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Phytochemical Investigation of the Aerial Parts of *Salvia rhytidea* Benth

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ABSTRACT

The genus *Salvia* is a rich source of structurally diverse terpenoids. *Salvia rhytidea* Benth. from the Lamiaceae family is one of the endemic species in the southeast of Iran. Significant biological activities such as anti-inflammatory, cytotoxic, antioxidant, antibacterial, and antifungal effects have been previously reported for the plant. There has been no phytochemical study on the aerial parts of *S. rhytidea*, with the exception of an analysis of the essential oil. The aim of this study was to carry out a comprehensive phytochemical investigation on aerial parts of *S. rhytidea*. The ethyl acetate (EtOAc) extract of the aerial parts of the plant was separated by different chromatographic methods on the silica gel and Sephadex LH-20 columns, and preparative thin layer chromatography (Prep TLC) to afford eleven (1-11) metabolites. The structure elucidation of the compounds was performed by extensive 1D and 2D-NMR spectroscopy and comparing their spectra with those reported in the literature. The process resulted in the isolation and purification of four flavonoids, salvigenin (1), eupatorin (2), cirsiliol (3), and cirsimaritin (4), three pentacyclic triterpenes, *α*-amyrin (5), lupeol (6), and ursolic acids (8), two labdane diterpenoids, sclareol (9) and 6*β*-hydroxysclareol (10), and two steroidal compounds, *β*-sitosterol (7) and daucosterol (11). All of these metabolites are described here for *S. rhytidea* for the first time. In previous studies, several biological properties have been reported for these compounds. Hereupon, *S. rhytidea* has good potential to conduct further studies in the pharmaceutical and cosmetic fields.

Keywords: Flavonoid, Terpenoid, Steroid, NMR

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INTRODUCTION

Secondary metabolites as organic compounds produced by plants, animals, fungi, and bacteria are important in herbivores [1]. Traditionally, humans used them as medicines, recreational drugs, and pigments [2]. Recently, medicinal plants have gained more attention in the food, drug, cosmetics, and hygiene industries due to their high economic value and bioactive metabolites [3].

The genus *Salvia* is the largest member of the Lamiaceae family and has a worldwide distribution, especially in Central Asia, the Mediterranean, Pacific Islands, Africa, and America [4]. Iran as one of the main origins of the *Salvia* genus has 60 species with 17 endemics. *Salvia* species are known as “Maryam-Goli” in Persian and exhibit various therapeutic activities [5]. The genus is famous for its traditional uses in the treatment of some diseases like colds, bronchitis, aches, infections and hemorrhage [6]. Among the Iranian Salvia, aerial parts of *Salvia hydrangea* are used as sedative and antispasmodic, *Salvia sclarea* as tonic, *Salvia macrosiphon* as antimicrobial, *Salvia mirzayanii* as anti-inflammatory, and *S. reuterana* as antianxiety herbal drugs [7-8]. Several Salvia species have economic importance because of their uses in pharmaceutical, food and perfume industries, and some species are grown in gardens as ornamental plants [9]. Prior phytochemical studies have reported that sage is one of the rich sources of bioactive metabolites such as diterpenoids, triterpenoids, sesquiterpenoids, sesterterpenoids, and flavonoids [10-12]. The most abundant diterpenoids in the genus are abietanes and rearranged abietanes [11-13]. Labdane diterpenoids are rather rare in Salvia species, although they are frequently found in other genera of the Lamiaceae [12-14]. Some triterpenoids with highly unusual carbon skeletons were also reported from this genus [15].

S. rhytidea Benth. as one of the Persian endemic species has been used as blood flow promotor, antidiabetic, antifungal, antibacterial, and antioxidant [16-17]. Previous studies on *S. rhytidea* have demonstrated that the roots of this plant contain tanshinone and abietane diterpenoids with antifungal and antimicrobial activity [18-19]. Other works analyzed the essential oil of the plant using GC-MS and found that it contains high amounts of monoterpenoids [20-21]. However, no phytochemical studies have been done on aerial parts of this sage. For the first time, we report here the results of phytochemical investigations on the EtOAc extract of aerial parts of *S. rhytidea*.

MATERIALS AND METHODS

Plant Material

Aerial parts of *S. rhytidea* were collected from Bardsir city in Kerman province, during the flowering stage in June 2019. The plant was authenticated by the taxonomist Dr. Mansour Mirtadzadini and a voucher specimen (MPH-2243) was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

Extraction and Fractionation

Air-dried aerial parts of *S. rhytidea* (3.0 kg) were powdered and then macerated with 5 liters of EtOAc for four times (4 x 5 L) to afford 195 g dried EtOAc extract. A part of extract (120 g) was separated on a silica gel column with a gradient of *n*-hexane-EtOAc(100:0 to 0:100), and then an increasing concentration of MeOH (up to 30%), as a mobile phase [22]. Based on TLC analysis, the fractions with similar patterns were pooled to yield 15 combined fractions.

Fr.4 (6.5 g) was submitted to a silica gel CC (500 g, 4.5×100 cm), eluted with *n*-hexane-CH_2Cl_2-$CO(CH_3)_2$ (93:5:2) to obtain six subfractions (Fr.4.1-Fr.4.6). Fr.4.2 and Fr.4.5 were purified by trituration with MeOH to afford compounds **5** (32 mg) and **6** (25 mg), respectively. Fr.6 (900 mg) was separated on silica gel CC (250 g, 3×60 cm), using *n*-hexane-CH_2Cl_2-$CO(CH_3)_2$ (80:15:5) as mobile phase to give four subfractions (Fr.6.1-Fr.6.4). Fr.6.3 was triturated with MeOH to get compound **7** (100 mg). Fr.9 (5 g) was purified on a silica gel CC (500 g, 4.5×100 cm), using *n*-hexane-$CHCl_3$-$CO(CH_3)_2$ (60:30:10) as mobile phase to give six subfractions (Fr.9.1-Fr.9.6). Fr.9.2 to Fr.9.4 were mixed and further separated on silica gel (250 g, 3×60 cm) using CH_2Cl_2-MeOH (97:3) as mobile phase to yield compounds 1 (500 mg), 2 (4.7 mg), and 8 (90 mg). Fr.11 (600 mg) was further separated via silica gel CC (150 g, 2.5×80 cm) using CH_2Cl_2-$CO(CH_3)_2$ (90:10) as eluent to obtain four subfractions (Fr.11.1-Fr.11.4). Fr.11.2 to Fr.11.4 were mixed (120 mg) and then loaded on Prep-TLC eluted with CH_2Cl_2-MeOH (95:5) to give compounds 3 (2 mg) and 4 (5.9 mg). Fr.13 (400 mg) was further separated via Sephadex LH-20 (2×80 cm) using MeOH (100%) as eluent to give ten subfractions (Fr.13.1-Fr.13.10). Fr.13.4 to Fr.13.7 were mixed (179 mg) and further purified on silica gel column (80 g, 2×80 cm) using CH_2Cl_2-MeOH-CH_2O_2 (90:8:2) as mobile phase to yield compounds 9 (28 mg) and 10 (3.2 mg). About 300 mg of compound 11 was separated and purified from Fr.14 by trituration with MeOH.

Structure Elucidation

1D and 2D-NMR experiments were measured on a Bruker Avance II 600 (600.19 MHz for ^1H and 150.91 MHz for ^{13}C) spectrometer. $CDCl_3$, CD_3OD and DMSO-d_6 were used as deuterated solvents.

RESULTS

In this study, for the first time we investigated the chemical composition of *S. rhytidea*, a plant that was used extensively in folk medicine of southeast parts of Iran [23-24]. The aerial parts of *S. rhytidea* were extracted with EtOAc. Fractionation of the extract by a combination of open-column chromatography on silica gel, Sephadex LH-20, and Prep TLC afforded eleven compounds (1−11) (Fig.1). Structures of the compounds were established by high-field NMR technique (^1H &^{13}C NMR, ^1H-^1H COSY, HSQC, HMBC) and comparing the data with those reported in the literature.

1D and 2D NMR spectra of some of the compounds are given in the supplementary material appendix. Accordingly, the structures were identified as salvigenin (1) [25], eupatorin (2) [26], cirsiliol (3) [27], cirsimaritin (4) [28], *α*-amyrin (5) [29], lupeol (6) [30], *β*-sitosterol (7) [31], ursolic acid (8) [32], sclareol (9) [33], 6*β*-hydroxysclareol (10) [12], and daucosterol (11) [34].

For instance, the identification of compound 10 is described in the following. Compound 10 as a white powder was assigned by ^1H &^{13}CNMR, HSQC, COSY and HMBC spectra. The ^{13}C-NMR showed 20 carbon signals as categorized by the HSQC spectrum to five methyls (δ_C 15.9, 23.1, 25.0, 27.3 and 32.8), seven methylenes (δ_C 17.9, 18.7, 41.2, 43.5, 45.6, 51.5, and 109.9), four methines (δ_C 56.4, 61.3, 66.2, 146.3) and four quaternary carbons (33.2, 38.7, 71.2, and 71.5). ^1H-NMR spectrum showed resonances for three olefinic protons at δ_H 5.84 (1H, dd, *J*=17.4, 10.7 Hz), 5.10 (1H, dd, *J*=17.4, 2.1 Hz) and 4.90 (1H, dd, *J*=10.7, 2.1 Hz), and five methyl protons at δ_H 1.18 (3H, s, H-17), 1.11 (3H, s, H-19), 1.10 (3H, s, H-16), 1.06 (3H, s, H-20), 0.9 (3H, s, H-18). Fig. 2 represents the HMBC (**a**) and ^1H-^1H COSY (**b**) spectra and Key correlations of **10** (**c**). In the COSY spectrum (Fig 2, B), correlations observed between δ_H 5.84 with 4.90 and 5.10, δ_H 4.25 with 1.45 and 0.77, and δ_H 1.39 with 1.66 and 0.94. From these elements, the structural features were reminiscent of a labdane diterpenoid. HMBC correlations (Fig 2, C) from H_2-1(δ_H 0.81 and 1.54) to C-3 (δ_C 43.5) and C-5 (δ_C 56.4), from H_2-2 (δ_H 1.33 and 1.60) to C-3 (δ_C 43.5) and C-10 (δ_C 38.7), from H-5 (δ_H 0.77) to C-4 (δ_C 33.2), C-10 (δ_C 38.7), C-19 (δ_C 23.1), and C-20 (δ_C 15.9), from H_2-7 (δ_H 1.45 and 1.82) to C-5 (δ_C 56.4), C-6 (δ_C 66.2), C-8 (δ_C 71.2), and C-9 (δ_C 61.3), from H_2-11 (δ_H 1.19 and 1.39), C-8 (δ_C 71.2), and C-9 (δ_C 61.3), from H-14 (δ_H 5.84) to C-12 (δ_C 45.6), C-13 (δ_C 71.5), and C-16 (δ_C 27.3), and from H_2-15 (δ_H 4.90 and 5.10) to C-13 (δ_C 71.5), C-14 (δ_C 146.3) and C-16 (δ_C 27.3) confirmed the structure as 6*β*-hydroxysclareol (**10**).

NMR Data of the Isolated Compounds

Salvigenin (**1**): mp. 185-188 ºC, ^1H NMR (600.19 MHz, $CDCl_3$), δ 12.79 (1H, s, OH-5), 7.85 (2H, d, J= 8.6 Hz, H-2′, 6′), 7.03 (2H, d, J=8.6 Hz, H-3′, 5′), 6.58 (1H, s, H-8), 6.55 (1H, s, H-3), 3.98 (3H, s, OMe), 3.94 (3H, s, OMe),

3.90 (3H, s, OMe), ^{13}C NMR (150.91 MHz, $CDCl_3$) δ 164.4 (C-2), 104.5 (C-3), 183.1 (C-4), 153.6 (C-5), 133.0 (C-6), 159.1 (C-7), 90.9 (C-8), 153.4 (C-9), 106.5 (C-10), 123.9 (C-1′), 128.4 (C-2′, 6′), 114.9 (C-3′, 5′), 163.0 (C-4′), 61.2 (OMe), 56.7 (OMe), 55.9 (OMe).

Eupatorin (**2**): mp. 194-196 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$-CD_3OD) δ 7.45 (1H, dd, J=8.5, 2.2 Hz, H-6′), 7.37 (1H, d, J=2.2 Hz, H-2′), 6.95 (1H, d, J=8.5 Hz, H-5′), 6.60 (1H, s, H-8), 6.55 (1H, s, H-3), 3.96 (3H, s, H-4′), 3.93 (3H, s, H-7), 3.87 (3H, s, H-6); ^{13}C NMR (150.91 MHz, $CDCl_3$-CD_3OD) δ 166 (C-2), 104 (C-3), 147 (C-5), 133 (C-6), 158 (C-7), 92 (C-8), 154 (C-9), 107 (C-10), 123.5 (C-1′), 113 (C-2′), 147 (C-3′), 152 (C-4′), 112.5 (C-5′), 119 (C-6′).

Cirsiliol (**3**): mp. 279-282 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$-CD_3OD) δ 7.36 (1H, dd, J=8.3, 2.2 Hz, H-6′), 7.34 (1H, d, J=2.2 Hz, H-2′), 6.89 (1H, d, J=8.3 Hz, H-5′), 6.60 (1H, s, H-8), 6.52 (1H, s, H-3), 3.95 (3H, s, H-7), 3.88 (3H, s, H-6); ^{13}C NMR (150.91 MHz, $CDCl_3$-CD_3OD) δ 166 (C-2), 104 (C-3), 147 (C-5), 133 (C-6), 159 (C-7), 91.5 (C-8), 154(C-9), 107 (C-10), 123.5 (C-1′), 114 (C-2′), 146 (C-3′), 150 (C-4′), 116 (C-5′), 120 (C-6′).

Cirsimaritin (4): mp. 265-267 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$-CD_3OD) δ 7.78 (2H, d, J=8.8 Hz, H-2′, 6′), 6.90 (2H, d, J=8.8 Hz, H-3′, 5′), 6.60 (1H, s, H-8), 6.55 (1H, s, H-3), 3.95 (3H, s, H-7), 3.86 (3H, s, H-6); ^{13}C NMR (150.91 MHz, $CDCl_3$-CD_3OD) δ 166 (C-2), 104 (C-3), 147 (C-5), 133 (C-6), 157.7 (C-7), 92 (C-8),154 (C-9), 107 (C-10), 123 (C-1′), 129 (C-2′), 117 (C-3′), 162 (C-4′).

***α*-amyrin** (**5**): mp. 184-186 °C, ^{1}H NMR (600.19 MHz, $CDCl_3$) δ 3.21 (1H, dd, J= 10.5 and 5.1 Hz, H-3), 0.72 (1H, d, J = 11.1, H-5), 1.53 (1H, m, H-9), 5.28 (1H, t, J = 3.3 Hz, H-12), 2.82 (1H, dd, J = 14.1, 4.6, H-18), 0.78 (3H, s, H-23), 0.98 (3H, S, H-24), 0.93 (3H, s, H-25), 0.99 (3H, s, H-26), 1.05 (3H, s, H-27), 0.77 (3H, s, H-28), 0.77 (3H, s, H-29, 0.89 (3H, s, H-30) ; ^{13}C NMR (150.91 MHz, $CDCl_3$) δ 38.5 (C-1), 27.1 (C-2), 79.0 (C-3), 39.0 (C-4), 55.3 (C-5), 18.3 (C-6), 31.5 (C-7), 41.0 (C-8), 47.6 (C-9), 37.5 (C-10), 22.9 (C-11), 122.4 (C-12), 144.1 (C-13), 41.0 (C-14), 27.9 (C-15), 26.4 (C-16), 32.5 (C-17), 40.9 (C-18), 39.2 (C-19), 39.6 (C-20), 31.0 (C-21), 38.6 (C-22), 15.7 (C-23), 27.9 (C-24), 15.5 (C-25), 16.8 (C-26), 23.2 (C-27), 28.5 (C-28), 17.5 (C-29), 21.4 (C-30).

Lupeol (**6**): mp. 212-214 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$) δ, 3.13 (1H, m, H-3), 0.68 (1H, m, H-5), 1.22 (1H, s, H-9), 2.31 (1H, m, H-19), 0.91 (3H, s, H-23), 0.71 (3H, s, H-24), 0.77 (3H, s, H-25), 0.97 (3H, s, H-26), 0.89 (3H, s, H-27), 0.74 (3H, s, H-28), 4.50 (1H, brs, H-29), 4.62 (1H, brs, H-29), 1.61 (3H, s, H-30); ^{13}C NMR (150.91 MHz, $CDCl_3$) δ 38.5 (C-1), 27.1 (C-2), 79.3 (C-3), 39.0 (C-4), 55.6 (C-5), 18.3 (C-6), 33.5 (C-7), 41.0 (C-8), 50.2 (C-9), 37.5 (C-10), 22.9 (C-11), 26.8 (C-12), 37.6 (C-13), 42.0 (C-14), 27.5 (C-15), 35.26 (C-16), 44.1 (C-17), 49..0 (C-18), 48.4 (C-19), 151.6 (C-20), 31.0 (C-21), 39.6 (C-22), 28.0 (C-23), 15.3 (C-24), 16.3 (C-25), 16.1 (C-26), 14.9 (C-27), 18.2 (C-28), 109.8 (C-29), 19.6 (C-30).

***β*-sitosterol** (**7**): mp. 132-134 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$) δ 5.39 (1H, m, H-6), 3.56 (1H, m, H-3), 1.05 (3H, s, Me-19), 0.96 (3H, d, J=6.5 Hz, Me-21), 0.89 (3H, t, J=7.4 Hz, Me-29), 0.87 (3H, d, J=6.7 Hz, Me-26), 0.85 (3H, d, J=6.7Hz, Me-27), 0.72 (3H, s, Me-18); ^{13}C NMR (150.91 MHz, $CDCl_3$) δ 37.7 (C-1), 32.3 (C-2), 72.2 (C-3), 42.8 (C-4), 141.2 (C-5), 122.1 (C-6), 32.1 (C-7), 32.3 (C-8), 50.6 (C-9), 36.9 (C-10), 21.5 (C-11), 40.2 (C-12), 42.8 (C-13), 57.2 (C-14), 24.7 (C-15), 28.7 (C-16), 56.5 (C-17), 12.4 (C-18), 19.8 (C-19), 36.6 (C-20), 19.2 (C-21), 34.4 (C-22), 26.5 (C-23), 46.2 (C-24), 29.6 (C-25), 20.2 (C-26), 19.5 (C-27), 23.5 (C-28), 12.3 (C-29).

Ursolic acid (**8**): mp. 266-268 ºC, ^{1}H NMR (600.19 MHz, DMSO-d_6) δ 5.13 (1H, m, H-12), 4.31 (1H, brs, OH), 3.00 (1H, m, H-3), 2.10 (1H, d, J=11.25 Hz, H-18), 1.04 (3H, s, Me-27), 0.92 (3H, d, J=6.5 Hz, Me-30), 0.89 (3H, s, Me-24), 0.87 (3H, s, Me-25), 0.81 (3H, d, J=6.25 Hz, Me-29), 0.75 (3H, s, Me-26), 0.68 (3H, s, Me-23); ^{13}C-NMR (DMSO-d6) δ 39.2 (C-1), 27.8 (C-2), 77.7 (C-3), 39.2 (C-4), 55.6 (C-5), 18.9 (C-6), 33.6 (C-7), 40.0 (C-8), 47.9 (C-9), 37.4 (C-10), 23.7 (C-11), 125.4 (C-12), 139.0 (C-13), 42.5 (C-14), 28.4 (C-15), 24.7 (C-16), 47.7 (C-17), 53.2 (C-18), 39.4 (C-19), 39.3 (C-20), 31.1 (C-21), 37.2 (C-22), 29.1 (C-23), 16.1 (C-24), 16.9 (C-25), 17.8 (C-26), 24.1 (C-27), 179.1 (C-28), 17.9 (C-29), 21.9 (C-30).

Sclareol (**9**): mp. 99-102 ºC, ^{1}H NMR (600.19 MHz, $CDCl_3$) δ 5.83 (1H, dd, J=11, 16 Hz, H-14), 5.31 (1H, dd, J=2, 16 Hz, H-15), 4.73 (1H, dd, J=2, 11 Hz, H-15′), 1.30 (3H, s, Me-13), 1.18 (3H, s, Me-17), 0.99 (3H, s), 0.83 (6H, s); ^{13}C NMR (150.91 MHz, $CDCl_3$) δ 39.5 (C-1), 18.2 (C-2), 42.0 (C-3), 31.5 (C-4), 55.9 (C-5), 20.3 (C-6), 43.9 (C-7), 74.1 (C-8), 59.7 (C-9), 38.4 (C-10), 21.7 (C-11), 45.4 (C-12), 79.0 (C-13), 145.5 (C-14), 110.2 (C-15), 23.7 (C-16), 27.0 (C-17), 33.2 (C-18), 25.4 (C-19), 16.5 (C-20).

6β-hydroxysclareol (**10**): mp. 109-111 ºC, ^{1}H NMR (600.19 MHz, DMSO-d_6) δ 5.84 (1H, dd, J=17.4, 10.7 Hz, H-14), 5.10 (1H, dd, J=17.4, 2.1 Hz, H-15), 4.90 (1H, dd, J=10.7, 2.1 Hz, H-15), 1.18 (3H, s, H-17), 1.11 (3H, s, H-19), 1.10 (3H, s, H-16), 1.06 (3H, s, H-20), 0.9 (3H, s, H-18); ^{13}C NMR (150.91 MHz, DMSO-d_6) δ 41.2 (C-1), 17.9 (C-2), 43.5 (C-3), 33.2 (C-4), 56.4 (C-5), 66.2 (C-6), 51.5 (C-7), 71.2 (C-8), 61.3 (C-9), 38.7 (C-10), 18.7 (C-11), 45.6 (C-12), 71.5 (C-13), 146.3 (C-14), 109.9 (C-15), 27.3 (C-16), 25.0 (C-17), 32.8 (C-18), 23.1 (C-19), 15.9 (C-20).

Daucosterol (**11**): mp. 274-276 ºC, ^{1}H NMR (600.19 MHz, pyridine-d_5) δ 5.32 (1H, m, H-6), 4.99 (1H, d, J = 7.7 Hz, H-1′), 4.50 (1H, dd, J = 11.6, 2.1 Hz, H-6′β), 4.40 (1H, dd, J = 11.7, 5.2 Hz, H-6′α), 4.23 (2H, m, H-3′,4′), 4.00 (1H, t, J = 7.9 Hz, H-2′), 3.93 (1H, m, H-5′), 3.90 (1H, m, H-3), 0.96 (3H, d, J=6.4 Hz, Me-21), 0.91 (3H, s, Me-19), 0.87 (3H, t, J=7.3 Hz, Me-29), 0.85 (3H, d, J = 6.8 Hz, Me-26), 0.83 (3H, d, J = 6.9 Hz, Me-27), 0.64 (3H, s, Me-18); ^{13}C NMR (150.91 MHz, pyridine-d_5) δ 38.8 (C-1), 33.5 (C-2), 79.8 (C-3), 41.3 (C-4), 142.2 (C-5), 123.2 (C-6), 31.6 (C-7), 33.4 (C-8), 51.7 (C-9), 38.2 (C-10), 22.7 (C-11), 40.7 (C-12), 43.8 (C-13), 58.1 (C-14), 25.8 (C-15), 29.9 (C-16), 57.6 (C-17), 13.5 (C-18), 20.7 (C-19), 37.7 (C-20), 20.3 (C-21), 35.5 (C-22), 27.7 (C-23), 47.4 (C-24), 30.7 (C-25), 21.3 (C-26), 20.5 (C-27), 24.7 (C-28), 13.3 (C-29),103.9 (C-1′), 76.7 (C-2′), 79.9 (C-3′), 73.0 (C-4′), 79.4 (C-5′), 64.1 (C-6′).

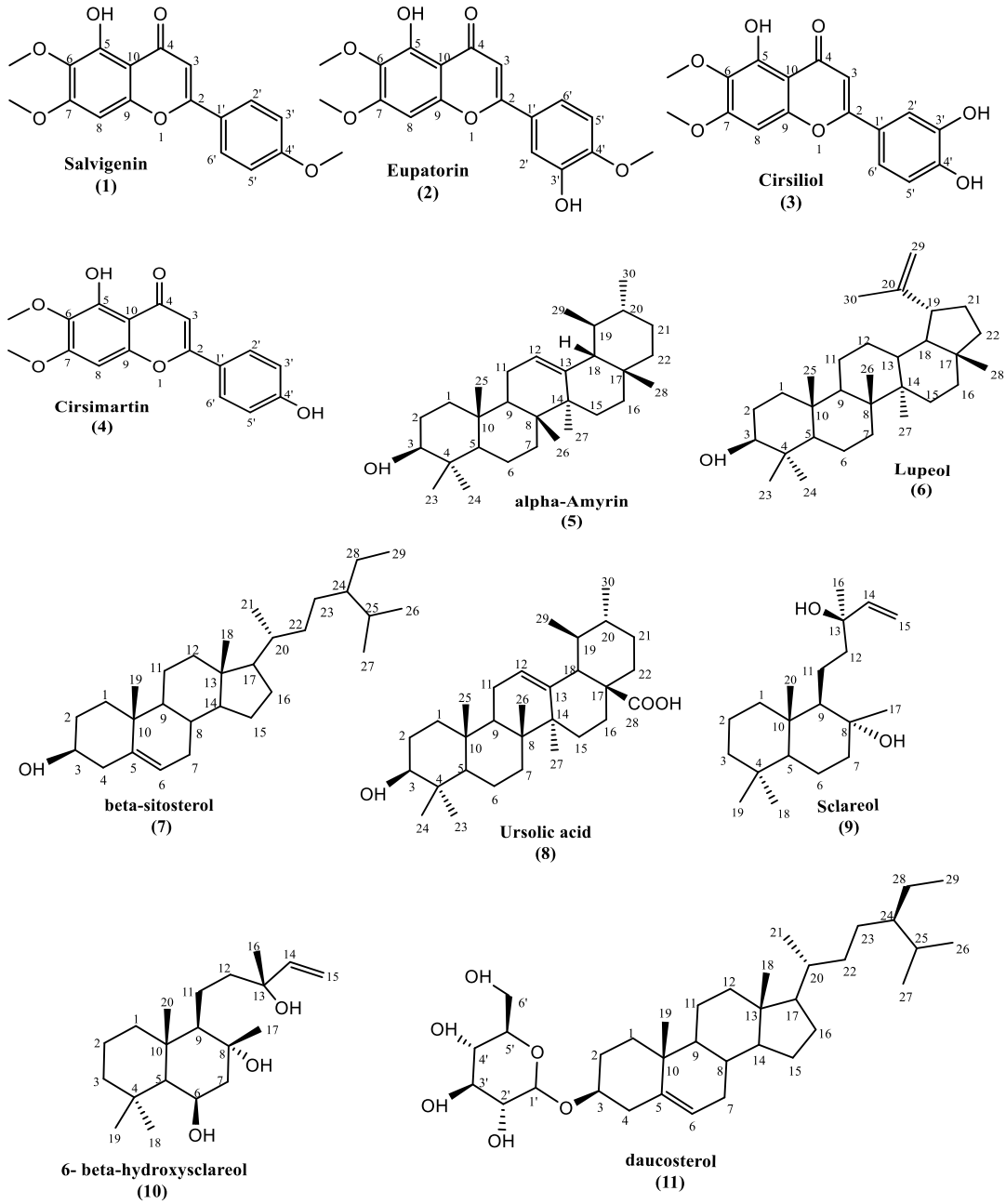

Fig. 1 The chemical structure of compounds **1-11**.

DISCUSSION

S. rhytidea from the Lamiaceae family is an endemic plant that grows in the southeast of Iran. Generally, bioactive effects of this plant such as antioxidant, antidiabetic, and antifungal have been reported previously [16-17]. Two phytochemical studies on the roots of this plant introduced abietane and rearranged abietane diterpenoids as bio-effective agents. In one of the studies, two diterpenoid derivatives with anticancer activity, namely sahandinone and miltirone were isolated from ether extract of roots of *S. rhytidea* [16]. In the other study, 1-deoxo-aurocadiol, ferruginol, taxodione, arucadiol, microstegiol, and 7α-etoxyroyleanone were reported from the petroleum ether extract of roots of this plant [19].

With respect to uses of *S. rhytidea* in folk medicine and experiments that have been accomplished to investigate its biological properties, we decided to study the chemical composition of the crude extract. This process resulted in isolation of eleven known compounds including four flavonoids, three pentacyclic triterpenes, two labdane diterpenoids, and two steroids. Ursane and oleanane-type triterpenoids are common in *Salvia* species while lupane-type triterpenoids and labdane diterpenoids were only found in some species, and *S. rhytidea* is a new source of these terpenoids. In the past few decades, Salvia constituents have attracted considerable attention from medicinal chemists and clinicians as antimicrobial, antioxidant, antitumor, and antifeeding agents. Many natural Salvia constituents from different species, as well as hemisynthetic derivatives, have been tested by many research groups. A few have shown very potent activity against bacteria and tumor cell lines [13].

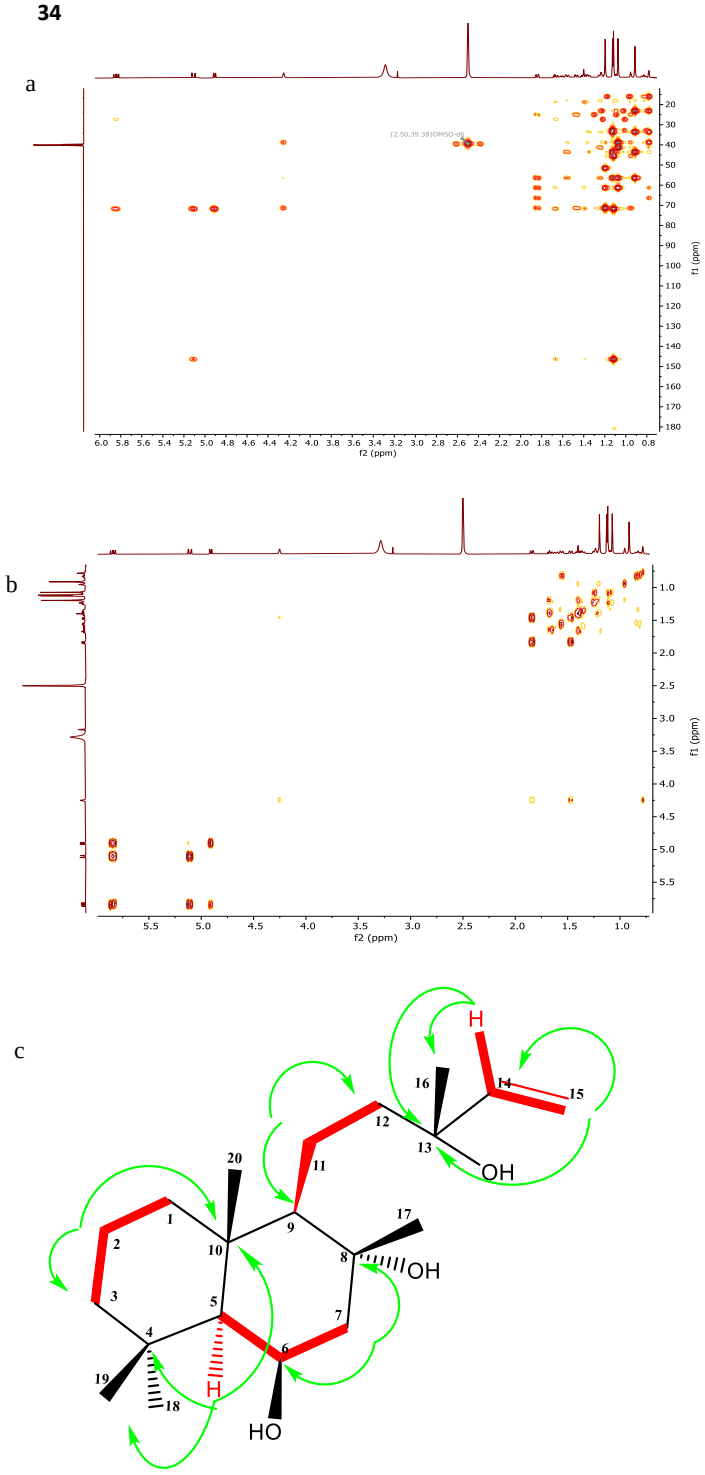

Fig. 2 HMBC (a) and ^{1}H-^{1}H COSY (b) spectra and Key correlations of 6β-hydroxysclareol (10) (c), ^{1}H-^{1}H COSY ( ) and HMBC ( ) .

Flavonoids are widely identified as a class of natural products with cancer protective properties through multifactorial pathways [35]. Salvigenin has been found to have anti-proliferative, anti-inflamatory and cytotoxic effects in different cellular models of cancer [36]. Eupatorin has been shown to exhibit anti-proliferative, antiangiogenesis, anti-inflammatory and cytotoxic properties in cell culture studies in vitro and in vivo [37-38]. Also, cirsiliol has been shown to exhibit hypnotic and sedative effects due to its ability to function as a competitive ligand for the BDZ-R (benzodiazepine receptors) [27]. In previous neuro-pharmacological research, cirsimaritin has been found to demonstrate central nervous system (CNS) activity such as anxiolysis, antidepressant and antinociception effects in mouse models [39].

Diterpenoids are bioactive compounds that are widely used in drug development and clinical research [40]. For the first time in 1931, a labdane diterpene, sclareol was isolated from *S. sclarea* L. This metabolite has been reported to demonstrate antibacterial and antifungal activity and is used commercially in the perfumery and tobacco industries [41]. 6*β*-hydroxysclareol together with five other sclareol derivatives were isolated from the extract of aerial parts of *S. reuterana*. These compounds were evaluated for the inhibitory activity in MCF-7 and Hela cell lines. The finding of the structure–activity relationship (SAR) investigation has shown that the presence of double bond in the sclareol skeletons is a key and important feature that affects the change in the cytotoxic activity of metabolites [12].

Triterpenes are one of the most widespread classes of bioactive natural compounds with antioxidant, anticancer, anti-inflammatory, hepatoprotective, and anti-HIV activities [42]. Lupeol, a metabolite belonging to the pentacyclic triterpene group, has been shown to have antioxidant, anti-diabetic, anti-inflammatory, and anti-mutagenic effects [43]. *α*-amyrin, as a pentacyclic triterpene, has been found to demonstrate a wide spectrum of activity including hepatoprotective, antihyperglycemic, anti-ulcer, anti-tumor, and anti-inflammatory properties [44]. In terms of health effects, ursolic acid has been noted for its antihyperlipidemic, anti-inflammatory, antitumor activities in laboratory animals [45].

Phytosterols have been distinguished with a slight difference at the C-24 position known as stigmasterol, *β*-sitosterol and campesterol. According to in vivo research, a diet containing 2% mixed phytosterols has been found to reduce prostate, breast and colon cancers with suppresses the proliferation and induces the cell cytotoxicity [46]. Furthermore, *β*-sitosterol and daucosterol (β-sitosterol glycoside), which are sterols derived from plants, have been found to exhibit antineoplastic, immunomodulating and anti-inflammatory properties [47].

With regard to excessive amount of salvigenin, ursolic acid, and *β*-sitosterol in *S. rhytidea*, therapeutic effects of the plant may be related to the presence of these compounds. Nevertheless, we cannot attribute biological properties of *S. rhytidea* to one of these metabolites. These compounds have also been found plentifully in other species of Lamiaceae family and exact assessment of its biological effects in these species has not been reported. Therefore, we believe that the biological effects of *S. rhytidea* are due to all existing compounds on this plant.

CONCLUSION

Phytochemical investigation of the EtOAc extract of *S. rhytidea* aerial parts resulted in isolation and structure elucidation of eleven compounds (1–11) from this species for the first time. Our results showed that *S. rhytidea* is a rich source of flavonoids, triterpenoids, and labdane diterpenoids. All these types of compounds have a good history for use as anti-cancer and anti-oxidant reagents. Hereupon, *S. rhytidea* has a good potential to conduct further studies in the food and pharmaceutical fields.

Authors Contributions

MMF designed and coordinated the project. AS performed the extraction, isolation and structural identification of the compounds. MA provided the NMR instrumental facilities. AS and MMF wrote the manuscript. All authors reviewed the manuscript.

Conflict of Interest

Authors declare no conflict of interest in this study.

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REFERENCES

1. Adedeji A.A., Babalola O.O. Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. Planta 2020; 252: 61.
2. Olanrewaju O.S., Ayangbenro A.S., Babalola O.O., Glick B.R. Plant health: feedback effect of root exudates-rhizobiome interactions. Appl Microbiol Biotechnol. 2019; 103: 1155-1166.
3. Ramírez R.D., Passari A.K., Ruiz V.B., Rodríguez S.R., Sánchez S., Demain A.L. Impact of novel microbial secondary metabolites on the pharma industry. Appl Microbiol Biotechnol. 2022; 106: 1855-1878.
4. Walker J.B., Sytsma K.J., Treutlein J., Wink M. Salvia (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. Am J Bot. 2004; 91: 1115-1125.
5. Askari S.F., Avan R., Tayarani Z., Sahebkar A., Eghbali S. Iranian *Salvia* species: a phytochemical and pharmacological update. Phytochemistry 2021; 183: 112619.
6. Bahadori M.B., Valizadeh H., Asghari B., Dinparast L., Farimani M.M., Bahadori Sh. Chemical composition and antimicrobial, cytotoxicity, antioxidant and enzyme inhibitory activities of *Salvia spinosa* L. J. Funct. Foods 2015; 18: 727-736.
7. Zargari A. Medicinal plants. University of Tehran Pub., Tehran, Iran. 1990; 4.
8. Farimani M.M., Mazarei Z. Sesterterpenoids and other constituents from *Salvia lachnocalyx* Hedge. Fitoterapia 2014; 98: 234-240.
9. Kahraman A., Celep F., Dogan M. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). S. Afr. J. Bot. 2010; 76: 187-195.
10. Farimani M.M., Bahadori M.B., Koulaei S.A., Salehi P., Ebrahimi S.N., Khavasi H.R., Hamburger M. New ursane triterpenoids from *Salvia urmiensis* Bunge: absolute configuration and anti-proliferative activity. Fitoterapia 2015; 106: 1-6.
11. Farimani M.M., Khodaei B., Moradi H., Aliabadi A., Ebrahimi S.N., De Mieri M., Kaiser M., Hamburger M. Phytochemical study of *Salvia leriifolia* roots: rearranged abietane diterpenoids with antiprotozoal activity. J. Nat. Prod. 2018; 81: 1384-1390.
12. Farimani M.M., Miran M. Labdane diterpenoids from *Salvia reuterana*. Phytochemistry. 2014; 108: 264-269.
13. Wu Y.B., Ni Zh.Y., Shi Q.W., Dong M., Kiyota H., Gu Y.Ch., Cong B. Constituents from *Salvia* species and their biological activities. Chemical Reviews 2012; 112: 5967-6026.
14. Farimani M.M., Taleghani A., Aliabadi A., Aliahmadi A., Esmaeili M.A., Sarvestani N.N., Khavasi H.R., Smieško M., Hamburger M., Ebrahimi S.N. Labdane diterpenoids from *Salvia leriifolia*: Absolute configuration, antimicrobial and cytotoxic activities. Planta Med. 2016; 1279-1285.
15. Tabefam M., Farimani M.M., Danton O., Ramseyer J., Nejad Ebrahimi S., Neuburger M., Kaiser M., Salehi P., Potterat O., Hamburger M. Antiprotozoal isoprenoids from *Salvia hydrangea*. J. Nat. Prod. 2018; 81: 2682-2691.
16. Jassbi A.R., Eghtesadi F., Hazeri N., Ma'sumi H., Valizadeh J., Chandran J.N., Schneider B., Baldwin I.T. The roots of *Salvia rhytidea*: a rich source of biologically active diterpenoids. Nat. Prod. Res. 2017; 31: 477-481.
17. Fooladi S., Ansari M., Sharififar F., Pournourmohammadi S., Rad B.L., Mohamadi N. Effect of *Salvia rhytidea* Benth. extract on serum glucose, gut alphaglucosidase in healthy and streptozotocin-induced diabetic rats. J. Ayurvedic Herb. Med. 2016; 2: 40-42.
18. Salari S., Bakhshi T., Sharififar F., Naseri A., Almani P.G. Evaluation of antifungal activity of standardized extract of *Salvia rhytidea* Benth.(Lamiaceae) against various *Candida* isolates. J Mycol Med. 2016; 26: 323-330.
19. Eghtesadi F., Farimani M.M., Hazeri N., Valizadeh J. Abietane and nor-abitane diterpenoids from the roots of *Salvia rhytidea*. Springer Plus. 2016; 5: 1-6.
20. Rustaiyan A.H., Akhgar M.R., Masoudi S., Nematollahi F. Chemical composition of essential oils of three Salvia species growing wild in Iran: *Salvia rhytidea* Benth, *S. limbata* CA Mey. and *S. palaestina* Benth. J. Essent. Oil Res. 2005; 17: 522-524.
21. Habibi Z., Yousefi M., Aghaie H.R., Salehi P., Masoudi S., Rustaiyan A.H. Chemical composition of essential oil of *Salvia persepolitana* Boiss. and *Salvia rhytidea* Benth. from Iran. J. Essent. Oil Res. 2008; 20: 1-3.
22. Soroury S., Alilou M., Gelbrich T., Tabefam M., Danton O., Ebrahimi S.N., Kaiser M., Hamburger M., Stuppner H., Farimani M.M. Unusual derivatives from *Hypericum scabrum*. Sci. Rep. 2020; 10: 22181.
23. Zahabi Z.F., Sharififar F., Almani P.G., Salari S. Antifungal activities of different fractions *of Salvia rhytidea* Benth as a valuable medicinal plant against different *Candida* species in Kerman province (Southeast of Iran). Gene Rep. 2020; 19: 100624.
24. Ansari M., Sharififar F., Arabzadeh A.M., Mehni F., Mirtadzadini M., Iranmanesh Z., Nikpour N. *In vitro* evaluation of anti-herpes simplex-1 activity of three standardized medicinal plants from Lamiaceae. Anc. Sci. Life. 2014; 34: 33.
25. Ulubelen A., Öztürk S., Işildatici S. A new flavone from *Salvia triloba* Lf (Labiatae). J Pharm Sci. 1968; 57: 1037-1038.
26. González-Cortazar M., Salinas-Sánchez D.O., Herrera-Ruiz M., Román-Ramos D.C., Zamilpa A., Jiménez-Ferrer E., Ble-González E.A., Álvarez-Fitz P., Castrejón-Salgado R., Pérez-García M.D. Eupatorin and Salviandulin-A, with Antimicrobial

and Anti-Inflammatory Effects from *Salvia lavanduloides* Kunth Leaves. Plants. 2022; 11: 1739.

27. Marder M., Viola H., Wasowski C., Wolfman C., Waterman PG., Medina JH., Paladini AC. Cirsiliol and caffeic acid ethyl ester, isolated from *S. guaranitica*, are competitive ligands for the central benzodiazepine receptors. Phytomedicine 1996; 3: 29-31.
28. Hawas U.W., El-Desoky S.K., Kawashty S.A., Sharaf M. Two new flavonoids from *Origanum vulgare*. Nat. Prod. Res. 2008; 22: 1540-1543.
29. Okoye N.N., Ajaghaku D.L., Okeke H.N., Ilodigwe E.E., Nworu C.S., Okoye F.B.C. beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharm. Biol. 2014;52: 1478-1486.
30. Magalhães C.G., Ferrari F.C., Guimarâes D.A., Silva G.D., Duarte L.P., Figueiredo R.C. Maytenus salicifolia Reissek, Celastraceae: triterpenes isolated from stems and antioxidant property of extracts from aerial parts. Rev Bras Farmacogn. 2011;21:415-419.
31. Bin Sayeed M.S., Rezaul Karim S.M., Sharmin T., Morshed M.M. Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: a plant-derived orphan phytosterol. Med. 2016; 3: 29.
32. Seebacher W., Simic N., Weis R., Saf R., Kunert O. Complete assignments of 1H and 13C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. Magn Reson Chem. 2003;41:636-638.
33. Ncube E.N., Steenkamp P.A., van der Westhuyzen C.W., Steenkamp L.H., Dubery I.A. Metabolomics-guided analysis of the biocatalytic conversion of sclareol to ambradiol by Hyphozyma roseoniger. Catalysts 2022; 12: 55.
34. Abdollahnezhad H., Bahadori M.B., Pourjafar H., Movahhedin N. Purification, characterization, and antioxidant activity of daucosterol and stigmasterol from *Prangos ferulacea*. Lett. Appl. Biosci. 2021; 10: 2174-2180.
35. Raffa D., Maggio B., Raimondi M.V., Plescia F., Daidone G. Recent discoveries of anticancer flavonoids. Eur. J. Med. Chem. 2017; 142: 213-228.
36. Mansourizadeh F., Sepehri H., Khoee S., Farimani M.M., Delphi L., Tousi M.Sh. Designing Salvigenin–loaded mPEG-b-PLGA@ Fe_3O_4 nanoparticles system for improvement of Salvigenin anti-cancer effects on the breast cancer cells, an in vitro study. J Drug Deliv Sci. Technol. 2020; 57: 101619.
37. Sarvestani N.N., Sepehri H., Delphi L., Farimani M.M. Eupatorin and salvigenin potentiate doxorubicin-induced apoptosis and cell cycle arrest in HT-29 and SW948 human colon cancer cells. Asian Pac. J. Cancer Prev. 2018; 19: 131.
38. Tousi M.Sh., Sepehri H., Khoee S., Farimani M.M., Delphi L., Mansourizadeh F. Evaluation of apoptotic effects of mPEG-b-PLGA coated iron oxide nanoparticles as a eupatorin carrier on DU-145 and LNCaP human prostate cancer cell lines. J. Pharm. Anal. 2021; 11: 108-121.
39. Abdelhalim A., Karim N., Chebib M., Aburjai T., Khan I., Johnston G.AR., Hanrahan J. Antidepressant, anxiolytic and antinociceptive activities of constituents from *Rosmarinus officinalis*. J. Pharm. Pharm. Sci. 2015; 18: 448-459.
40. Hu Zh., Liu X., Tian M., Ma Y., Jin B., Gao W., Cui G., Guo J., Huang L. Recent progress and new perspectives for diterpenoid biosynthesis in medicinal plants. Med. Res. Rev. 2021; 41: 2971-2997.
41. Choudhary M.I., Siddiqui Z.A., Hussain S. Structure elucidation and antibacterial activity of new fungal metabolites of sclareol. Chem. Biodivers. 2006; 3: 54-61.
42. Muffler K., Leipold D., Scheller M.Ch., Haas Ch., Steingroewer J., Bley Th., Neuhaus H.E., Mirata M.A., Schrader J., Ulber R. Biotransformation of triterpenes. Process Biochem. 2011; 46: 1-15.
43. Siddique H.R., Saleem M. Beneficial health effects of lupeol triterpene: a review of preclinical studies. Life Sci. 2011; 88: 285-293.
44. Singh D., Arya P., Sharma A., Dobhal M., Gupta R. Modulatory potential of α-amyrin against hepatic oxidative stress through antioxidant status in wistar albino rats. J. Ethnopharmacol. 2015; 161: 186-193.
45. Moghaddam F.M., Farimani M.M., Salahvarzi S., Amin Gh. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. Evid. Based Complementary Altern. Med. 2007; 4: 95-98.
46. Bradford P.G., Awad A.B. Phytosterols as anticancer compounds. Mol Nutr Food Res. 2007; 51: 161-170.
47. Esmaeili M.A., Farimani M.M. Inactivation of PI3K/Akt pathway and upregulation of PTEN gene are involved in daucosterol, isolated from *Salvia sahendica*, induced apoptosis in human breast adenocarcinoma cells. S. Afr. J. Bot. 2014; 93: 37-47.