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Antioxidant abietane diterpenoids from *Salvia barrelieri*

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Abstract

The root extract of endemic Algerian *Salvia* species *Salvia barrelieri* Ettling and its diterpenoids were investigated for potential antioxidant activity. From its acetone extract, a new natural abietane diterpenoid 7-oxoroyleanone-12-methyl ether (**1**) and six known diterpenoids 7α-acetoxyroyleanone-12-methyl ether (**2**), royleanone (**3**), horminone (**4**), 7-acetylhorminone (**5**), cryptojaponol (**6**) and inuroyleanol (**7**) were isolated, and their structures were elucidated by spectroscopic means. Among the diterpenoids, the new diterpenoid 7-oxoroyleanone-12-methyl ether (**1**) showed highest superoxide anion scavenging activity while inuroyleanol (**7**) showed both the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and inhibition of lipid peroxidation in β-carotene–linoleic acid system. These findings indicate that *S. barrelieri* extract as well as isolated abietane diterpenes, particularly inuroyleanol are promising antioxidants which can be used as food additives.

Keywords: Antioxidant activity; *S. barrelieri*; Lamiaceae; Abietane diterpenoids; 7-Oxoroyleanone-12-methyl ether

1. Introduction

Traditionally used herbs and spices have much more attention in the food industry due to their antioxidant and antibacterial properties. Nowadays, in most countries there are some limitations in using synthetic antioxidant compounds in the food products because of their side effects, therefore natural sources have became more important to find proper and safe food antioxidants. *Salvia* species can be one of natural sources for this purpose with good culinary qualities, and their extracts are commonly used to increase the shelf life of foods (Gu & Weng, 2001). *Salvia* is the largest genus in mint family (Labiatae = Lamiaceae) with over 900 species in the world throughout, particularly in the countries near to Equator zone from America to China (Stanley & Williams, 1973). The name *Salvia* (sage) is derived from salvare which means healer in Latin. In Europe, particularly in Mediterranean countries, infusions of several *Salvia* species are commonly used, and commercial samples mainly consist of *S. officinalis* L. and/or *S. triloba* L. (= *S. fruticosa* Mill.) (Ulubelen, 2000; Ulubelen & Topçu, 1998). Besides their antioxidant (Tepe, Sokmen, Akpulat, & Sokmen, 2006), antiseptic (Daniela, 1993) and antibacterial (Ulubelen, 2003) properties they possess antifungal (Kobayashi, Nishino, Tomita, & Fukushima, 1987), antiviral (Tada, Okuno, Chiba, Ohnishi, & Yoshii, 1994), cytotoxic (Lin, Wang, Huang, Huang, & Cordell, 1989; Ulubelen, Topcu, Chai, & Pezzuto, 1999), carminative, diuretic, hypoglycemic (Jimenez, Risco, Ruiz, & Zarzuelo, 1986), hemostatic, wound healing, spasmolytic, tranquilizer and sedative activities. Keller lists more than 60 different ailments for which sage is claimed to be therapeutic (Keller, 1978). Particular species, such as *S. milthiorrhiza* Bunge in China have been used in the treatment of some heart diseases

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angina pectoris and myocardial infarction (Chang & But, 1986).

In a recent study, sage and some other spices were used to preserve turkey meat as antioxidant, and it was reported that the addition of sage and the other spices retarded the process of oxidation which proved sage to be more effective than the mixture of the other spices (Karpinska, Borowski, & Danowska-Oziewicz, 2001). Abietane diterpenoids carnosic acid, royleanonic acid, carnosol and rosmanol have good antioxidant abilities isolated from sage and rosemary plants (Gu & Weng, 1997; Wellwood & Cole, 2004). The oxidation cascade of carnosic acid, which causes formation of other phenolic and quinone abietanes in Labiatae plants, particularly in *Salvia* and *Rosmarinus* species was proposed by Wenkert, Fuchs, and McChesney (1965), and antioxidant activity mechanism studies on abietanes have been still continuing (Masuda, Inaba, & Takeda, 2001; Masuda et al., 2002; Santos-Gomes, Seabra, Andrade, & Fernandes-Ferreira, 2002). In Algeria, 23 *Salvia* species are growing, four being endemic (Quezel & Santa, 1962). Recently, one of Algerian species *S. jaminiana* has been investigated for the antibacterial activity of the constituents (Kabouche et al., 2005). In this study, *S. barrelieri* Ettling was investigated for the first time for diterpenoid constituents and their antioxidant activities.

2. Materials and methods

2.1. Plant material

Whole plant of *Salvia barrelieri* Ettling (Labiatae = Lamiaceae) was collected from Ammoucha-Setif district in Northeastern Algeria in June 2004. It was identified by Prof. Gérard De Bélair from Annaba-University (Algeria) and Prof. Kerim Alpinar at Faculty of Pharmacy, Istanbul University where it was deposited and its voucher specimen number is ISTE 81939.

2.2. Extraction and isolation

The roots separated from the whole plant and then dried and powdered (750 g). They were extracted with acetone (10 L) in a Soxhlet, filtered and evaporated to dryness in vacuo for the isolation of diterpenoids. The residue (AESB = Acetone Extract of *Salvia barrelieri*) (12 g) was fractionated on a silica gel column eluting with cyclohexane, a gradient of EtOAc was added up to 100% followed by MeOH. Similar fractions were combined. The major fraction (7.6 g) was rechromatographed on a silica gel column eluting with cyclohexane and a gradient of EtOAc up to 100%. The fraction, obtained by elution of EtOAc (4–6%) was chromatographed on a silica gel prep. TLC plate using toluene/diethyl ether/(95/5) system to isolate 7-acetylhorminone (**5**) (50 mg). The column fraction corresponding to the gradient of EtOAc 3–4% was fractionated on a silica gel column eluting with cyclohexane and a gradient of dichloromethane up to 100%. The fraction obtained by elution of solvent mixture of cyclohexane/dichloromethane (60/40) was purified on a silica gel prep. TLC using cyclohexane/dichloromethane (50/50) system to afford 7α-acetoxyroyleanone-12-methyl ether (**2**) (15 mg). The fraction corresponding to the gradient of cyclohexane/dichloromethane 80/20 was fractionated on a silica gel column eluting with cyclohexane and a gradient of dichloromethane up to 100%. The fraction corresponding to the gradient of cyclohexane/dichloromethane 70/30 was chromatographed on the silica gel prep. TLC eluted with cyclohexane/diethyl ether (96/4) to isolate royleanone (**3**) (20 mg), horminone (**4**) (30 mg), cryptojaponol (**6**) (15 mg) and inuroyleanol (**7**) (10 mg). The fraction corresponding to the gradient cyclohexane/dichloromethane 50/50 was chromatographed in the similar conditions to afford the new compound 7-oxoroyleanone-12-methyl ether (**1**) (15 mg).

2.3. Spectral measurements

Instruments: The UV spectra were recorded on Shimadzu UV-1601 in MeOH (at Faculty of Pharmacy, Istanbul University), IR spectra on Perkin–Elmer One B in $CHCl_3$ (at Istanbul Technical University), NMR spectra on a Varian Mercury-Vx 400 spectrometer operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR (TMS as an internal standard) (at Boğaziçi University), including APT, HSQC and HMBC experiments, and EIMS spectra on a GC-MS spectrometer (at National Metrology Institute, TUBITAK). Optical rotations were determined using an Optical Activity Ltd., AA-5 polarimeter (at Faculty of Pharmacy, Istanbul University).

7-Oxoroyleanone-12-methyl ether (***1***): Amorphous solid $[\alpha]_D^{20}$ – 97.1° ($CHCl_3$; c 0.100); IR ν_{max} ($CHCl_3$): 3403, 2927, 2853, 1703, 1664, 1626, 1456, 1416, 1368, 1337, 1295, 1248, 1196, 1145, 1105, 1064, 914, 751, 667 cm^{-1}. EIMS (probe) 70 eV, *m/z* (rel. int.): 344 $[M]^+$ (48) for $C_{21}H_{28}O_4$, 329 $[M - Me]^+$ (70), 311 $[M - H_2O]^+$(13), 273 (26), 261 (48), 246 (36), 232 (24), 206 (15), 166 (30), 148 (100), 134 (16), 114 (21), 90 (28), 82 (42), 68 (80), 54 (86); ^1H and ^{13}C NMR (see Table 1).

7α-Acetoxyroyleanone-12-methyl ether (7-acetylhorminone-12-methyl ether) (***2***): Amorphous solid, $[\alpha]_D^{20}$ 0° ($CHCl_3$, c 0.010); IR ν_{max} ($CHCl_3$): 3358, 2927, 2870, 1740, 1644, 1462, 1371, 1235, 1209, 1137, 1011, 946, 894, 759, 668 cm^{-1}. EIMS (probe) 70 eV, *m/z* (rel. int.): 388 $[M]^+$ (1) for $C_{23}H_{32}O_5$, 346 $[M - Ac + H]^+$ (11), 328 $[M - HOAc]^+$ (26), 313 $[328\text{-}Me]^+$ (19), 295 $[313\text{-}H_2O]^+$ (12), 285 (14), 257 (14), 243 (18), 229 (20), 203 (16), 187 (14), 167 (42), 149 (100), 119 (15), 109 (31), 91 (32), 83 (48), 69 (67), 57 (78), 55 (94); ^1H and ^{13}C NMR (see Table 1).

2.4. Antioxidant activity

In this study, mainly three methods, DPPH radical scavenging activity, β-carotene bleaching method and superoxide anion scavenging activity are carried out.

Table 1
^{1}H and ^{13}C NMR[a] data of compounds **1** and **2** in $CDCl_3$, *J* values are given in parentheses

Position	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1β	2.73 tt (3.0; 13.5)	36.85	2.62 dd (3.5; 12.8)	36.25
1α	1.16 dt (3.2; 14.0)		1.13 dt (3.5; 12.8)	
2β	1.75 dddd (2.0; 3.5; 13.0; 14.0)	18.24	1.76 dddd (2.0; 3.5; 13.0; 14.2)	16.24
2α	1.53 m		1.57 m	
3β	1.46 dt (3.0; 14.0)	40.71	1.53 dt (3.0; 14.5)	41.51
3α	1.23 m		1.24 m	
4	–	33.28	–	33.29
5	1.80 dd (4.5; 14.0)	49.16	1.47 dd (1.5; 13.0)	46.63
6α	2.63 dd (4.5; 18.0)	35.54	1.66 ddd (4.2; 13.0; 14.5)	25.23
6β	2.54 dd (14.0; 18.0)		1.94 brd (14.5)	
7β	–	196.94	5.91 dd (1.7; 4.1)	64.75
8	–	130.72	–	137.32
9	–	159.12	–	152.84
10	–	39.97	–	39.53
11	–	185.03	–	183.81
12	–	156.39	–	156.89
13	–	136.43	–	136.17
14	–	185.03	–	186.35
15	3.21 sept. (7.0)	24.62	3.17 sept. (7.0)	24.99
16	1.21 d (7.0)	20.28	1.17 d (7.0)	20.98
17	1.26 d (7.0)	20.28	1.22 d (7.0)	20.64
18	0.94 s	32.62	0.90 s	33.37
19	0.98 s	21.22	0.89 s	22.04
20	1.44 s	18.23	1.30 s	19.13
OMe	3.88 s	60.37	3.78 s	60.87
$CO\underline{C}H_3$	–	–	2.02 s	21.48
$\underline{C}OCH_3$	–	–	–	169.55

[a] Carbons were determined based on APT, HSQC and HMBC experiments (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR).

2.4.1. Chemicals

L-ascorbic acid (L-AA), butylated hydroxytoluene (BHT), acetone and methanol were obtained from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, β-carotene, polyoxyethylene sorbitan monopalmitate (Tween 40), and α-tocopherol (TOC) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT) and phenazine methosulphate (PMS) were obtained from Fluka Chemical Co. (Fluka GmbH, Steinheim, Germany, packed in Switzerland). All other chemicals and solvents were analytical grade.

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

The antioxidant activity of the extract and compounds (**1**–**7**) was evaluated by β-carotene–linoleic acid model system (Miller, 1971). 0.5 mg of β-carotene in 1 mL of chloroform was added to 25 μL of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After chloroform was evaporated 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. BHT and α-tocopherol were used as standards. The bleaching rate (*R*) of β-carotene was calculated according to the following equation:

$$R = \ln(a/b)/t$$

where ln is the natural log, *a* is the absorbance at time 0, *b* is the absorbance at time *t* (120 min) (Cheung, Cheung, & Ooi, 2003). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using following equation:

$$AA = [(R_{control} - R_{sample})/R_{control}] \times 100$$

2.4.3. Free radical scavenging activity

Radical scavenging activity of the extract and compounds (**1**–**7**) was determined using DPPH as a reagent (Kirby & Schmidt, 1997) with some modification. Briefly, a 0.1 mM solution of DPPH radical in methanol was prepared and then, 4 mL of this solution was mixed with 1 mL of sample solutions in methanol. Finally, the samples were incubated for 30 min in a dark room at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm using a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. BHT

and α-tocopherol were used as standards. The percent DPPH scavenging effect was calculated using the following equation:

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

where A_{control} is the absorbance of the control, and A_{sample} is the absorbance of the sample.

2.4.4. Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of the extract and compounds (**1–5, 7**) was based on the method described by Liu, Ooi, and Chang (1997) with slight modification. Superoxide radicals are generated in PMS-NADH-NBT systems by oxidation of nicotinamide adenine dinucleotide (NADH) and assayed by the reduction of nitroblue tetrazolium (NBT). The superoxide radicals were generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and sample solutions. The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. L-ascorbic acid, BHT and α-tocopherol were used as standards. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion radical generation was calculated using the following formula:

$$\text{Inhibition}(\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the control, and A_{sample} is the absorbance of the sample.

2.4.5. Statistical analysis

Experimental results concerning this study were means ± S.D. of three parallel measurements. 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.

3. Results and discussion

From the acetone extract of the roots of *S. barrelieri* seven abietane diterpenoids were isolated, and their structures were elucidated as a new natural abietane 7-oxoroyleanone-12-methyl ether (**1**) and six known abietane diterpenoids 7α-acetoxyroyleanone-12-methyl ether (**2**), royleanone (**3**), horminone (**4**), 7-acetylhorminone (**5**), cryptojaponol (**6**), and inuroyleanol (7-oxo-11,14-dihydroxy-12-methoxy-8,11,13-abietatriene) (**7**) (Fig. 1).

Compound **1** gave a molecular ion peak at m/z 344, $[M - Me]^+$ signal was shown at m/z 329. In the IR spectrum, a carbonyl signal was observed at 1704 cm^{-1} besides the absorption peaks at 1665 and 1627 cm^{-1} for *p*-quinone moiety. In the ^1H NMR ($CDCl_3$) spectrum (Table 1), methyl singlets at δ 0.94, 0.98 and at δ 1.44 and two methyl doublets at δ 1.21 and 1.26 were observed. The latter two doublets (each $J = 7.0$ Hz) together with a methine signal at δ 3.21 ($J = 7.0$ Hz) are indicative of an isopropyl group attach to a quinone group rather than an aromatic ring. Besides the isopropyl group, the presence of three methyl singlets in the ^1H NMR and 21 carbon signal in the ^{13}C NMR spectra (Table 1) revealed that an abietane diterpenoid structure (Topcu & Ulubelen, 1996). A methoxy signal was present at δ 3.88 while the characteristic H-1β proton signal was observed at δ 2.73 as tt ($J = 3.0$ and 13.5 Hz). The signal at δ 2.54 as dd ($J = 14.0$ and 18.0 Hz) and 2.63 ($J = 4.5$ and 18.0 Hz) were assigned to C-6 protons while H-5 appeared at δ 1.80 as dd. The observation of H-6 signals with large J values, and H-5 with a very characteristic doublet of doublet were indicative of the presence of a keto group at C-7. The observation of the keto carbonyl at 196.94 ppm in the ^{13}C NMR spectrum was verified its placement to be at C-7 which should be conjugated to the *p*-quinone moiety rather than being an

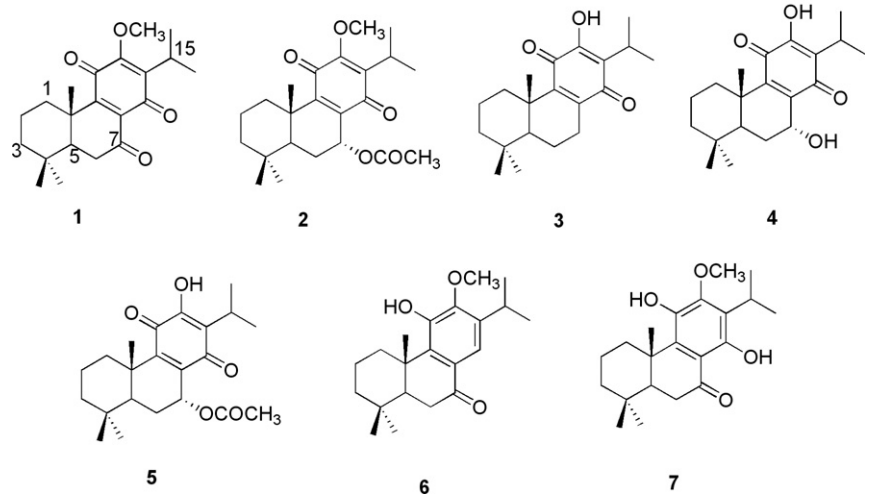

Fig. 1. Chemical formulae of abietane diterpenes.

isolated oxo group. Both quinone carbonyls were seen at 185.03 ppm, and the methoxy carbon at 60.37 ppm. All the spectral data indicated that the structure of the diterpenoid **1** to be 7-oxoroyleanone-12-methyl ether (**1**) which was previously synthesized by Matsumoto and Harada (1979). However, it was isolated from nature for the first time in this study.

Compound **2** has recently presented as a new compound from *Hyptis verticillata* (Bakir et al., 2006), structural analysis was only based on single crystal X-ray analysis. However, none of the other spectral data including MS, ^{1}H and ^{13}C NMR, and IR data were given in the study. Therefore, the spectral data, excluding X-ray analysis are now given herein. It gave a molecular ion peak at m/z 388 in its EI-MS spectrum. The presence of an acetoxy (1740 and 1235 cm^{-1}) and quinone moiety (1650, 1645 and 1605 cm^{-1}) was observed in the IR spectrum. ^{1}H and ^{13}C NMR spectra revealed the abietane quinonoid diterpene structure of **2** carrying the acetoxy group (δ_H 2.02 and δ_C 21.48) as well as a methoxy group (δ_H 3.78 and δ_C 60.87). The placement of the acetoxy group at C-7 clearly followed by ^{1}H NMR spectrum with appearance of H-7 signal at δ 5.91 as dd ($J = 1.7$ and 4.1 Hz), attributed to its β stereochemistry as seen in horminone, instead of α stereochemistry as in taxoquinone where appeared as a multiplet or brodened singlet rather than a well observed narrow dd (Tezuka et al., 1998). All the ^{1}H and ^{13}C NMR spectral data verified its structure as 7α-acetoxyroyleanone-12-methyl ether (7-acetylhorminone-12-methyl ether) (**2**) (Bakir et al., 2006).

The structures of the other known compounds were identified as royleanone (**3**) (Katti, Rüedi, & Eugster, 1982), horminone (**4**) (Katti et al., 1982), 7-acetylhorminone (**5**) (Hensch, Rüedi, & Eugster, 1975), cryptojaponol (**6**) (Wenkert, Campello, McChesney, & Watts, 1974), and inuroyleanol (**7**) (Bhat, Kalyanamaran, Kohl, & De Souza, 1975) based on their spectral (^{1}H and ^{13}C NMR and Mass) analyses and TLC comparison with standard samples.

Fig. 2 shows the antioxidant activity of the extract of *S. barrelieri* (AESB) and compounds (**1**–**7**) comparing with α-tocopherol and BHT, which was determined by the β-carotene bleaching method. The activity was increased as dose dependent. In this system, the extract exhibited highest antioxidant activity surpassing all the tested abietanes and positive controls. Among the isolated diterpenoids inuroyleanol (**7**) exhibited highest activity being close effectiveness to the standards both BHT and α-tocopherol. None of the tested abietanes (**1**–**7**) showed greater antioxidant than BHT and α-tocopherol, and 7-acetylhorminone (**5**) showed the lowest antioxidant activity. Results were found statistically significant ($p < 0.05$).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dins, Cunha, & Almeida, 1997). The AESB showed higher activity than to those of isolated pure compounds except inuroyleanol (**7**). Inuroyleanol (**7**) exhibited high activity unlike other abietanes and showed stronger

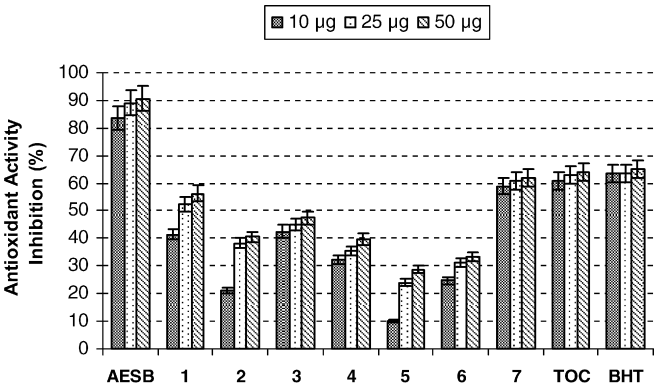

Fig. 2. Antioxidant activity results (% Inhibition) of diterpenes (**1**–**7**), acetone extract of *Salvia barrelieri* (AESB) and α-tocopherol (TOC), butylated hydroxytoluene (BHT) by the β-carotene bleaching method. Values are mean ± S.D., $n = 3$. $p < 0.05$, significantly different with student's t-test.

effect than known standards BHT and α-tocopherol in all concentrations (Fig. 3) and their IC_{50} values are found to be 7.26 ± 0.06, 7.73 ± 0.56 and 16.09 ± 0.68 µg/mL, respectively. Results were found statistically significant ($p < 0.05$). Scavenging effect of abietanes, the AESB and standards on the DPPH radical decreased in order of **7** > α-tocopherol > BHT > AESB > **1** > **2** > **3** > **4** > **5** > **6** at all concentrations (10 µg/mL, 25 µg/mL and 50 µg/mL) demonstrating a linearity with increasing concentrations.

Superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion. Fig. 4 shows the percentage inhibition of superoxide anion radical generation by 12.5 µg/mL of acetone extract (AESB), the isolated diterpenoids, except compound **6**, in comparison with same concentration of L-ascorbic acid, BHT and α-tocopherol. All tested abietanes exhibited higher superoxide radical scavenging activity than α-tocopherol. All tested abietanes also showed higher superoxide radical scavenging activity than BHT, except inuroyleanol (**7**). Even, compound **1** (%65.3895 ± 2.24) has a competition with a well-known antioxidant L-ascorbic acid (%65.9712 ± 1.00). Results were found statistically significant ($p < 0.05$). Superoxide

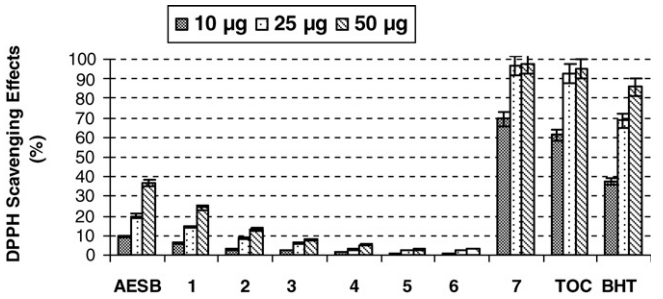

Fig. 3. Free radical scavenging activity of diterpenes (**1**–**7**), AESB, and standards by DPPH radical. Values are mean ± S.D., $n = 3$. $p < 0.05$, significantly different with student's t-test.

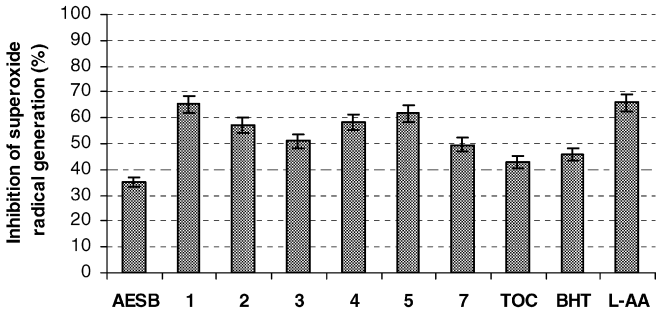

Fig. 4. Superoxide anion radical scavenging activity of 12.5 μg/mL concentration of diterpenes (**1–5, 7**), AESB and standards by the PMS-NADH-NBT method. L-ascorbic acid (L-AA). Values are mean ± S.D., $n = 3$. $p < 0.05$, significantly different with student's t-test.

radical anion scavenging activity of the tested abietanes, the AESB and standards are in following order:

L-ascorbic acid >**1** > **5** > **4** > **2** > **3** > BHT > **7** > α-tocopherol > AESB.

4. Conclusion

Among abietane diterpenes, inuroyleanol (**7**) showed the highest DPPH scavenging activity as well as the highest inhibition on lipid peroxidation in β-carotene–linoleic acid system. In contrast, inuroyleanol (**7**) revealed the lowest superoxide anion scavenging activity while compound **1** showed the highest activity with an equal antioxidant potent to the well known antioxidant L-ascorbic acid, even more active than BHT and α-tocopherol. Royleanone (**3**) showed higher antioxidant activity in the two systems than both horminone (**4**) and 7-acetylhorminone (**5**). Considering these results in both DPPH and β-carotene–linoleic acid systems we can conclude that quinonoid or fully substituted C ring phenolic abietane diterpenoids having no substituent or different than a keto group at C-7 would be decreased activity. In DPPH free radical scavenging test method, 7α-acetoxyroyleanone-12-methyl ether (**2**), which is a methyl derivative of 7-acetylhorminone (**5**) at C-12, showed higher activity as much 4–5 times of (**5**) as, this effect can be attributable to the higher scavenging power of the methoxy group at C-12. In fact, in the literature, the higher activity of carnosic acid, which possessed *ortho*-dihydroxyl groups on aromatic ring C with comparison of royleanonic acid having hydroxy-*p*-benzoquinone moiety, has been previously explained by inhibition of the oxidation through donating H atoms to scavenge free radicals (Miura, Kikuzaki, & Nakatani, 2002; Wei & Ho, 2006) in the two systems (DPPH and β-carotene–linoleic acid). Therefore, *ortho*-dihydroxy phenols or one being hydroxyl and other a methoxyl can form more stable radicals by donating H atoms as observed for inuroyleanol (**7**), thus this type of abietanes are expected to be more active antioxidants than abietanes which contain the monohydroxy phenol or *p*-quinoid C ring abietanes.

In fact, inuroyleanol (**7**) was found to be active in the investigated three systems competing with the standards BHT and α-tocopherol. However, due to its hydroquinone structure it showed lesser superoxide anion radical scavenging potential compared to the other tested abietanes which all have *p*-quinoid C ring. This can be explained based on behavior of quinone compounds where superoxide radical scavenging process is reversible depending on the redox potential (Murakami & Zs.-Nagy, 1990) through electron transfer from superoxide to the quinone moiety.

These findings indicate that *Salvia* extracts should be investigated for their toxicity besides antioxidant activities for their potential use in food industry.

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