

## 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  $\beta$ -amyryn 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:** *Salvia cassia*; antioxidant activity; anticholinesterase activity; fatty acid composition;  $\beta$ -amyryn 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.  $\beta$ -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,  $\text{CH}_2\text{Cl}_2$  and  $\text{CH}_3\text{OH}$  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  $\beta$ -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  $\text{CH}_2\text{Cl}_2$  yielded oleanolic acid (**2**). Combined fractions 30-46 eluted with  $\text{CH}_2\text{Cl}_2$  were refractionated on *Si Gel* column and yielded apigenin 7, 4'-dimethyl ether (**3**) (benzene 25:  $\text{CH}_2\text{Cl}_2$  75 solvent system) and salvigenin (**4**) (solvent  $\text{CH}_2\text{Cl}_2$ ), after rechromatography on *Sephadex LH-20* (Sigma-Aldrich, Cat. No. LH2010) columns in MeOH and preparative chromatography on *Si Gel* plates (*Silica gel* 60  $\text{F}_{254}$ , Merck Cat. No. 105554). The combined fractions 47-52 eluted with  $\text{CH}_2\text{Cl}_2$ - $\text{CH}_3\text{OH}$  (90:10 – 80:20) systems were refractionated on a *Si Gel* column. Fraction 10 (from 47-52) eluted with PE: $\text{CH}_2\text{Cl}_2$  (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);  $\beta$ -Carotene, linoleic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA),  $\alpha$ -tocopherol, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from E. Merck (Darmstadt, 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

<sup>1</sup>H NMR spectra were recorded on Varian Mercury-VX at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C (APT) in CDCl<sub>3</sub>; 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<sup>-1</sup> 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  $\alpha$ -tocopherol were used as standards.

#### 2.7.2. $\beta$ -Carotene-Linoleic Acid Assay

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

#### 2.7.3. Metal Chelating Activity

The ferrous ion-chelating capacity of the extracts was estimated by the Fe<sup>2+</sup>-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 $\beta$ -Amyrin Fatty Acid Esters (**1a**, **1b**, **1c**, **1d**):

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

## 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 <sup>b</sup> ( $\mu$ g QE/mg extract)	Amount of flavonoids ( $\mu$ g QE/mg extract) <sup>c</sup>
Crude ethanol extract	39.87 $\pm$ 1.12	27.95 $\pm$ 0.36
Dichloromethane extract	21.34 $\pm$ 0.22	41.43 $\pm$ 0.65
Water extract	44.65 $\pm$ 0.31	23.23 $\pm$ 0.43

<sup>a</sup>Average of three parallel measurements. <sup>b</sup>PE, pyrocatechol equivalents. <sup>c</sup>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,  $\beta$ -carotene-linoleic acid test system for lipid peroxidation inhibition determination, and Fe<sup>+2</sup>-ferrozine system for metal chelation capacity determination (Tables 2-4).

**Table 2.** DPPH free radical scavenging capacity of the *Salvia cassia* extracts (Inhibition %)<sup>a</sup>

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 <sup>b</sup>	63.18±1.38	93.93±0.05	94.48±0.39	94.79±0.50
BHT <sup>b</sup>	55.02±0.60	83.54±0.56	92.62±0.02	94.42±0.28

<sup>a</sup>Average of three parallel measurements. <sup>b</sup>Reference compounds.

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

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 <sup>b</sup>	63.54± 0.93	63.66± 0.58	65.22± 0.91	66.22± 1.00
BHA <sup>b</sup>	90.49± 0.53	92.00± 0.01	95.12±1.29	96.54±0.01
α-Tocopherol <sup>b</sup>	60.91±0.07	72.92±0.79	88.17±0.73	84.23±0.92

<sup>a</sup>Average of three parallel measurements. <sup>b</sup>Reference compounds.

**Table 4.** Ferrous ion chelating capacity of the *Salvia cassia* extracts. (Inhibition %)<sup>a</sup>

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 <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>
Quercetin <sup>c</sup>	4.00±0.33	5.00±0.08	7.38±0.83	14.37±0.30
(+)-Catechin <sup>c</sup>	0.27±0.52	0.53±0.99	0.91±0.95	1.77±0.70

<sup>a</sup>Average of three parallel measurements. <sup>b</sup>NA: not active. <sup>c</sup>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 ( $73.05 \pm 1.61$  at  $200 \mu\text{M}$ ). 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  $\beta$ -amyrin fatty acids also showed inhibition on AChE enzyme (Table 5). As manoyloxide showed low activity against AChE at  $200 \mu\text{M}$  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<sup>a</sup>

	Inhibition % against AChE <sup>a</sup>				Inhibition % against BChE <sup>a</sup>			
	25 $\mu\text{M}$	50 $\mu\text{M}$	100 $\mu\text{M}$	200 $\mu\text{M}$	25 $\mu\text{M}$	50 $\mu\text{M}$	100 $\mu\text{M}$	200 $\mu\text{M}$
Crude ethanol extract	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>
Dichloromethane extract	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	$6.93 \pm 0.92$	$8.50 \pm 0.29$	$13.65 \pm 1.32$	$24.46 \pm 2.45$
Water extract	$33.05 \pm 0.62$	$43.60 \pm 0.55$	$59.90 \pm 0.86$	$73.05 \pm 1.61$	$22.35 \pm 1.02$	$39.98 \pm 1.09$	$48.78 \pm 0.99$	$52.89 \pm 1.66$
FAE <sup>c</sup> mixture	$38.80 \pm 0.22$	$44.80 \pm 0.75$	$55.29 \pm 0.79$	$50.39 \pm 0.80$	$17.04 \pm 0.67$	$38.18 \pm 0.75$	$38.89 \pm 0.78$	$41.85 \pm 0.82$
Oleanolic acid	$49.52 \pm 1.03$	$55.16 \pm 0.98$	$78.36 \pm 1.13$	$82.85 \pm 0.88$	$14.02 \pm 0.07$	$18.90 \pm 0.14$	$22.36 \pm 0.43$	$26.91 \pm 0.82$
Manoyloxide	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	$16.75 \pm 0.23$	NA <sup>b</sup>	NA <sup>b</sup>	$20.14 \pm 0.38$	$28.32 \pm 0.34$
Salvigenin <sup>9</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	NA <sup>b</sup>	$4.85 \pm 0.54$	$5.67 \pm 0.36$	$7.96 \pm 0.67$	$14.19 \pm 0.51$
Apigenin 7,4'-dimethyl ether <sup>9</sup>	$1.78 \pm 0.87$	$3.78 \pm 0.58$	$7.35 \pm 0.58$	$12.35 \pm 0.99$	$30.59 \pm 0.73$	$35.69 \pm 1.29$	$42.31 \pm 0.36$	$50.31 \pm 0.94$
Galanthamine <sup>d</sup>	$69.38 \pm 1.10$	$75.98 \pm 0.95$	$78.19 \pm 0.48$	$83.41 \pm 0.53$	$40.59 \pm 0.88$	$48.73 \pm 0.90$	$65.02 \pm 0.44$	$74.22 \pm 0.89$

<sup>a</sup>Average of three parallel measurements. <sup>b</sup>Not active. <sup>c</sup>Fatty acid esters mixture. <sup>d</sup>Standard drug.

### 3.2. Phytochemical Analysis

Since the antioxidant activity of the dichloromethane extract was found to be fairly high for the  $\text{Fe}^+$ -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  $^1\text{H}$  and  $^{13}\text{C}$  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 indices (RI) and percentages are shown in Table 6.

**Table 6.** Identified compounds from the fraction of 17-21 of the *Salvia cassia* dichloromethane extract<sup>a</sup>

RI	Compound <sup>a</sup>	%
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

<sup>a</sup>The acids were analyzed after methylation of the sample.

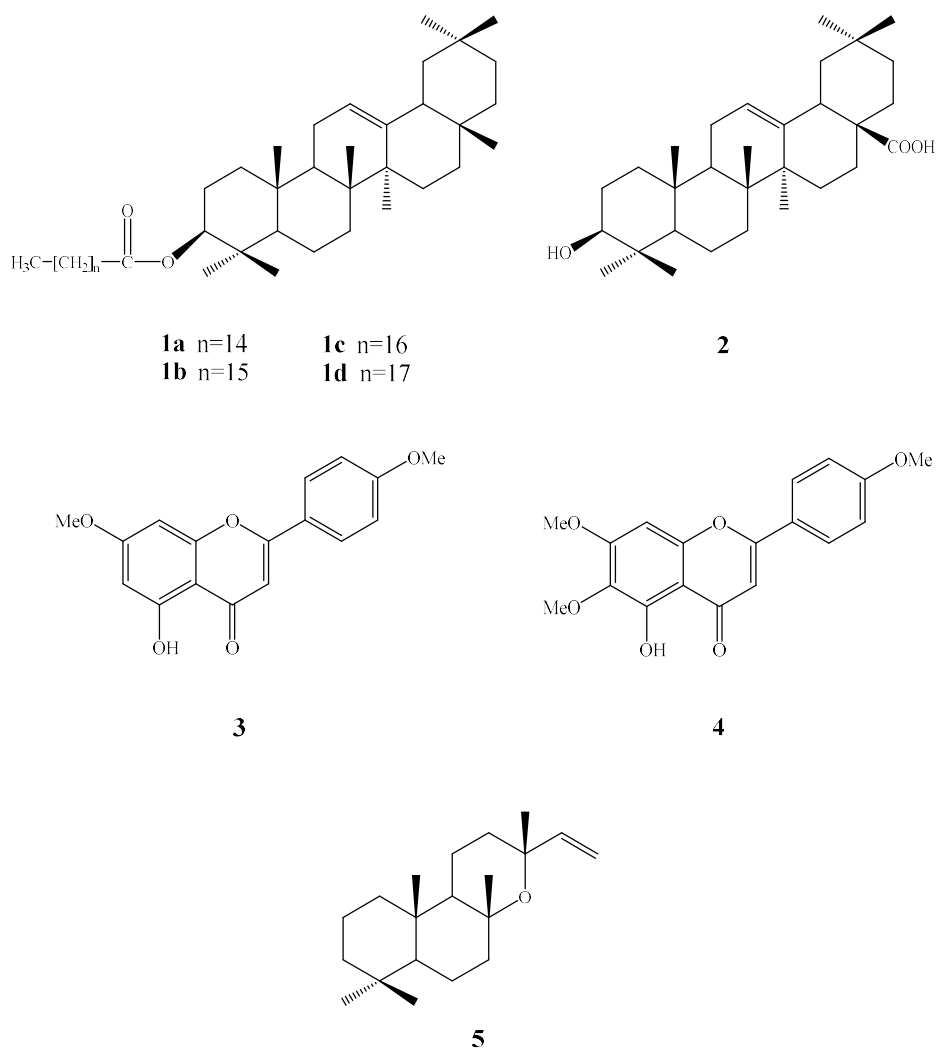
The rest of the dichloromethane extract of *Salvia cassia* was fractionated on silica gel columns, repeatedly. Further purifications were achieved by chromatography on Sephadex LH-20 columns and by preparative TLC.

The process yielded a series of fatty acid esters of  $\beta$ -amyryn (**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 <sup>1</sup>H NMR and <sup>13</sup>C NMR techniques and through comparison with literature values.

The <sup>1</sup>H NMR and <sup>13</sup>C NMR DEPT data for **1** correlated with  $\Delta^{12}$ -pentacyclic triterpenoid ring structure with eight methyl groups at  $\delta_c$  28.6, 14.4, 15.8, 17.0, 26.2, 28.3, 33.6, 23.9 ppm and  $\delta_H$  0.82, 0.86 (four overlapping peaks), 0.95, 0.96, 1.12 ppm, one vinylic proton peak at  $\delta_H$  5.17 and methyne carbon at  $\delta_c$  122.0 ppm, and a one-proton doublet of doublets at  $\delta_H$  4.49 with a methyne carbon at  $\delta_c$  80.8 ppm indicating an ester linkage. The long chain fatty acid moiety was suggested by an intense broad singlet at  $\delta$  1.24 (methylene chain) and a two proton triplet at  $\delta$  2.28 ( $J=6.8$  Hz) ( $\alpha$ -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  $\beta$ -amyryl hexadecanoate (**1a**),  $\beta$ -amyryl heptadecanoate (**1b**),  $\beta$ -amyryl octadecanoate (**1c**) and  $\beta$ -amyryl nonadecanoate (**1d**), which differ from each other with only a CH<sub>2</sub> 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  $\Delta^{12}$  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].  $\beta$ -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 salviagin (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,  $\alpha$ -amyryl, was identified by  $^1\text{H}$  NMR and  $^{13}\text{C}$  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  $^1\text{H}$  NMR and 100 MHz for  $^{13}\text{C}$  NMR, in  $\text{CDCl}_3$ ,  $\delta$  in ppm and  $J$  in Hz)

C, H position	$\delta_{\text{C}}$		$\delta_{\text{H}}$
1	38.5	CH <sub>2</sub>	
2	27.1	CH <sub>2</sub>	
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 <sub>2</sub>	
7	32.8	CH <sub>2</sub>	
8	37.4	q	
9	47.8	CH	
10	37.1	q	
11	23.7	CH <sub>2</sub>	
12	122.0	CH	5.17 br s
13	145.2	q	
14	41.9	q	
15	26.2	CH <sub>2</sub>	
16	26.4	CH <sub>2</sub>	
17	32.2	q	
18	47.4	CH	
19	47.0	CH <sub>2</sub>	
20	31.3	q	
21	34.9	CH <sub>2</sub>	
22	38.0	CH <sub>2</sub>	
23	28.6	CH <sub>3</sub>	0.86, s
24	14.4	CH <sub>3</sub>	0.86, s
25	15.8	CH <sub>3</sub>	0.95, s
26	17.0	CH <sub>3</sub>	0.96, s
27	26.2	CH <sub>3</sub>	1.12, s
28	28.3	CH <sub>3</sub>	0.82, s
29	33.6	CH <sub>3</sub>	0.86, s
30	23.9	CH <sub>3</sub>	0.86, s
C=O	175		
long chain methylenes	29.95-29.41	CH <sub>2</sub>	1.24 br s
$\alpha$ -methylene	35.1	CH <sub>2</sub>	2.28 t, $J=6.8$ Hz

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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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## References

- [1] F. Celep and A. Kahraman (2012). *Salvia* L., In: Türkiye Bitkileri Listesi (Damarlı Bitkiler), ed: A. Güner, S. Aslan, T. Ekim, M. Vural, M.T. Babaç, Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.
- [2] T. Baytop (1999). Türkiye’de Bitkiler ile Tedavi, Geçmişte ve Bugün. Nobel Press, İstanbul, 142-144.
- [3] B. Bozan, N. Öztürk, M. Koşar, Z. Tunalier and K.H.C. Başer (2002). Antioxidant and free radical scavenger activities of eight *Salvia* species, *Chem. Nat. Compd.* **38**, 198-200.
- [4] G. Topçu, A. Ertaş, U. Kolak, M. Öztürk and A. Ulubelen (2007). Antioxidant activity tests on novel triterpenoids from *Salvia macrochlamys*, *Arkivoc* **7**, 195-208.
- [5] I. Kıvrak, M.E. Duru, M. Öztürk, N. Mercan, M. Harmandar and G. Topçu (2009). Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*, *Food Chem.* **116**, 470-479.
- [6] U. Kolak, A. Kabouche, M. Ozturk, Z. Kabouche, G. Topcu and A. Ulubelen (2009). Antioxidant Diterpenoids from the Roots of *Salvia barrelieri*, *Phytochem. Anal.* **20**, 320-327.
- [7] A. Ulubelen and G. Topçu (1998). Chemical and biological investigations of *Salvia* species growing in Turkey. In: *Studies in Natural Products Chemistry*, ed: Atta-ur Rahman, Elsevier, New York, **20**, pp.659-718.
- [8] A. Ulubelen (2003). Cardioactive and antibacterial terpenoids from some *Salvia* species, *Phytochemistry* **24**, 1386-1387.
- [9] G. Topçu and A. C. Gören (2007). Biological activity of diterpenoids isolated from Anatolian Lamiaceae plants, *Rec. Nat. Prod.* **1**, 1-16.
- [10] N. Gürsoy, B. Tepe and H. A. Akpulat (2012). Chemical composition and antioxidant activity of the essential oils of *Salvia palaestina* (Bentham) and *S. ceratophylla* (L.), *Rec. Nat. Prod.* **6**, 278-287.
- [11] N. Tan, D. Satana, B. Şen, E. Tan, H. B. Altan, B. Demirci and M. Uzun (2016). Antimycobacterial and antifungal activities of selected four *Salvia* species, *Rec. Nat. Prod.* **10**, 593-603.
- [12] U. Kolak, I Hacibekiroğlu, M. Öztürk, F. Özgökçe, G. Topçu and A. Ulubelen (2009). Antioxidant and anticholinesterase constituents of *Salvia pocolata*, *Turk. J. Chem.* **33**, 813-823.
- [13] G. Tel, M. Öztürk, M.E. Duru, M. Harmandar and G. Topçu (2010). Chemical composition of the essential oil and hexane extract of *Salvia chionantha* and their antioxidant and anticholinesterase activities, *Food Chem. Toxicol.* **48**, 3189-3193.
- [14] M.E. Duru, G. Tel and M. Öztürk (2012). Chemical composition, antioxidant and anticholinesterase activities of the essential oil of *Salvia chrysophylla* Stapf. *Rec. Nat. Prod.* **6**, 175-179.
- [15] I.E. Orhan, F.S. Şenol, T. Erçetin, A. Kahraman, F. Celep, G. Akaydın, B. Şener and M. Doğan (2013). Assessment of anticholinesterase and antioxidant properties of selected sage (*Salvia*) species with their total phenol and flavonoid contents, *Ind. Crop. Prod.* **41**, 21-30.
- [16] F.S. Şenol, I. Orhan, F. Celep, A. Kahraman, M. Doğan, G. Yılmaz and B. Şener (2010). Survey of 55 Turkish *Salvia* taxa for their acetylcholinesterase inhibitory and antioxidant activities, *Food Chem.* **120**, 34-43.
- [17] I.E. Orhan, F.S. Şenol, N. Öztürk, G. Akaydın and B. Şener (2012). Profiling of *in vitro* neurobiological effects and phenolic acids of selected endemic *Salvia* species, *Food Chem.* **132**, 1360-1367.
- [18] M.J.R. Howes, N.S.L. Perry and P.J. Houghton (2003). Plants with traditional uses and activities relevant to the management of Alzheimer’s disease and other cognitive disorders, *Phytother. Res.* **17**, 1-18.
- [19] N.S.L. Perry, G. Court, N. Bidet, J. Court and E.K. Perry (1996). Cholinergic activities of European herbs and potential for dementia therapy, *J. Geriat. Psychiat.* **11**, 1063-1069.
- [20] N.S.L. Perry, P.J. Houghton, J. Sampson, A.E. Theobald, S. Hart, M. Lis-Balchin, J.R.S. Hoult, P. Evans, P. Jenner, S. Milligan and E.K. Perry (2001). *In vitro* activities of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer’s disease, *J. Pharm. Pharmacol.* **53**, 1347-1356.
- [21] K. Slinkard and V.L. Singleton (1977). Total phenol analyses: Automation and comparison with manual methods, *Am. J. Enol. Viticult.* **28**, 49-55.
- [22] M.I.N. Moreno, M.I. Isla, A.R. Sampietro and M.A. Vattuone (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina, *J. Ethnopharmacol.* **71**, 109-114.
- [23] M.S. Blois (1958). Antioxidant determinations by the use of a stable free radical, *Nature* **181**, 1199-1200.

- [24] G.J. Marco (1968). A rapid method for evaluation of antioxidants, *J. Am. Oil. Chem. Soc.* **45**, 594-598.
- [25] E.A. Decker and B. Welch (1990). Role of ferritin as a lipid oxidation catalyst in muscle food, *J. Agric. Food Chem.* **38**, 674-677.
- [26] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherston (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochemical Pharmacol.* **7**, 88-95.
- [27] W. Seebacher, N. Simic, R. Weis, R. Saf and O. Kunert (2003). Complete assignments of <sup>1</sup>H and <sup>13</sup>C-NMR resonances of oleanolic acid, 18 alpha-oleanolic acid, ursolic acid and their 11-oxo derivatives, *Magn. Res. Chem.* **41**, 636-638.
- [28] C. Zdero, F. Bohlmann and R.M. King (1992). Ent-Labdanes, manoyloxiide and helipterol derivatives from *Chrysocephalum ambiguum*, *Phytochemistry* **31**, 1631-1638.
- [29] S.B. Mahato and A.P. Kundu (1994). <sup>13</sup>C NMR Spectra of pentacyclic triterpenoids - A compilation and some salient features, *Phytochemistry*, **37**, 1517-1575.
- [30] B. Sen-Utsukarci, B. Gurdal, M. Bilgin, D. Satana, B. Demirci, N.Tan and Afife Mat (2019). Biological activities of various extracts from *Salvia cassia* Sam. ex Rech.f. and chemical composition of its most active extract, *Rec.Nat.Prod.* **13(1)**, 24-36.
- [31] G. Janicsak, I. Zupko, I. Mathe and J. Hohmann (2010). Comparative study of the antioxidant activities of eleven *Salvia* species, *Nat. Prod. Commun.* **5**, 227-230.
- [32] V.O. Elias, B.R.T. Simoneit, A.S. Pereira and J.N. Cardoso (1997). Mass spectra of triterpenyl alkanoates, novel natural products, *J. Mass. Spec.* **32**, 1356-1361.

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