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Antioxidant potential and phenolic constituents of *Salvia cedronella*

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Abstract

The acetone extract of the aerial parts of the plant *Salvia cedronella* Boiss. was screened for its total phenolic content and flavonoid content. The antioxidant potential was evaluated, *in vitro*, by using three different assays; β-carotene–linoleic acid test system for total antioxidant activity, DPPH for free radical scavenging activity, Fe^{2+}–ferrozine test system for metal chelation. A high content of phenolics, potent radical scavenging ability and significant iron chelating effect were observed. However, the inhibition of lipid peroxidation was not significant in β-carotene–linoleic acid test system. A phytochemical analysis yielded a new coumarin, 3-methoxy-4-hydroxymethyl coumarin, together with *p*-hydroxyphenylethyl docosanoate, and two triterpenoids oleanolic acid and betulinic acid.

Keywords: *Salvia cedronella*; Antioxidant activity; 3-Methoxy-4-hydroxymethyl coumarin; *p*-Hydroxyphenylethyl docosanoate

1. Introduction

Synthetic antioxidants such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole) and TBHQ (tertiary butylhydroquinone) have commonly been used to prevent oxidative deterioration of fats and oily foods. Nowadays, however, the scientists have casted some toxicological doubts on synthetic antioxidants due to their adverse side effects, and people are more concerned about food safety and quality. Since herbs and spices are a major source of natural antioxidants, antioxidant activity of Lamiaceae (Labiatae) plants which are widely grown in Turkey were investigated (Topçu,, Ertaş, Kolak, Öztürk, & Ulubelen, 2007). *Salvia* genus is represented by 94 taxa in Turkey, half of them being endemic (Güner, Özhatay, Ekim, & Başer, 2000; Hedge, 1982). We have been studying "*Salvia* genus" for many years and obtained various bioactive compounds (Topçu, 2006; Ulubelen & Topçu, 1998) from over 50 species. Since antiquity, *Salvia* species have been well known plants and widely used as folk medicines with antibacterial (Ulubelen, Öksüz, Topçu, Gören, & Voelter, 2001), antituberculosis (Ulubelen, Topçu, Lotter, Wagner, & Eris, 1994), antiviral, cytotoxic (Topçu et al., 2003; Topçu & Ulubelen, 1999), cardiovascular (Ulubelen, 2003; Ulubelen et al., 2002), liver protective, and other properties (Zhou, Zuo, & Chow, 2005). Sage is also used to preserve foods, especially meat and cheese, due to its antioxidant properties, as well as being employed as a spice for flavoring (Daniela, 1993).

Phytochemical investigations have shown that *Salvia* species are mainly rich in diterpenoids (Topçu & Ulubelen, 2007; Ulubelen & Topçu, 1992; Ulubelen, Topçu, Sönmez, Choudhary, & Atta-ur-Rahman, 1995; Ulubelen, Topçu, & Tan, 1992) and triterpenoids (Topçu, 2006; Topçu, Türkmen, Schilling, & Kingston, 2004; Topçu, Ulubelen, & Eris, 1994) as well as in flavonoids (Topçu, Tan, Ulubelen, Sun, & Watson, 1995) and other phenolic compounds (Lu & Foo, 2002). Phenolics are non-essential dietary components which have been associated with inhibition of atherosclero-

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sis and cancer, and the phenolic content of the plant extracts was found to be well correlated with their antioxidant activity (Velioglu, Mazza, Gao, & Oomah, 1998). In the course of our search for new bioactive compounds from *Salvia* species, the total content of phenolic compounds and flavonoids of the acetone extract of *Salvia cedronella*, an endemic species in Turkey, was determined. The extract showed a high content of total phenolics, whereas the flavonoid content was found to be quite poor. In general, the scavenging properties of phenolic compounds have been well documented by the researchers (Miura, Kikuzaki, & Nakatani, 2002; Piccinelli, DeSimone, Passi, & Rastrelli, 2004), and there are several antioxidant activity studies on *Salvia* species (Kabouche, Kabouche, Öztürk, Kolak, & Topçu, 2007; Topçu et al., 2007; Weng & Wang, 2000) including a screening on the antioxidant potentials of some *Salvia* extracts and essential oils from Turkey (Tepe et al., 2004; Tepe, Sokmen, Akpulat, & Sokmen, 2006). In this study, the acetone extract of the aerial parts of *S. cedronella* was evaluated by three methods for total antioxidant, DPPH free radical scavenging and metal chelating activities. Fractionation of the extract led to the isolation of an uncommon phenolic ester (**1**), which has not previously been isolated from a *Salvia* species, and a new coumarin (**2**), together with the known triterpenoids oleanolic acid and betulinic acid.

2. Materials and methods

2.1. Chemicals and spectral measurements

Ferrous chloride, chloroform, methanol, pyrocatechol, quercetin (Q) were obtained from E. Merck (Darmstadt, Germany). Folin–Ciocalteu's reagent (FCR), β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and α-tocopherol (TOC), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (Ferrozine) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

UV–VIS measurements were recorded on a Shimadzu UV-1601 (Kyoto, Japan). IR: Perkin Elmer 1600 FTIR. ^{1}H NMR and ^{13}C NMR: Varian Mercury-Mx at 400 MHz for ^{1}H and 100 MHz for ^{13}C, with TMS as internal standard. EIMS: VG Zab Spec GC–MS spectrometer.

2.2. Plant material, preparation of extracts and isolation of compounds

S. cedronella Boiss. was collected and identified by Dr. Tuncay Dirmenci and Dr. Turgut Kılıç from Burdur in Turkey (Hedge, 1982) in July 2003. A voucher specimen (T.D. 2301) was deposited at the Special Collection of Dr. Tuncay Dirmenci at the Biology Department, Balikesir University, Turkey.

The air-dried aerial parts of the plant (580 g) were extracted with acetone to give 8.8 g of crude extract. The latter was chromatographed on a silica gel column, eluted with solvent systems of increasing polarity from Petroleum Ether, Petroleum ether-CH_2Cl_2, CH_2Cl_2-MeOH to MeOH. Sixty fractions, each 200 mL were collected. Fractions were monitored by TLC and similar fractions were combined. Fractions 29–35 (CH_2Cl_2-MeOH, 95:5) were rechromatographed on a silica gel column. Further purification by preparative thin layer chromatography afforded compounds **1** (38 mg) and **2** (11 mg), and the triterpenoid oleanolic acid. Fractions 36–38 (CH_2Cl_2-MeOH, 90:10) were combined and rechromatographed on a silica gel column. The triterpenoid, betulinic acid was obtained after further purification by preparative thin layer chromatography.

2.2.1. 2-(4-Hydroxyphenyl)ethanol ester of docosanoic acid (*1*)

IR ν (KBr): 3420, 2915, 2847, 1730.5, 1480, 1290, 1160 cm^{-1}. UV λ_{max} (MeOH) (log ε): 227 (4.2), 240 (3.0), 278 (3.8) nm. EIMS *m/z* (relative intensity): 459.2 $[M-1]^+$ (13), 442 $[M-H_2O]^+$ (22), 367 $[M-C_6H_4OH]^+$ (11), 339 (13), 323 (45), 295 (12), 165 (66), 121 $[M-OOC{-}C_{20}H_{40}CH_3]^+$ (100), 93 $[121-C_2H_4]^+$ (7), 91 (14). ^{1}H NMR and ^{13}C NMR data: see Table 1, Figs. 3 and 4.

2.2.2. 3-Methoxy-4-hydroxymethyl coumarin (*2*)

UV λ_{max} (MeOH) (log ε): 207 (4.8), 227 (4.2), 275 (3.2) and 302 (3.0) nm. ^{1}H NMR and ^{13}C NMR data: see Table 2, Figs. 5 and 6.

Table 1
^{1}H NMR and ^{13}C NMR data for compound **1** (400 MHz and 100 MHz, in CD_3OD, *J* in Hz)

C	δ_H (*J*)	δ_C
1′		130.21
2′, 6′	6.67 br d (8.19)	130.16
3′, 5′	7.00 br d (8.19)	115.47
4′		154.27
7′	2.78, 2H, t (7.02)	34.70
8′	4.15, 2H, t (7.02)	64.01
1		173.21
α	2.20, 2H, t (7.4)	34.62
β	1.58, 2H, br s	32.13
γ	1.56, 2H, br s	30.17
$(CH_2)_{17}$	1.24, 34H, br s	29.16
CH_3	0.81, 3H, t (6.82)	14.52
OH	4.60, br s	

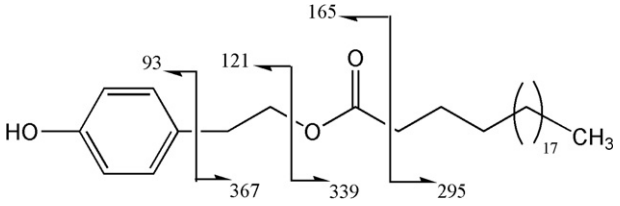

Fig. 1. Mass fragmentation pattern of **1**.

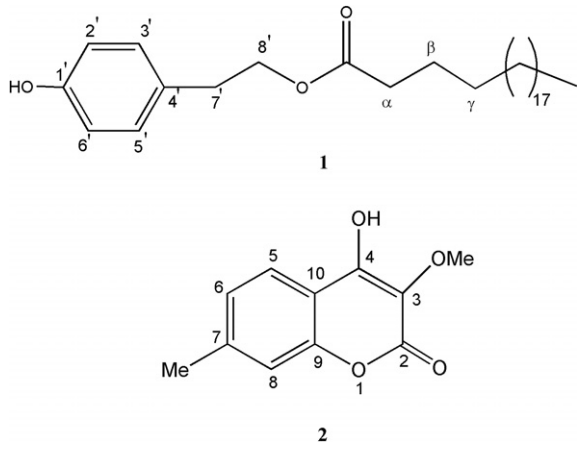

Fig. 2. *p*-Hydroxyphenylethyl docosanoate (**1**) and 3-methoxy-4-hydroxy-7-methylcoumarin (**2**).

2.3. Determination of total phenolic concentration

The concentrations of phenolic content in extract were expressed as microgram of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton (1977). One milliliter of the solution (contains 1 mg) of the extracts in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 min, 3 mL of sodium carbonate (2%) were added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard pyrocatechol graph:

$$\text{Absorbance} = 0.08237\ \text{pyrocatechol}\ (\mu g) + 0.00058 \quad (R^2 : 0.9985)$$

2.4. Determination of total flavonoid concentration

Measurement of flavonoid concentration of the extract was based on the method described by Park, Koo, Ikegaki, and Contado (1997) with a slight modification and results were expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg) extracts in methanol was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.06648\ \text{quercetin}\ (\mu g) - 0.01586 \quad (R^2 : 0.9972)$$

2.5. Determination of the antioxidant activity with the β-carotene bleaching method

The antioxidant activity of the *S. cedronella* extract was evaluated using β-carotene–linoleic acid model system

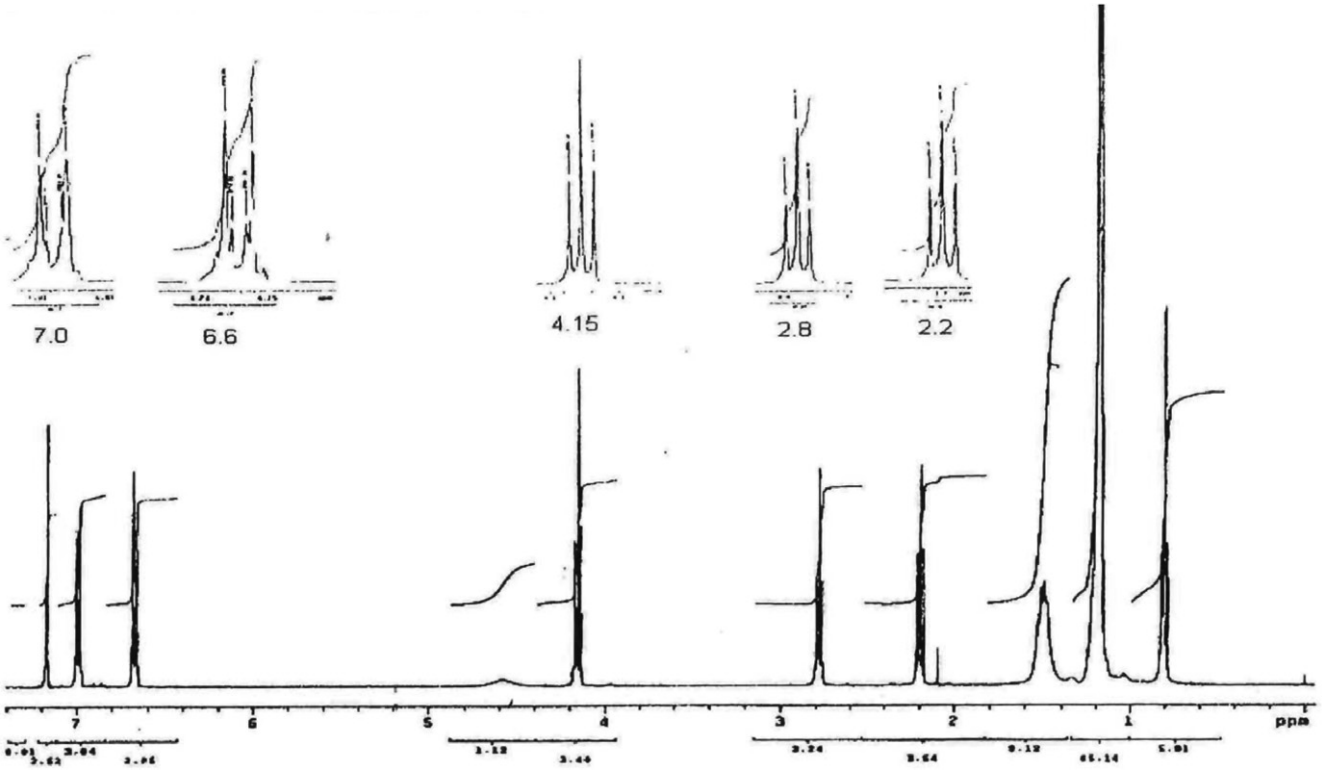

Fig. 3. ^{1}H NMR spectrum of compound **1**.

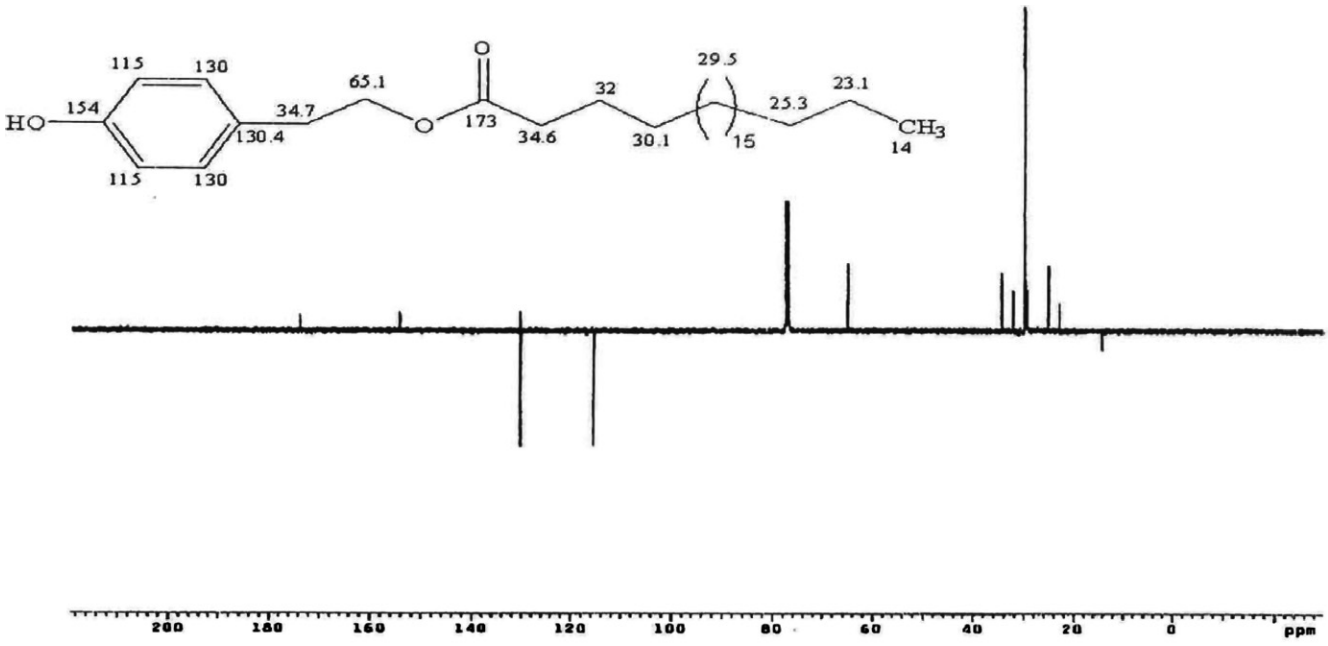

Fig. 4. ^{13}C NMR spectrum of compound **1**.

Table 2
^{1}H NMR and ^{13}C NMR data of compound **2** (400 MHz and 100 MHz, in CD_3OD, *J* in Hz)

C	δ_H (*J*)	δ_C
2	–	160.13
3	–	130.29
4	–	150.35
5	6.95, d (8.5)	114.22
6	7.54, dd (8.5;1.95)	124.45
7	–	146.34
8	7.52, d (1.95)	110.48
9	–	150.64
10	–	114.22
4-OH	6.06, s	–
3-OMe	3.92, s	57.33
7-Me	2.56, s	27.56

(Kabouche et al., 2007; Miller, 1971). β-Carotene (0.5 mg) in 1 mL of chloroform was added to 25 μL of linoleic acid, and 200 mg of Tween-40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, were added by vigorous shaking. Four thousand microliters of this mixture were transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50 °C. A blank, devoid of β-carotene, was prepared for background subtraction. Quercetin, BHT, BHA and α-tocopherol were used as standards.

2.6. Free radical scavenging activity

The free radical scavenging activity was determined by the DPPH assay described by Blois (1958). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation (Gülçin, Oktay, Kireçci, & Küfrevioğlu, 2003):

$$\text{DPPH Scavenging Effect } (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.7. Metal chelating activity

The chelating activity on Fe^{2+} was measured as reported by Decker and Welch (1990). The extracts were added to a solution of 2 mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture reached equilibrium, the absorbance was determined at 562 nm, results were given as percentage inhibition (Gülçin et al., 2003).

2.8. Statistical analysis

All data on all antioxidant 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, *p* values <0.05 were regarded as significant.

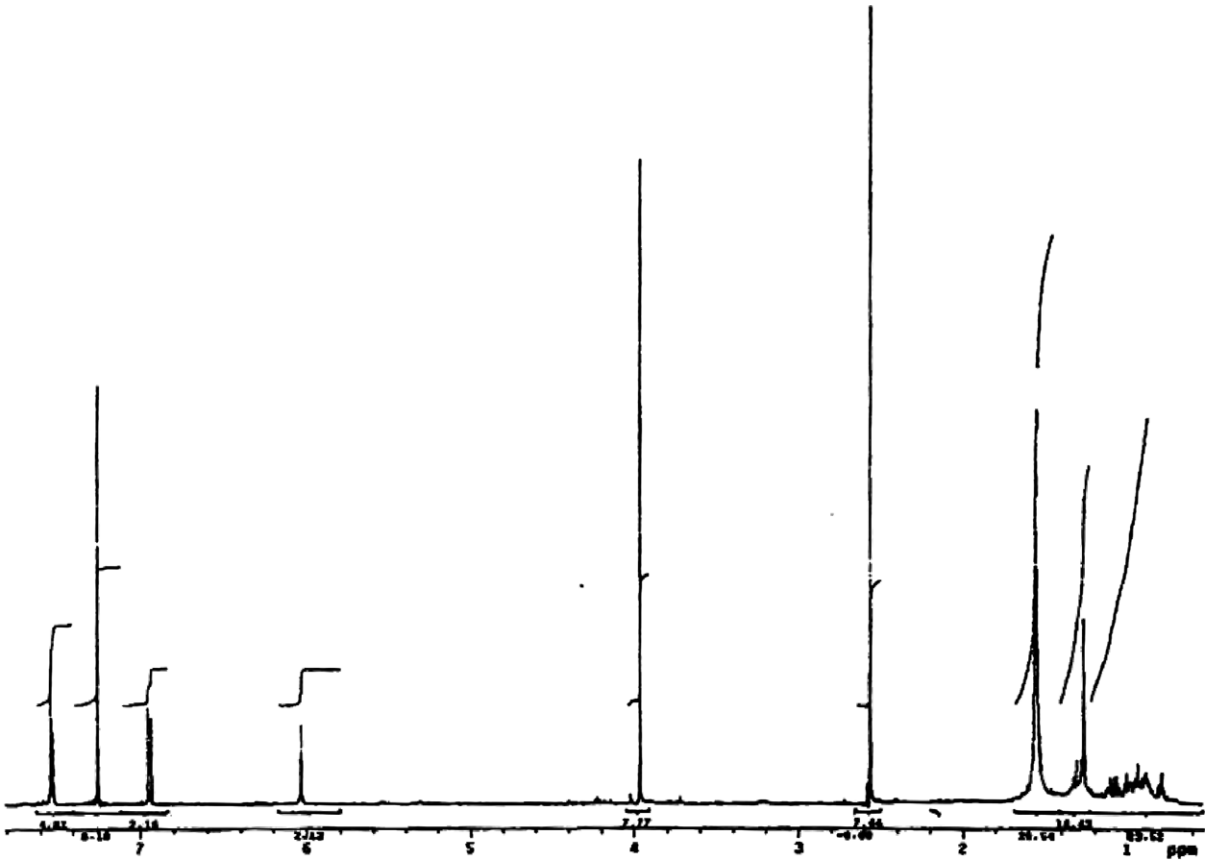

Fig. 5. ^{1}H NMR spectrum of compound **2**.

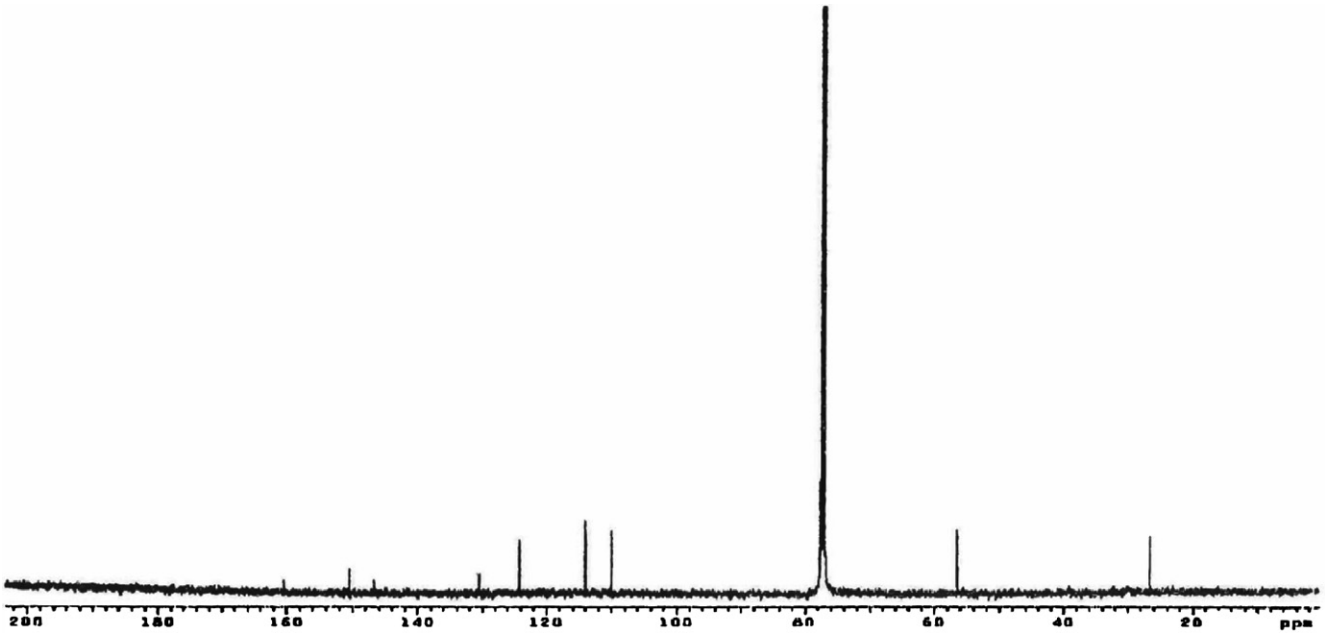

Fig. 6. ^{13}C NMR spectrum of compound **2**.

3. Results and discussion

Since *Salvia* species are known to be rich in phenolics (Lu & Foo, 2002), the content of total phenolic compounds as well as the content of flavonoids in the acetone extract of the aerial parts of *S. cedronella* were investigated. Measurements showed that the extract has a high content of total phenolics, whereas the flavonoid content to be quite poor

(Table 3). The antioxidant activity of the extract was evaluated by three methods: total antioxidant, free radical scavenging and metal chelating activities. The extract exhibited a high DPPH free radical scavenging activity competing with the standards BHT, and α-tocopherol (Table 4) except for 10 μg/mL concentration. A moderate metal chelating effect with ferrous ions was observed in comparison to the standards quercetin and (+)-catechine (Table 5). The radical scavenging activity and the metal chelating effects were dependent on concentration and both were increased with increased amount of sample. If we make comparison with catechine, the extract showed higher metal chelating activity which can be related to its high phenolic constituent. The total antioxidant activity of the *Salvia* extract was measured as % inhibition of lipid peroxidation in the β-carotene–linoleic acid system, and was compared with the most commonly used standard antioxidants BHT, BHA, α-tocopherol, and quercetin. However, comparisons showed no potent total antioxidant activity (Table 6) for the extract.

A phytochemical analysis of the acetone extract of the aerial parts of the plant *S. cedronella* yielded two phenolic compounds. One of the phenolics proved to be an ester of a long chain fatty acid, and has been identified as *p*-hydroxyphenylethyldocosanoate (**1**). The other phenolic was identified as a new coumarin, 3-methoxy-4-hydroxymethyl coumarin (**2**). The known triterpenoids, oleanolic acid and betulinic acid were also isolated.

Table 3
Total phenolic and flavonoid contents of *S. cedronella* extract[a]

Sample	Phenolic content μg pyrocatechol/mg extract	Flavonoid content μg quercetin/mg extract
Extract	116.64 ± 1.31	24.44 ± 0.45

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

Table 4
DPPH radical scavenging activity (%) of *S. cedronella* extract, BHT, and α-tocopherol[a]

Sample	10 μg	25 μg	50 μg	100 μg
Extract	32.79 ± 0.51	76.86 ± 0.87	94.61 ± 0.01	95.39 ± 0.10
BHT	55.02 ± 0.60	83.54 ± 0.56	92.62 ± 0.02	94.42 ± 0.28
α-Tocopherol	63.18 ± 1.38	93.93 ± 0.05	94.48 ± 0.39	94.79 ± 0.50

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

Table 5
The metal chelating effect (%) of *S. cedronella* extract, quercetin and (+)-catechine on ferrous ions[a]

Sample	50 μg	100 μg	200 μg	400 μg
Extract	0.71 ± 0.66	1.86 ± 0.13	1.61 ± 0.01	6.07 ± 1.00
Quercetin	4.00 ± 0.33	5.00 ± 0.08	7.38 ± 0.83	14.37 ± 0.30
(+)-Catechine	0.27 ± 0.52	0.53 ± 0.99	0.91 ± 0.95	1.77 ± 0.70

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

Table 6
Total antioxidant activity (%) of *S. cedronella* extract, BHT, BHA, α-tocopherol, and quercetin in β-carotene–linoleic acid system[a]

Sample	10 μg	25 μg	50 μg	100 μg
Extract	13.71 ± 0.90	16.66 ± 3.11	27.04 ± 0.89	54.67 ± 0.95
BHT	63.54 ± 0.93	63.66 ± 0.58	65.22 ± 0.91	66.22 ± 1.00
BHA	90.49 ± 0.53	92.00 ± 0.01	95.12 ± 1.29	96.54 ± 0.01
α-Tocopherol	60.91 ± 0.07	72.92 ± 0.79	88.17 ± 0.73	84.23 ± 0.92
Quercetin	82.55 ± 1.95	86.80 ± 1.57	91.76 ± 0.59	93.73 ± 0.50

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

The IR spectrum of **1** displayed an ester carbonyl at 1730.5 cm^{-1} and an aromatic hydroxyl at 3420 cm^{-1} as a weak absorption band. The UV spectrum showed a substituted aromatic system with maxima at 240 and 278 nm. The ^{1}H NMR spectrum (Table 1) indicated the presence of an aromatic moiety attached to a large hydrocarbon chain. In the aromatic region, two pairs of *ortho* coupled doublets ($J = 8.19$ Hz) at δ 6.67 and 7.00, suggested a *para* substituted benzene ring. Methylene signals were observed at δ 2.78 (2H, t, $J = 7.02$ Hz, Ar-CH_2—) and δ 4.15 (2H, t, $J = 7.02$ Hz, —CH_2O—). In addition, the peaks at δ 2.20 (2H, t, $J = 7.4$ Hz, O—CO—CH_2—), δ 1.58 (2H, br s, 2 × CH_2), 1.56 (2H, br s, 2 × CH_2), δ 1.20 (34 H, br s, 17 × CH_2), δ 0.81 (3H, t, $J = 6.82$ Hz, CH_3) indicated a long chain acid (docosanoic acid) esterified with the *p*-hydroxyphenylethanol. The $[M]^+$ at *m*/*z* 460 was in agreement with the molecular formula $C_{30}H_{52}O_3$. The mass fragments at *m*/*z* 339, 295, 165 and 121 confirmed the location of the carbonyl group (Fig. 1). Particularly, formation of the peak at *m*/*z* 121 (100%) indicated the loss of the esterified portion from the molecule. The ^{13}C NMR (APT) spectrum of **1** was in agreement with the given structure (Table 1). Methine carbon peaks observed at δ 130.16 and δ 115.47 corresponding to two pairs of unsubstituted aromatic carbons. The peaks at δ 154.27 and δ 130.21 are quaternary aromatic carbon peaks. Compound **1** also showed signals for an ester carbonyl at δ 173.21, a methylene attached to oxygen function at δ 64.01, an end methyl group at δ 14.52. Methylene signals were also observed for a benzylic carbon at δ 34.70 (C-7′), and fatty acid ester carbons at δ 34.62 (C-α), 32.13 (C-β), 30.17 (C-γ) and 29.16 (for $(CH_2)_{17}$). The length of the fatty acid methylene chain was determined as 20 carbons, based on ^{1}H NMR and mass spectral data. In view of the data, **1** was identified as *p*-hydroxyphenylethyl docosanoate (**1**) (Fig. 2).

Phenolic esters of long chain fatty acids are not common in plant material (Houghton, 1989; Marco, Sanz-Cervera, Garcia-Lliso, Susana, & Garcia-Jacas, 1994) and this is the first time that **1** has been isolated from a *Salvia* species. These compounds have been proposed as forming the

building blocks of the complex polymeric material suberin. Suberin impregnates the cell walls of cork tissue in plants and has a passive defence role (Houghton, 1989).

Compound **2** showed intense blue fluorescence under UV light at 365 nm on TLC plates. It had UV absorption bands at 207, 227, 275 and 302 nm. The blue fluorescence and the UV spectrum are characteristic of a coumarin skeleton. Coumarins are recognized by two distinct doublets with high coupling constants ($J = 9.5$ Hz), in the low field region of the ^{1}H NMR spectrum, resonating between δ 6.1–6.5 (H-3) and δ 7.5–8.3 (H-4) (Murray, Mendez, & Brown, 1982). The fact that these doublets were not observed for **2**, indicates that C-3 and C-4 should be substituted. The ^{1}H NMR spectrum showed the presence of an aromatic methyl group (δ 2.56, 3H, s), a methoxyl group (δ 3.92, 3H, s) and a hydroxyl group (δ 6.06, OH, s, D_2O exch.) (Table 2). In addition, ^{1}H NMR spectrum of **2** afforded signals for three aromatic protons at δ 7.54 (1H, dd, $J = 8.5$ and 1.95 Hz), δ 7.52 (1H, d, $J = 1.95$ Hz) and δ 6.95 (1H, d, $J = 8.5$ Hz). The three aromatic protons showed *ortho* (8.5 Hz), *meta* (1.95 Hz) and *ortho–meta* (8.5 and 1.5 Hz) couplings to each other showing that the benzene ring of the coumarin bears only one substituent, either at C-6 or C-7. It is known that in the presence of an oxygenated substituent such as OH or OMe on C-6, the UV maxima would be shifted from 275 and 310 nm to longer wavelengths (Parmalr, Rathore, Singh, Jain, & Gupta, 1985). This is not the case with **2**. In the ^{1}H NMR spectrum, for coumarins bearing OH or OMe at C-7, the *ortho* protons are shielded and H-6 resonates at δ 6.6–6.8, H-8 resonates at δ 6.5–6.9 and H-5 resonates at δ 7.2–7.5. However, in case of 7-CH_3, *ortho* protons resonate at around δ 7.6. In view of these data, the methyl group should be attached to C-7. The two substituents on the pyrone ring are —OMe and —OH. The —OH signal at δ 6.1 rules out hydrogen bonding, which should be a factor if —OH substitution is at C-3. Intramolecular H-bonded aromatic hydroxyl protons resonate at around δ 7.0. As a result, the —OMe has been assigned to C-3 and —OH to C-4. The ^{13}C NMR spectrum (Table 2) showed three quaternary carbon atoms appearing at δ 150.35 (C-4), δ 130.29 (C-3) and δ 146.34 (C-7), and three methine carbon atoms at δ 124.45 (C-6), δ 114.22 (C-5) and δ 110.48 (C-8). Based on HMQC correlations, C-5, C-6 and C-8 signals were unambiguously assigned (Fig. 7). The carbonyl functionality at C-2 is observed at δ 160.13 while, other two quaternary carbons C-9 and C-10 are assigned to 150.64 and 114.22 based on HMBC experiments, respectively. Thus, the compound has been characterised as a new coumarin, 3-methoxy-4-hydroxy-7-methylcoumarin (**2**) (Fig. 2).

4. Conclusions

It is concluded that, the acetone extract of the aerial parts of *S. cedronella* showed a high radical scavenging ability in a dose dependent manner in DPPH as well as metal chelation in Fe^{2+}–ferrozin test system, which

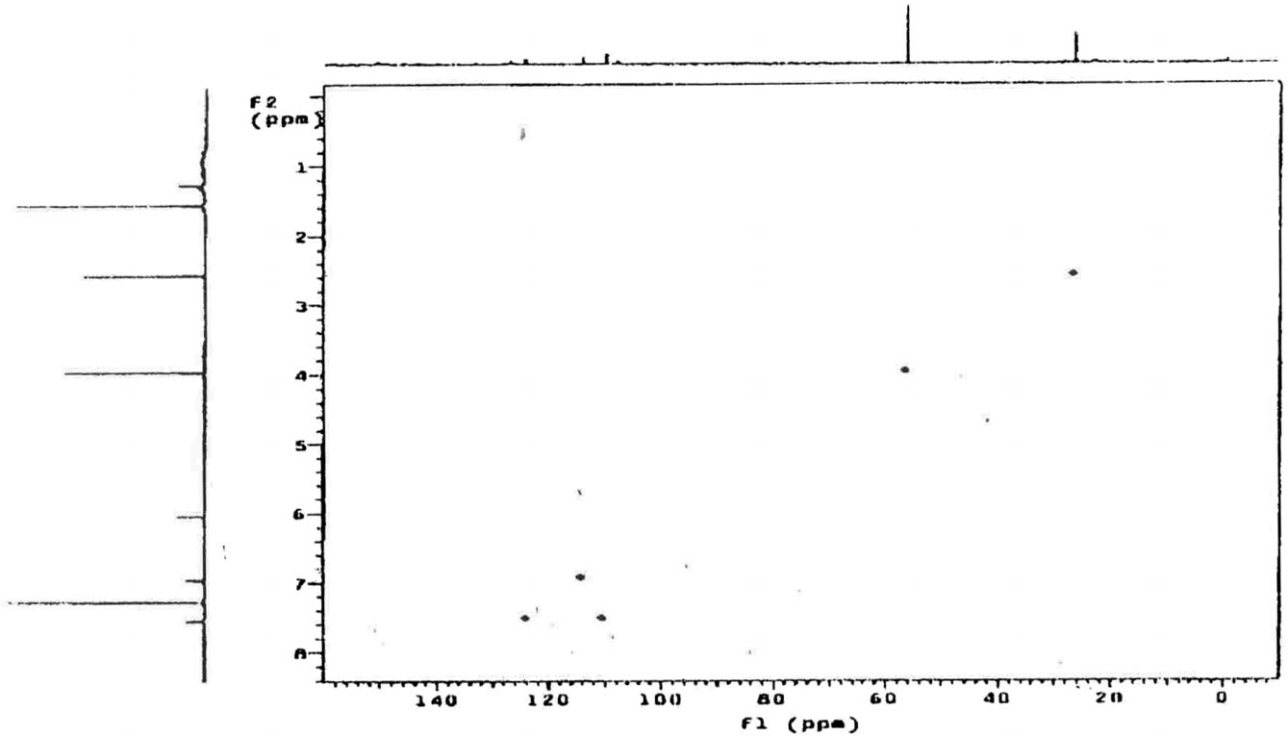

Fig. 7. HMQC spectrum of compound **2**.

competes with the standards BHT and α-tocopherol, particularly at high concentrations (50 and 100 μg/mL). However, total antioxidant activity which measured as inhibition of lipid peroxidation by β-carotene–linoleic acid system was not found to be comparable with the standards BHT, BHA, α-tocopherol and quercetin. It is noteworthy that the antioxidant activity of the standards were showed almost no difference in β-carotene–linoleic acid system (Table 6) at all doses while the acetone extract of *S. cedronella* showed a dose dependent activity which indicated that one or some compounds in the extract might be responsible for this total antioxidant activity rather than whole extract.

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