88
Compound 11	4:6	0.90



TLC of compounds 1,2 & 3 | TLC of compounds 4, 5 & 6 | TLC of Compounds 7, 8 & 9 | TLC of Compounds 10& 11.

Figure 1: Thin Layer Chromatography profile of the isolated chemical components.

Plant-derived natural products have long been and will continue to be extremely important as sources of medicinal agents and models for the design, synthesis, and semisynthesis of novel substances for treating humankind's diseases. Thus it is reasonable to expect that new plant sources of valuable and pharmaceutically interesting materials remain to be discovered and developed.[1] Methanol extract of the *C. phlomidis* leaf was subjected to the isolation by column chromatography for the isolation of the new chemical constituents in the plant with the cytotoxic potential. Methanol extract was chromatographed on a silica gel column and gradient elution technique was followed using chloroform: methanol as the mobile phase in different ratios (Table 1, 2 and Figure 1)**.** Nine compounds (1, 2, 3, 4, 6, 7, 8, 9 and 11) isolated from the methanol extract of the *C. phlomidis* leaf were characterized spectrally, the details are as follows.

Compound 1

IR (KBr) cm^{-1}: 1411.09 (C-C Arm str), 1368.39 (C-H rock in alkanes), 1183.74 (C-O str in esters), 912.32 (=CH bending in alkenes); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.94 (3H, -CH3), 1.97 (2H, -CH2), 2.32 (2H, -CH2), 4.10 (2H, -CH2), 5.15 (1H, -CH), 5.42 (1H, -CH), 7.46 (2H, -CH), 7.58 (1H, -CH), 8.03 (2H, -CH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 14.30 (-CH3), 20.53 (-CH2), 27.26 (-CH2), 63.38 (-CH2), 122.93 (-CH in ethylene), 128.00 (2 x –CH in benzene), 129.60 (2 x –CH in benzene), 130.08 (-CH in benzene), 132.40 (-CH in benzene), 134.83 (-CH in ethylene), 166.00 (-C=O in carboxyl); Mass (m/z): 204.26 (M+).

Compound 2

IR (KBr) cm^{-1}: 3449.64 (OH str of alcohol), 2923.15 (CH_2 of Alkanes), 2712.34 (CH str of aldehyde); ^1H NMR ($CDCl_3$, 200 MHz): δ 3.82 & 3.93 (2H, -CH2), 3.97 (1H, -CH), 4.47 (1H, -OH), 6.15 (1H, -OH), 9.66 (1H, -CHO); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 64.96 (-CH2), 74.68 (-CH), 202.00 (-C=O in carbonyl); Mass (m/z): 90.08(M+).

Compound 3

IR (KBr) cm^{-1} : 2934.56 and 2863.04 (-CH str of alkane); ^1H NMR ($CDCl_3$, 200 MHz): δ 0.92 (6H, -CH3), 1.29 (28H, -CH2); ^{13}C NMR ($CDCl_3$, 200 MHz): **δ** 14.10 (2 x -CH3), 22.73 (2 x -CH2), 29.00 (6 x -CH2), 29.42 (2 x -CH2), 29.71 (2 x -CH2), 31.98 (2 x –CH2); Mass (m/z): 226.44 (M+).

Compound 4

IR (KBr) cm^{-1}: 3324.41 (-OH of phenol), 1378.44 (-CH3 of alkane); ^1H NMR ($CDCl_3$, 200 MHz): δ 0.85 (3H, -CH3), 1.69 (2H, -CH2), 2.84 (2H, -CH2), 6.43 (1H, -CH), 7.30 (1H, -CH), 9.65 (2H, -OH), 12.50 (1H, -OH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 13.85 (-CH3), 17.91 (-CH2), 44.30 (-CH2), 105.50 (-CH in benzene), 115.55 (-CH in benzene), 120.00 (-CH in benzene), 142.40 (-C in benzene), 155.60 (-CH in benzene), 165.12 (-C in benzene), 200.37 (-C in carbonyl); Mass (m/z): 196.19 (M+).

Compound 6

IR (KBr) cm^{-1}: 3477.39 (OH str in phenol), 803.14 (=CH bending in alkenes); ^1H NMR ($CDCl_3$, 200 MHz): δ 3.86 (3H, CH3), 7.00 (1H, -CH), 7.08 (1H, -CH), 7.47 (1H, -CH), 7.77 (1H, -CH), 7.78 (1H, -CH), 7.82 (1H, -CH), 9.44 (1H, OH), 9.81 (1H, OH), 10.26 (1H, OH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 55.20 (-CH3), 103.10 (-CH in benzene), 109.04 (-CH in benzene), 113.30 (-CH in benzene), 115.00 (-CH in benzene), 119.80 (-CH in benzene), 121.88 (-C in benzene), 129.50 (-CH in benzene), 132.00 (-C in benzene), 138.54 (-C in ethylene), 144.10 (-C in ethylene), 146.23 (-C in benzene), 152.40 (-C in benzene), 156.74 (-C in benzene), 159.10 (-C in benzene), 172.32 (-C=O in carbonyl); Mass (m/z): 300.26 (M+).

Compound 7

IR (KBr) cm^{-1}: 3657.86 (-OH of hydroxyl), 3024.27 (=CH str of alkenes), 1662.96 (C=C str in alkenes), 1458.73 (-CH3 and –CH2 in alkanes), 971.11 (=CH bending of alkenes); ^1H NMR ($CDCl_3$, 200 MHz): δ 1.67 & 1.84 (2H, -CH2), 2.08 & 2.10 (2H, CH2), 2.04 & 2.27

(2H, -CH2), 3.92 (1H, -CH), 4.94 (1H, -OH), 5.61 (2H, -CH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 24.20 (-CH2), 31.30 (-CH2), 34.80 (-CH2), 67.40 (-CH2), 121.38 (-CH in ethylene), 126.51 (-CH in ethylene); Mass (m/z): 98.14 (M+).

Compound 8

IR (KBr) cm^{-1}: 1462.11 (C-C aromatic str), 1292.55, 1114.76, 1070.09 (C-O str in ester). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.98 (6H, -CH3), 7.51 (2H, -CH), 7.69 (2H, -CH); ^{13}C NMR ($CDCl_3$, 200 MHz): **δ** 52.80 (2 x –CH3), 128.93 (2 x – CH in benzene), 131.38 (2 x –CH in benzene), 132.40 (2 x –C in benzene), 166.70 (2 x – C in carboxyl). Mass (m/z): 222.09 (M+).

Compound 9

IR (KBr) cm^{-1}: 3042.78 (=CH str in alkenes), 2874.31 (CH str in alkanes), 1238.09, 1143.94 (C-O str in ester); ^{1}H NMR ($CDCl_3$, 200 MHz): **δ** 0.89 (3H, -CH3), 1.13 (6H, -CH3), 1.22 (2H, CH2), 1.27 (2H, -CH2), 1.29 (2H, CH2), 1.31 (2H, CH2), 1.33 (4H, CH2), 1.36 (2H, CH2), 1.62 (2H, CH2), 2.01 (2H, CH2), 2.03 (1H, -CH), 2.29 (2H, -CH2), 2.76 (2H, -CH2), 4.99(1H, -CH), 5.30 (1H, -CH), 5.32 (1H, -CH), 5.34 (1H, -CH), 5.37 (1H, -CH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 14.12 (-CH3), 22.18 (2 x –CH3), 22.65 (-CH2), 25.25 (-CH2), 25.60 (-CH2), 27.29 (-CH2), 27.31 (-CH2), 29.10 (-CH2), 29.31 (-CH2), 29.34 (2 x -CH2) , 29.76 (-CH2), 31.68 (-CH2), 34.72 (-CH2), 67.70 (-CH methiene), 128.10 (-CH in ethylene), 128.14 (-CH in ethylene), 130.20 (-CH in ethylene), 130.39 (-CH in ethylene), 173.28 (-COO in carboxyl); Mass (m/z): 322 (M+).

Compound 11

IR (KBr) cm^{-1} : 2914.67 (CH str of alkanes), 1734.94 (C=O str in esters), 1382.67 (CH3 in alkanes), 1181.42, 1108.56 (C-O str in esters); ^{1}H NMR ($CDCl_3$, 200 MHz): **δ** 0.88 (6H, -CH3), 1.26 (38H, -CH2), 1.29 (8H, -CH2), 1.33 (6H, -CH2), 1.44 (2H, -CH2), 1.59 (-CH2), 1.63 (2H, -CH2), 1.98 (4H, -CH2), 2.25 (2H, -CH2), 4.04 (2H, -CH2), 5.33 (2H, -CH); ^{13}C NMR ($CDCl_3$, 200 MHz): δ 14.24 (2 x -CH3), 22.79 (2 x -CH2), 25.22 (-CH2), 25.40 (-CH2), 27.40 (2 x -CH2), 28.94 (-CH2), 29.0 (-CH2), 29.3 (3 x -CH2), 29.4 (-CH2), 29.56 (12 x -CH2), 29.70 (3 x -CH2), 29.90 (2 x -CH2), 31.96 (2 x -CH2), 34.29 (-CH2), 64.46 (-CH2), 129.90 (-CH), 130.20 (-CH), 173.93 (=CO in carboxyl). Mass (m/z): 562.42(M+).

Table 3: List of the compounds identified by the spectral studies.

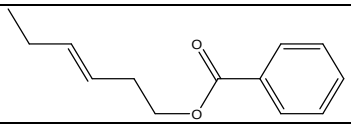
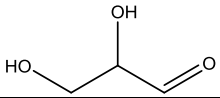
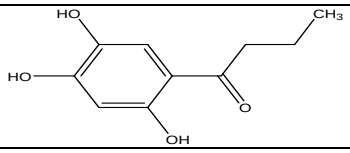
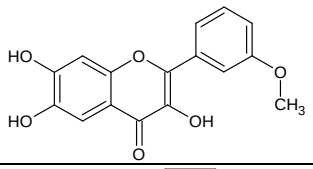
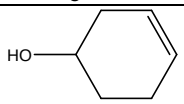
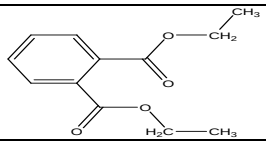
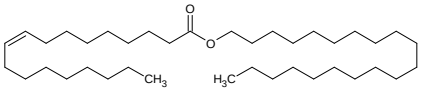
Compound No.	Compound Name	Structure	Molecular Weight	Molecular Formula
1	3-Hexen-1-yl benzoate		204.26	$C_{13}H_{16}O_2$
2	2,3-dihydroxypropanal		90.07	$C_3H_6O_3$
3	Hexadecane		226.44	$C_{16}H_{34}$
4	1-(2,4,5 trihydroxy phenyl)-1-butanone		196.2	$C_{10}H_{12}O_4$
6	3,6,7- trihydroxy-2-(3-methoxy phenyl)- 4H-chromen-4-one		300.26	$C_6H_{12}O_6$
7	3- cyclohexen-1-ol		98.15	$C_6H_{10}O$
8	1,2-benzenedicarboxylic acid, diethyl ester		222.23	$C_{12}H_{14}O_4$
9	Isopropyl Linoleate		322.52	$C_{21}H_{38}O_2$
11	Oleic acid, eicosyl ester (oleic acid ester)		562.99	$C_{38}H_{74}O_2$

Table 4: Antioxidant activity of the isolated compounds of methanol extract of *C. phlomidis* in DPPH free radical scavenging assay.

Compounds	% Inhibition				IC_{50}
	50 µg/mL	125 µg/mL	500 µg/mL	1000 µg/mL	
Compound 1	40.78 ± 0.56	59.72 ± 0.64	63.59 ± 0.98	79.16 ± 0.72	79.58
Compound 2	34.15 ± 0.90	47.2 ± 0.88	54.68 ± 0.76	62.95 ± 0.60	126.9
Compound 3	32.53 ± 0.90	45.87 ± 0.88	54.28 ± 0.55	69.03 ± 0.71	123.62
Compound 4	41.27 ± 0.61	52.68 ± 0.86	57.49 ± 0.70	68.76 ± 0.84	96.38
Compound 6	49.62 ± 0.72	54.03 ± 0.42	61.97 ± 0.93	72.85 ± 0.59	63.16
Compound 7	30.69 ± 0.68	38.52 ± 0.37	46.15 ± 0.38	59.23 ±0.75	159.52
Compound 8	34.14 ± 0.56	53.47 ± 0.73	61.25 ± 0.31	77.90 ± 0.29	101.01
Compound 9	46.25 ± 0.23	58.1 ± 0.28	66.81 ± 0.73	70.15 ± 0.55	61.13
Compound 11	32.53 ± 0.36	46.15 ± 0.31	54.27 ± 0.58	63.84 ± 0.74	129.02

All values are Mean ± SD, n=3.

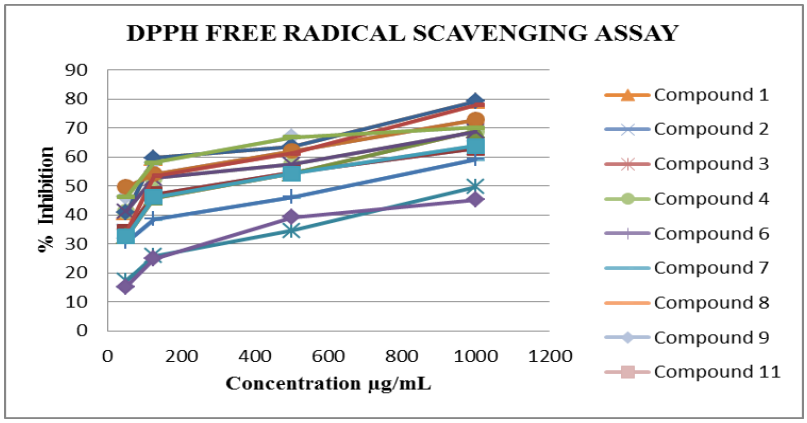


Figure 2: Percentage inhibition of the isolated compounds of methanol extract of *C. phlomidis* in DPPH free radical scavenging assay.

Nine compounds were characterized by spectral studies and were found to be 3-hexen-1-yl benzoate, 2,3-dihydroxypropanal, hexadecane, 1-(2,4,5 trihydroxyphenyl)-1-butanone, 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one,3-cyclohexen-1-ol,1,2-benzene dicarboxylic acid, diethyl ester, isopropyl linoleate, oleic acid eicosyl ester (oleic acid ester) (Table 3). In DPPH Free radical scavenging assay, compounds 6 (3, 6, 7-trihydroxy-2-(3-methoxy phenyl)- 4H- chromen-4-one) and compound 9 (Isopropyl linoleate) showed good antioxidant activity with an IC_{50} value of 63.16 µg/mL and 61.13 µg/mL respectively (Table 4 and Figure 2).

CONCLUSION

Nine compounds (1, 2, 3, 4, 6, 7, 8, 9 and 11) were isolated from the methanol extract of the *C. phlomidis* leaves and were characterized by spectral studies. Among these compounds 3-hexen-1-yl benzoate, 2,3-dihydroxypropanal, 1-(2,4,5 trihydroxyphenyl)-1-butanone, 3-cyclohexen-1-ol, isopropyl linoleate, oleic acid eicosyl ester are reported for the first time from *C. phlomidis* leaves. Compounds 6 and 9 showed good antioxidant activity with an IC_{50} value of 63.16 µg/mL and 61.13 µg/mL respectively.

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REFERENCES

1. Balandrin MF, Kinghorn AD and Fransworth NR. Plant-derived natural products in drug discovery and development an overview. In: Kinghorn AD and Balandrin MF (eds.). Human Medicinal Agents from Plants, American Chemical Society, 1993; 2-12.
2. Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharm Biol, 1999; 37(1): 80-91.
3. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 2005; 4(7): 685-688.
4. Rajasekaran A, Ponnusamy K. Antifungal activity of *Clerodendrum inerme* (L). and *Clerodendrum phlomidis* (L). Turk J Bio, 2006; 30(3): 139-142.
5. Prasad MP, Sushant S and Chikkaswamy BK. Phytochemical analysis, antioxidant potential, antibacterial activity and molecular characterization of *clerodendrum species.* International journal of molecular biology, 2012; 3(3): 71-76.
6. Srinivasa U, Rao JV, Krupanidhi AM, Babu PRS. Analgesic activity of leaves of *Clerodendrum phlomidis*. J Res Edu Indian Med, 2007; 13: 23-25.
7. Vadnere GP, Somani RS, Singhai AK. Studies on antiasthmatic activity of aqueous extract of *Clerodendrum phlomidis*. Pharmacolgyonline, 2007; 1: 487-494.
8. Rani S, Ahamed N, Rajaram S, Saluja R, Thenmozhi S, Murugesan T. Anti-diarrhoeal evaluation of *Clerodendrum phlomidis* Linn. Leaf extracts in rats. J Ethnopharmacol, 1999; 68(1-3): 315-319.
9. Prakash BN, Pandikumar P, Ignacimuthu S. Lysosomal membrane stabilization and anti-inflammatory activity of *Clerodendrum phlomidis* L.f., a traditional medicinal plant. J Ethnopharmacol, 2011; 135(3): 779-785.
10. Roy R, Singh UP, Pandey VB. Antifungal activity of some naturally occurring flavanoids. Orient J Chem, 1995; 11(2): 145-148.
11. Simonsen HT, Nordskjold JB, Smitt UW, Nyman U, Palpu P, Joshi P, Varughese G. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharmacol, 2001; 74(2): 195-204.
12. Chaturvedi GN, Subramaniyam PR, Tiwari SK, Singh KP. Experimental and clinical studies on diabetic mellitus evaluating the efficacy of an indigenous oral hypoglycaemic drug- Arani (*Clerodendrum phlomidis*). Ancient Sci Life, 1983; 3(4): 216-224.
13. Sharma N, Trivedi PC. Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita and Fusarium oxyporum*. Asian J Exp Sci, 2002; 16(1-2): 21-28.

14. Murugesan T, Saravanan KS, Lakshmi S, Ramya G, Thenmozhi K. Evaluation of psychopharmacological effects of *Clerodendrum phlomidis* Linn. extract. Phytomedicine, 2001; 8(6): 472-476.
15. Bhakuni DS et al. Chemical examination of *Clerodendron phlomidis* Linn. J Sci Indust Res, 1962; 21B: 48-49.
16. Subramanian SS, Nair AG. Scutellarein and pectolinaringenin from the leaves of *Clerodendron phlomidis* and *Duranta repens*. Phytochemistry, 1972; 11(10): 3095-3096.
17. Subramanian SS, Nair AG, Vedantham TN. (24S)-ethylcholesta-5, 22, 25-triene-3β-ol from four Clerodendron species. Phytochemistry, 1973; 12(8): 2078-2079.
18. Anam EM. Novel flavanone and chalcone glycosides from *Clerodendron phlomidis* (Verbanaceae). Indian J Chem, 1999; 38(10): 1307-1310.
19. Yogesh PB, Abhijit H, Siddharth S, Sudeshna S, Anil K & Nirup BM. New flavonoid glycosides and other chemical constituents from *Clerodendrum phlomidis* leaves: isolation and characterization. Natural Product Research, 2015; 29(19): 1850-1856.
20. Molyneux, P: The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for eestimating antioxidant activity: Songklanakarin J. Sci. Technol, 2004; 26: 211–219.