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Chemical Investigation and Bioactivity Screening of *Salvia cassia* Extracts

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Abstract: In this study the antioxidant and anticholinesterase activities of the crude ethanol extract, as well as the dichloromethane and water extracts obtained from partitioning of the crude ethanol extract, of the plant *Salvia cassia* Samuelss ex. Rech. Fil, were determined. The extracts were screened for their total phenolic and flavonoid contents. The dichloromethane extract, which showed higher inhibition of lipid peroxidation, higher metal chelating capacity and also a higher flavonoid content than the other extracts was analyzed for its constituents. GC-MS analysis resulted in the identification of a total of 30 long chain hydrocarbons. A series of *β*-amyrin fatty acid esters, a triterpene acid oleanolic acid, a diterpenoid manoyloxide, and two flavonoids apigenin 7, 4′-dimethyl ether and salvigenin were isolated by open column chromatography. Their structures were identified based on NMR and mass spectrometric techniques. The isolated compounds were also tested for their anticholinesterase activities. The triterpenoids as well as the water extract exhibited promising anticholinesterase activity.

Keywords: Keywords: *Salvia cassia;* antioxidant activity; anticholinesterase activity; fatty acid composition; β-amyrin fatty acid esters; flavonoids. © 2018 ACG Publications. All rights reserved.

1. Introduction

Salvia species have been used in traditional medicine since old ages, and therefore have attracted the attention of scientists. *Salvia* (sage) is the largest genus of the Lamiaceae family with about 900 *Salvia* species existing world-wide. Among the 110 taxa that grow in Turkey, about half are endemic [1]. The most widely used medicinal sage species, *Salvia officinalis* L., has found traditional applications as diuretic, antispasmodic, antiseptic and in the treatment of stomach disorders [2]. However in Turkey, *S. fruticosa* Mill. (syn: *S. triloba* L.) has been used as the medicinal sage. *Salvia* species have received special attention as sources of natural antioxidants [3-6]. Antimicrobial, particularly antituberculosis and antiviral, antidiabetic, antitumor activities of *Salvia* species have also been recorded [7-11].

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Salvia species have folkloric uses in the treatment of central nervous system disorders. They are giving promising results as acetyl- and butyrylcholinesterase enzyme inhibitors in the potential treatment of memory disorders including Alzheimer's disease [12-17]. Several clinical studies have been reported [18-20].

Salvia cassia Samuelss. ex. Rech. Fil is an endemic species to Anatolia, particularly south of Turkey as well as west Syria. In this study we describe the determination of the total phenolic and flavonoid contents and the evaluation of the antioxidant and anticholinesterase activities of the crude ethanol extract, as well as the dichloromethane and water extracts which were obtained from the partition of the crude ethanol extract. *β*-Carotene-linoleic acid test for determination of lipid peroxidation inhibitory activity has been run for the first time in this study.

This is the first study on the chemical composition and the fatty acid profile of *S. cassia*. Terpenoid and flavonoid constituents were isolated by chromatographic techniques and identified by spectroscopic methods. The fatty acid profile of the dichloromethane extract was screened by GC-MS.

2. Materials and Methods

2.1. Plant Material

Salvia cassia Samuelss. ex. Rech. Fil was collected in June 2010 from Hatay, Kırıkhan, by Dr. Gamze Kökdil, Mersin University Faculty of Pharmacy, Department of Pharmacognosy. A voucher specimen (AEF 21136) has been deposited at the Ankara University Herbarium.

2.2. Extraction and Isolation Methods

Air-dried leaves and stems (450 g) were extracted by ethyl alcohol. 34.0 g crude alcohol extract was obtained. It was left in the deep-freeze until fractionation. 29.0 g of the crude extract was partitioned repeatedly between water and dichloromethane and 9.0 g dichloromethane and 16.0 g water extracts were obtained. The dichloromethane extract was fractionated on a silica gel column (*Silica gel* 100, 0.063-0.200, Merck Cat. No. 110184) eluting with combinations of PE, benzene, CH_2Cl_2 and CH_3OH solvents in increasing polarities. Sixty fractions, 100 mL each, were collected. Fraction 16 eluted with benzene-dichloromethane (100:00 – 80:20) yielded a series of β-amyrin fatty acid esters (**1**). Fractions 17 through 21 eluted with benzene-dichloromethane (100:00 – 80:20) were subjected to GC-MS. Fraction 29, eluted with CH_2Cl_2 yielded oleanolic acid **(2)**. Combined fractions 30-46 eluted with CH_2Cl_2 were refractionated on *Si Gel* column and yielded apigenin 7, 4′-dimethyl ether **(3)** (benzene 25: CH_2Cl_2 75 solvent system) and salvigenin **(4)** (solvent CH_2Cl_2), after rechromatography on *Sephadex LH-20* (Sigma-Aldrich, Cat. No. LH2010) columns in MeOH and preparative chromatography on Si Gel plates (Silica gel 60 F_{254}, Merck Cat. No. 105554). The combined fractions 47-52 eluted with CH_2Cl_2-CH_3OH (90:10 – 80:20) systems were refractionated on a *Si Gel* column. Fraction 10 (from 47-52) eluted with PE:CH_2Cl_2 (1:1) solution, after purification on *Sephadex LH-20* column, yielded manoyloxide **(5)**.

2.3. Chemical Reagents

For the antioxidant activity tests, quercetin was obtained from E. Merck (Darmstadt, Germany); *β*-Carotene, linoleic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *α*-tocopherol, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from E. Merck (Darmstad, Germany). For anticholinergic tests, electric eel acetylcholinesterase (AChE), horse serum butyrylcholinesterase (BChE), acetylthiocholine iodide, butyrylthiocholine chloride and galanthamine were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

2.4. Spectroscopic Methods

^{1}H NMR spectra were recorded on Varian Mercury-VX at 400 MHz for ^{1}H and 100 MHz for ^{13}C (APT) in $CDCl_3$; UV-VIS spectra for antioxidant activity tests were performed on Shimadzu UV–1601 spectrophotometer in MeOH solvent; anticholinesterase activities were measured on SpectraLab 340PCF, Molecular Devices instrument; mass spectra were recorded on (+) AP-CIMS Thermo-LCQ Deca XP-Max spectrometer.

2.4.1. Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis of the fatty acids was performed using a Shimadzu GC-17 AAF, V3, 230V series gas chromatograph equipped with a FID and DB-1 fused silica capillary column [5]. For GC-MS detection, a Varian Saturn 2100 (EI, Quadrupole) was used [5]. Identification of components was based on GC retention indices and computer matching with the Wiley, NIST and TRLIB libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

2.5. Determination of Total Phenolic Content

The phenolic contents of the extracts were expressed as pyrocatechol equivalents (mg g^{-1} extract) according to the method of Slinkard and Singleton [21].

2.6. Determination of Total Flavonoid Content

Measurement of flavonoid concentration of the extracts was based on the method described by Moreno et al. [22], with a slight modification. The results were expressed as quercetin equivalents.

2.7. Measurements of Biological Activities

2.7.1. DPPH Free Radical-scavenging Activity

The free radical-scavenging activity was determined by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay described by Blois [23]. BHT and α-tocopherol were used as standards.

2.7.2. β-Carotene-Linoleic Acid Assay

The antioxidant activity was evaluated using the β-carotene-linoleic acid model system developed by Marco [24]. BHA, BHT and α-tocopherol were used as standards.

2.7.3. Metal Chelating Activity

The ferrous ion-chelating capacity of the extracts was estimated by the Fe^{2+}-ferrozine test system reported by Decker and Welch [25]. Quercetin and (+)-catechin were used as standards.

2.7.4. Inhibitory Activity Against AChE and BChE Enzymes

Inhibitory activity against AChE and BChE enzymes was determined by the use of the Ellman method [26]. AChE from electric eel and BChE from horse serum were used. Acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB (5, 5'-dithiobis (2-nitrobenzoic) acid) was used for the measurement of the anticholinesterase activity. Galanthamine was used as standard.

2.7.5. Statistical Analysis

All data on both antioxidant and anticholinesterase activity tests are mean values of triplicate analyses. The data were recorded as mean standard deviation. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Student's t-test and p values <0.05 were regarded as significant.

*2.8. Analysis of the Series of β-Amyrin Fatty Acid Esters (**1a, 1b, 1c, 1d**):*

β-amyryl-3-hexadecanoate **(1a)** (olean-12(13)-en-3β-ol hexadecanoate; β-amyryl-3-palmitate); β-amyryl-3-heptadecanoate **(1b)** (olean-12(13)-en-3β-ol heptadecanoate); β-amyryl-3-octa-decanoate **(1c)** (olean-12(13)-en-3β-ol octadecanoate; β-amyryl-3-stearate)**;** β-amyryl-3-nonadecanoate **(1d)** (olean-12(13)-en-3β-ol nonadecanoate). ^{1}H NMR (400 MHz, $CDCl_3$) and C-13 NMR (100 MHz, $CDCl_3$) was given in Table 7. APCI-MS: molecular ion peaks; *m/z* 665 ($M+H^+$; $C_{46}H_{81}O_2^+$; **1a**), 679 ($M+H^+$; $C_{47}H_{83}O_2^+$; **1b**), 693 ($M+H^+$; $C_{48}H_{85}O_2^+$; **1c**), 707 ($M+H^+$; $C_{49}H_{87}O_2^+$, **1d**), and prominent peaks; 425 (M^+- long chain acyl group; $C_{30}H_{49}O^+$; base peak), 407 (M^+- fatty acid - $2H^+$; oleanoyl carbocation - $2H^+$), 219 ($C_{16}H_{27}^+$, Δ^{12} oleanene retro-Diels Alder fragment of $C_{30}H_{49}^+$), 205 (219 - CH_3).

3. Results and Discussion

3.1. Biological Activities of the Extracts

3.1.1. Antioxidant Activities

The synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in food products for preservation for longer periods of time. Search for natural antioxidants has focused on plant species. In this study the phenolic and flavonoid contents of the crude ethanol, as well as the dichloromethane and water extracts obtained from the partition of the crude ethanol extract of *Salvia cassia,* were determined. Among the extracts, the dichloromethane extract had the richest total flavonoids content, whereas the water extract had the richest phenolics content (Table 1).

Table 1. Amount of the total phenolics and flavonoids in the *Salvia cassia* extracts

Sample	**Amount of PE[b] (µg QE/mg extract)**	**Amount of flavonoids (µg QE/mg extract)[c]**
Crude ethanol extract	39.87±1.12	27.95±0.36
Dichloromethane extract	21.34±0.22	41.43±0.65
Water extract	44.65±0.31	23.23±0.43

[a]Average of three parallel measurements.[b]PE, pyrocatechol equivalents. [c]QE, quercetin equivalents

The extracts were then screened for their antioxidant activities at four different concentrations, using three in vitro assays: DPPH method for free radical scavenging activity, β-carotene-linoleic acid test system for lipid peroxidation inhibition determination, and Fe^{+2}-ferrozine system for metal chelation capacity determination (Tables 2-4).

Table 2. DPPH free radical scavenging capacity of the *Salvia cassia* extracts (Inhibition %)[a]

Sample	10 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL
Crude ethanol extract	10.87±0,18	28,27±0.81	50.22±1.03	86.01±0.55
Dichloromethane extract	5.86±0.73	10.98±0.25	19.20±0.91	32.55±0.28
Water extract	12.82±1.01	31.39±0.30	58.71±0.40	93.11±0.24
α-Tocopherol[b]	63.18±1.38	93.93±0.05	94.48±0.39	94.79±0.50
BHT[b]	55.02±0.60	83.54±0.56	92.62±0.02	94.42±0.28

[a]Average of three parallel measurements. [b]Reference compounds.

Table 3. Inhibition of lipid peroxidation by the *Salvia cassia* extracts, in *β*-carotene-linoleic acid system (Inhibition %)[a]

Sample	10 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL
Crude ethanol extract	12.024±0.97	19.51±5.83	22.16±1.54	39.61±0.35
Dichloromethane extract	32.40±1.00	48.86±0.61	51.69±1.17	66.45±2.25
Water extract	5.22±1.30	15.06±1.86	21.10±0.35	25.98± 5.01
BHT[b]	63.54± 0.93	63.66± 0.58	65.22± 0.91	66.22± 1.00
BHA[b]	90.49± 0.53	92.00± 0.01	95.12±1.29	96.54±0.01
α-Tocopherol[b]	60.91±0.07	72.92±0.79	88.17±0.73	84.23±0.92

[a]Average of three parallel measurements. [b]Reference compounds.

Table 4. Ferrous ion chelating capacity of the *Salvia cassia* extracts. (Inhibition %)[a]

Sample	50 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL
Crude ethanol extract	1.12±0.23	2.42±0.39	7.17±0.01	14.88±0.73
Dichloromethane extract	37.97±1.06	55.89±1.23	60.00±1.68	59.62±1.29
Water extract	NA[b]	NA[b]	NA[b]	NA[b]
Quercetin[c]	4.00±0.33	5.00±0.08	7.38±0.83	14.37±0.30
(+)-Catechin[c]	0.27±0.52	0.53±0.99	0.91±0.95	1.77±0.70

[a]Average of three parallel measurements. [b]NA: not active. [c]Reference compound

The water extract showed remarkable free radical scavenging activity (93.11± 0.24% at 100 μg/mL) against DPPH, close to the standards α-tocopherol (94.79±0.50 at 100 μg/mL) and BHT (94.42±0.28 at 100 μg/mL). However, the dichloromethane extract exhibited much lower radical scavenging activity (Table 2). The lipid peroxidation inhibitory activity of the dichloromethane was close to the synthetic reference BHT, but less than the references BHA and α-tocopherol. Lipid peroxidation results for the water extract were low as compared to the dichloromethane extract (Table 3). The dichloromethane extract exhibited noticeable metal ion chelating capacity, higher than the reference quercetin and much higher than the crude ethanol extract (Table 4).

3.1.2. Anticholinesterase Activities

The three *S. cassia* extracts, as well as the terpenoids isolated from the dichloromethane extract, were tested for anticholinesterase activity. The water extract exhibited unexpectedly high activity, especially against the AChE enzyme (73.05±1.61 at 200 μM). Formerly reported results on AChE inhibition, carried out on different solvent extracts of *S. cassia*, did not show meaningful activity, while BChE inhibitory results of both studies are similar [17]. The method of extract preparation proves to be of significance in determination of activities.

Oleanolic acid, and to a lesser extent the mixture of β-amyrin fatty acids also showed inhibition on AChE enzyme (Table 5). As manoyloxide showed low activity against AChE at 200 μM it was not tested at lower concentrations (Table 5). We have reported the anticholinesterase activities of the isolated flavonoids salvigenin and apigenin 7, 4′-dimethyl ether in a former study [12].

Table 5. Anticholinesterase activities of the *Salvia cassia* extracts and isolated compounds[a]

	Inhibition % against AChE[a]				**Inhibition % against BChE[a]**			
	25 μM	**50 μM**	**100 μM**	**200 μM**	**25 μM**	**50 μM**	**100 μM**	**200 μM**
Crude ethanol extract	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
Dichloromethane extract	NA[b]	NA[b]	NA[b]	NA[b]	6.93±0.92	8.50±0.29	13.65±1.32	24.46±2.45
Water extract	33.05±0.62	43.60±0.55	59.90±0.86	73.05±1.61	22.35±1.02	39.98±1.09	48.78±0.99	52.89±1.66
FAE[c] mixture	38.80±0.22	44.80±0.75	55.29±0.79	50.39±0.80	17.04±0.67	38.18±0.75	38.89±0.78	41.85±0.82
Oleanolic acid	49.52±1.03	55.16±0,98	78.36±1.13	82.85±0.88	14.02±0.07	18.90±0.14	22.36±0.43	26.91±0.82
Manoyloxide	NA[b]	NA[b]	NA[b]	16.75± 0.23	NA[b]	NA[b]	20.14± 0.38	28.32±0.34
Salvigenin[9]	NA[b]	NA[b]	NA[b]	NA[b]	4.85±0.54	5.67±0.36	7.96±0.67	14.19±0.51
Apigenin 7,4′-dimethyl ether[9]	1.78±0.87	3.78±0.58	7.35±0.58	12.35±0.99	30.59±0.73	35.69±1.29	42.31±0.36	50.31±0.94
Galanthamine[d]	69.38±1.10	75.98±0.95	78.19±0.48	83.41±0.53	40.59±0.88	48.73±0.90	65.02±0.44	74.22±0.89

[a]Average of three parallel measurements. [b]Not active. [c]Fatty acid esters mixture. [d]Standard drug.

3.2. Phytochemical Analysis

Since the antioxidant activity of the dichloromethane extract was found to be fairly high for the Fe^{+}-ferrozine chelating and lipid-peroxidation inhibitory assays, it was further analyzed by phytochemical techniques in order to isolate pure compounds.

The dichloromethane extract was separated into its constituents by liquid chromatography on silica gel column. Both ^{1}H and ^{13}C NMR spectra indicated the presence of long chain hydrocarbons in fractions 17 through 21. Therefore a GC-MS analysis of 17-21 was undertaken and 30 long-chain hydrocarbons were determined. Among the identified fatty acids, the most abundant ones were found to be hexadecanoic acid (palmitic acid) (24.0%) and heptadecanoic acid (17.0%) (Table 6). Fifteen long-chain hydrocarbons which constitute 71.4 % of the total hydrocarbons, their retention indeces (RI) and percentages are shown in Table 6.

Table 6. Identified compounds from the fraction of 17-21 of the *Salvia cassia* dichloromethane extract[a]

RI	Compound[a]	%
1409	Octanedioic acid, dimethyl ester	1.8
1511	Nonanedioic acid, dimethyl ester	6.0
1706	Tetradecanoic acid, methyl ester	1.9
1830	6,10,14-Trimethyl-2-pentadecanone	3.4
1915	Hexadecanoic acid, methyl ester	24.0
2008	Heptadecanoic acid, methyl ester	17.0
2187	Octadecanoic acid, methyl ester	5.6
2209	Nonadecanoic acid, methyl ester	5.5
2310	Eicosanoic acid, methyl ester	1.3
2373	Eicosanoic acid, ethyl ester	1.2
2610	Octadecanoic acid, dihydroxy-, methyl ester	2.7
2700	Heptacosane	1.0

[a]The acids were analyzed after methylation of the sample.

The rest of the dichloromethane extract of *Salvia cassia* was fractionated on silica gel columns, repeatedly. Further purifications were achieved by chromatograpy on Sephadex LH-20 columns and by preparative TLC.

The process yielded a series of fatty acid esters of β-amyrin (**1**), the triterpene acid oleanolic acid (**2**)* [27], two flavonoids; apigenin 7, 4′-dimethyl ether (**3**) [12] and salvigenin (**4**) [12], and the diterpene manoyloxide [28] (**5**) (Figure 1). Compounds **2-5** were identified by ^{1}H NMR and ^{13}C NMR techniques and through comparison with literature values.

The ^{1}H NMR and ^{13}C NMR DEPT data for **1** correlated with Δ^{12}-pentacyclic triterpenoid ring structure with eight methyl groups at δ_C 28.6, 14.4, 15.8, 17.0, 26.2, 28.3, 33.6, 23.9 ppm and δ_H 0.82, 0.86 (four overlapping peaks), 0.95, 0.96, 1.12 ppm, one vinylic proton peak at δ_H 5.17 and methyne carbon at δ_C 122.0 ppm, and a one-proton doublet of doublets at δ_H 4.49 with a methyne carbon at δ_C 80.8 ppm indicating an ester linkage. The long chain fatty acid moiety was suggested by an intense broad singlet at δ 1.24 (methylene chain) and a two proton triplet at δ 2.28 (J=6.8 Hz) (α-methylene to ester carbonyl). C-13 NMR data of **1** were compared with C-13 NMR data of known triterpene skeletons and were found to correlate closely with those of the oleanane skeleton [29] (Table 7).

Compound **1** exhibited a series of [M+1] ion peaks at *m/z* 665, 679, 693 and 707, corresponding to β-amyryl hexadecanoate (**1a**), β-amyryl heptadecanoate (**1b**), β-amyryl octadecanoate (**1c**) and β-amyryl nonadecanoate (**1d**), which differ from each other with only a CH_2 unit, respectively. The fragmentation pattern in the APCI-MS spectrum is characteristic of pentacyclic triterpenoids with a double bond in their structure. The [M - fatty acid] peak is at *m/z* 409 (triterpene moiety), [M - acyl] peak at *m/z* 425 (base peak, triterpenol moiety) and retro-Diels Alder fragments related to pentacyclic triterpenoid Δ^{12} are observed at *m/z* 218 and 206.

Recently, the essential oil composition of *S.cassia* and its biological activity were reported [30] and in this study the total phenolic and flavonoid contents, as well as the antioxidant and the anticholinesterase activities of several extracts, of the endemic species *Salvia cassia*, have been evaluated. The dichloromethane extract of *Salvia cassia* which was found to be richer in flavonoids, also showed higher inhibition of lipid peroxidation and higher metal chelating capacity than the other extracts. The water extract which was richer in phenolics showed higher free radical scavenging capacity. Strong correlation between content of phenolics and free radical scavenging activity has been emphasized also in former studies [6,31]. β-Carotene-linoleic acid test, for determination of lipid peroxidation inhibitory activity, has been run for the first time in this study.

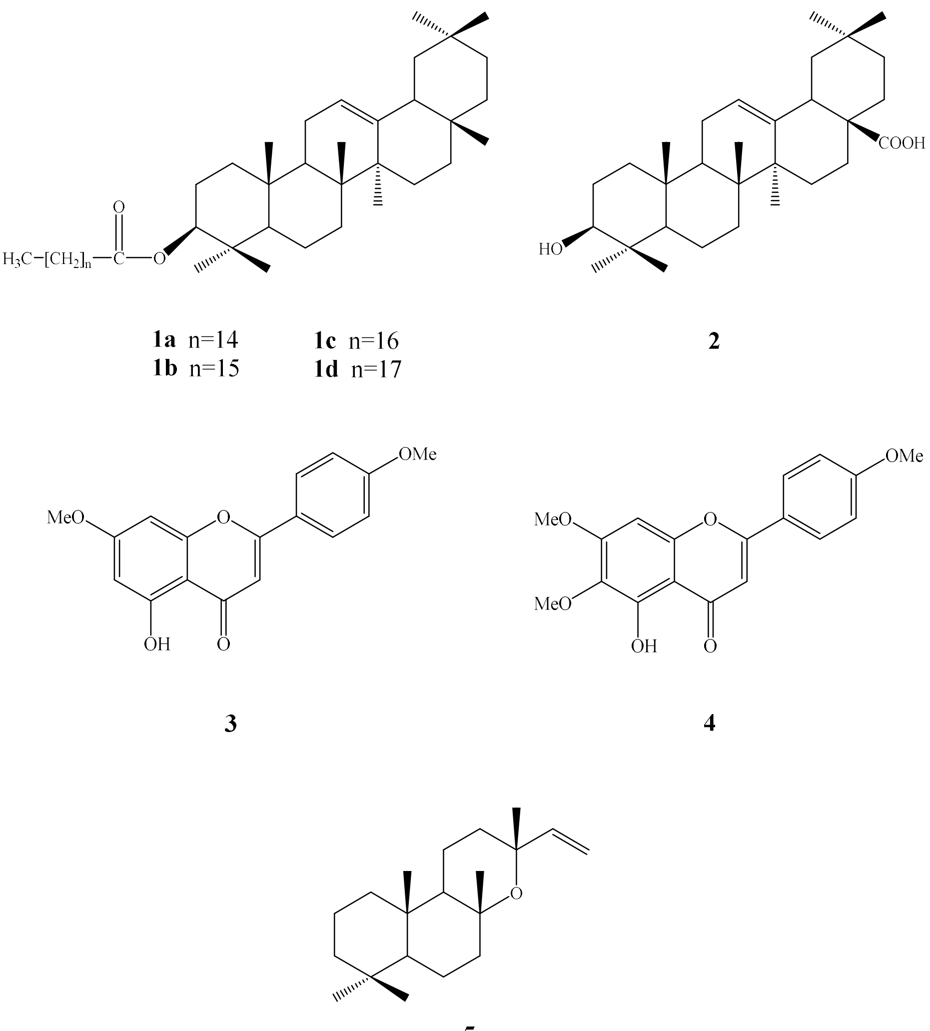

Figure 1. Chemical structures of the compounds isolated from the dichloromethane extract of *S. cassia.*

The antioxidant and anticholinesterase activities for the dichloromethane and water extracts, which were partitioned from the crude ethanol extract, were found to be higher than formerly reported results, on different extracts of *Salvia cassia* [17]. The water extract, which was partitioned from the crude ethanol extract, exhibited promising anticholinesterase activity. This result emphasizes the importance of the extraction method on the extent of activities.

This is the first phytochemical study of *Salvia cassia*. Terpenoid and flavonoid constituents were isolated by chromatographic techniques and identified by spectroscopic methods. Along with the known compounds, the triterpene acid oleanolic acid (**2**) [27], two flavonoids apigenin 7,4′-dimethyl ether (**3**) [12] and salvigenin (**4**) [12] and the diterpene manoyloxide (**5**) [28] (Figure 1), and a series of triterpene esters were described for the first time from *Salvia cassia*. The terpenoid moiety, α-amyryl, was identified by ^{1}H NMR and ^{13}C NMR techniques (Table 7). The long chain ester moieties were identified by MS analysis as hexa-, hepta-, octa-, and nona-decanoate esters. The odd carbon numbered esters are not common within the plant world, but have been reported in some studies [32]. The fatty acid esters are those of the free fatty acids, hexadecanoic acid, heptadecanoic acid, octadecanoic acid and nonadecanoic acid, amounting to nearly 50% of the whole fatty acid composition of *Salvia cassia*, reported as the result of the GC-MS analysis (Table 6).

Table 7. NMR data for compound **1** including **1a**, **1b**, **1c**, **1d** (at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, in $CDCl_3$, δ in ppm and J in Hz)

C, H position	δ_C		δ_H
1	38.5	CH_2	
2	27.1	CH_2	
3	80.8	CH	4.49 dd, J= 8.4, 6.8 Hz
4	39.9	q	
5	55.5	CH	
6	18.5	CH_2	
7	32.8	CH_2	
8	37.4	q	
9	47.8	CH	
10	37.1	q	
11	23.7	CH_2	
12	122.0	CH	5.17 br s
13	145.2	q	
14	41.9	q	
15	26.2	CH_2	
16	26.4	CH_2	
17	32.2	q	
18	47.4	CH	
19	47.0	CH_2	
20	31.3	q	
21	34.9	CH_2	
22	38.0	CH_2	
23	28.6	CH_3	0.86, s
24	14.4	CH_3	0.86, s
25	15.8	CH_3	0.95, s
26	17.0	CH_3	0.96, s
27	26.2	CH_3	1.12, s
28	28.3	CH_3	0.82, s
29	33.6	CH_3	0.86, s
30	23.9	CH_3	0.86, s
C=O	175		
long chain methylenes	29.95-29.41	CH_2	1.24 br s
α-methylene	35.1	CH_2	2.28 t, J=6.8Hz

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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