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Ent-kaurene Diterpenoids from *Sideritis lycia* with Antiviral and Cytotoxic Activities

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Abstract: The genus *Sideritis* (Lamiaceae) is represented by 45 species (54 taxa) in Anatolia with high endemism ratio (74%), and Turkey is one of the gene centers of the genus along with Spain. Acetone extract of the aerial parts of *Sideritis lycia* afforded eight known *ent*-kaurene diterpenoids, structures of which have been identified as linearol, isolinearol, isosidol, sidol, siderol, sideridiol, 7-*epi*-candicandiol and foliol through ^{1}H NMR, ^{13}C NMR and mass spectroscopic analyses. Cytotoxic and antiviral activities of the acetone extract, linearol, sidol and isosidol were investigated together with insecticidal activity of species. The antiviral index of linearol, isosidol and acetone extract of *S. lycia* were determined as 2.31, 2.01 and 2.58, respectively, except sidol. 7-*Epi*-candicandiol was found to be the most active diterpene against a series of cancer cell lines with ED_{50} values; KB (13.3 µg/mL), COL-2 (11.8 µg/mL), LU1 (17.9 µg/mL), LNCaP (14.9 µg/mL) and A2780 (9.0 µg/mL). Activity results of this study indicated that *ent*-kaurane diterpenes have potential to be considered as antiviral and cytotoxic lead compounds.

Keywords: *Sideritis lycia*; diterpenoid; NMR and mass spectroscopy; antiviral; cytotoxic activity; insecticidal activity.

1. Introduction

Lamiaceae is one of the largest families with 46 genera and 603 species in Flora of Turkey with about 12.000 taxa [1,2]. The genus *Sideritis* L. (Lamiaceae) includes approximately 150 species of annual and perennial plants distributed in different parts of the world, mainly in the Mediterranean basin. Turkey is one of the gene centers for the genus *Sideritis* along with Spain [1-5]. In Turkey, 45 *Sideritis* species, 38 being endemic, are growing naturally. Therefore, *Sideritis* L. is one of the most endemic genera of the Flora of Turkey [4-6]. Four of them (totally 5 taxa), which are annual, are included in Burgsdorfia and Hesiodia sections; these are *S. lanata* L., *S. romana* L. subsp. *romana*, *S. curvidens* Stapf, *S. montana* L. subsp. *montana*, and *S. montana* L. subsp. *remota* (d'Urv.) P.W. Ball ex Heywood [3,5,6]. The other species are perennial belonging to Empledocia section in Turkey. As a

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result of a detailed morphological and anatomical revisions of *Sideritis* species growing in Turkey, number of the species has increased to 45 (54 taxa) [1,8].

Sideritis species have been used as herbal tea, called mountain tea or valley tea in Turkey. They are widely used in treatment of gastrointestinal disorders, common cold and as diuretic [7]. They have been used as antispasmodic, gastro-protective, anti-inflammatory, antidepressant and anti-stress agents in Turkey and throughout Mediterranean region [9-11]. There are a number of biological activity studies, carried out by various groups in Turkey, Spain, Balkans and other countries [12-14], particularly for their antimicrobial [15], antiulcerative and anti-inflammatory [16-18], cytotoxic, apoptotic, antispasmodic [19], antioxidant, and antinociceptive activities [20,21].

Most of the chemical studies on Turkish *Sideritis* species, particularly for diterpenic structures have been carried out by Topcu's group including *Sideritis athoa* [22], *S. argyrea* [23], *S. dichotoma* [24], *S. sipylea* [24], *S. trojana* [25], *S. leptoclada* [26], *S. stricta* [27], *S. tmolea* [28], *S. condensata* [29], *S. arguta* [30], *S. congesta* [31], *S. niveotomentosa* [32], *S. brevibracteata* [33], and several other *Sideritis* species [34]. Antioxidant and anticholinesterase activities of *ent*-kaurane diterpenoids from *Sideritis arguta* and *S. congesta* were studied by Topcu group, and 7-*epi*-candicandiol and sideroxol showed the highest butyrylcholinesterase inhibitory activity among the screened compounds, including the standard compound galanthamine [30,31,33,35]. Insecticidal activity of some *Sideritis* species were also investigated in the previous studies [29, 36].

In Turkey, some of *Sideritis* species were studied phytochemically for their flavonoids, flavonoid glycosides and phenylpropanoids [37] rather than the pure di- and triterpenoids, which have potential anti-viral and cytotoxic activities [33,38]. In fact, those flavonoids and phenolics were found to be responsible for their anti-inflammatory, anti-ulcer and gastrointestinal activities. Monoterpenoids and iridoids are also other important secondary metabolites of *Sideritis* species, former ones were found in their essential oils, mostly studied by Baser group [9,39]. Its leaves are consumed as tea, called mountain tea, particularly called as dağ çayı or Kemer çayı in the region including South-West Aegean and Mediteranean area of Turkey. To prepare a tea from a *Sideritis* species (namely *Sideritis congesta*, *S. arguta*, *S. argyrea*, *S. libanotica* subsp. *linearis*, *S. pisidica* and *S. perfoliata*), the dried leaves and spikes and/or heads are left as one or two spikes in a cup of boiled water for 2-3 min until appearance of nice yellow color with a pleasant aroma.

There are few previous studies on *S. lycia*. While one of them was on its flavonoid constituents, the others were on its essential oil analysis, both carried out by Ezer et al. [40]. Separately, a study was released on isolation of polyphenolic compounds from *S. lycia* with their anti-inflammatory activity [41,42]. A very recent study also reported phenolic compounds and antioxidant activity of *S. lycia* and *S. libanotica* subsp. *linearis* from natural and cultivated plant materials [43]. However, there is no report on isolation and structure elucidation studies on *S. lycia* diterpenoids.

In this study, an endemic *Sideritis* species *S. lycia* Boiss. et Heldr. apud Bentham, grown in Antalya region, collected at Kemer was investigated for its diterpenoids. Isolation and structure elucidation of the diterpenoids, based on NMR and mass spectral analyses, along with antiviral, cytotoxic and insecticidal activities are reported.

2. Materials and Methods

2.1. General Experimental Procedures

The spectra were recorded on the following instruments; NMR: ^{1}H- and ^{13}C-NMRs were recorded on Varian 600 MHz and 150 MHz, respectively, in $CDCl_3$. For MS, VG ZabSpec High Resolution Mass Spectrometer and Bruker Daltonics microTOF-Q instruments were applied. Silica-gel 60 and Kieselgel 60F_{254} (E.Merck) were used for column chromatography and prepared (TLC) plates, respectively.

2.2. Plant Material

The aerial parts of *S. lycia* Boiss. et Heldr. apud Bentham were collected in Kemer (Antalya), Turkey (by Turgut Kilic) in June 2010, and the species was identified by Prof. Dr. K.H.C. Baser (Eskisehir). A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE 10143).

2.3. Extraction and Isolation

Dried and powdered aerial parts of *S. lycia* (1.5 kg) were extracted with hexane and acetone successively to yield 25 g and 30.2 g of the extracts, respectively. After checking them on TLC plates, the acetone extract appeared to be rich in diterpenoids. A small part of the acetone extract was separated for the activity tests, the remaining part was lyophilized and kept in deep-freeze. This part (30 g) was then dissolved in a minimum amount of acetone, and some silica gel powder was added into a porcelain mortar and gently blended using a pestle, to obtain a homogeneous mixture at room temperature. The dried mixture was then added to the top of a silica gel column (5 cm x 80 cm) chromatogtaphy. The initial elution was performed using hexane, after which a gradient of CH_2Cl_2 was added up to 100 %, which was followed by additions of acetone and then MeOH. The fractions 29-31 were combined and subjected to a silica gel column chromatography (2 cm x 40 cm), eluting with CH_2Cl_2: acetone (9:1) solvent system. Similar fractions were combined to afford five main fractions (A-F). While the fraction B afforded **1** (4.88 g), which was the main compound of the acetone extract, fraction C gave **2** (CH_2Cl_2:acetone (80:20), 53 mg), **5** (CH_2Cl_2:acetone (80:20), 25 mg) and **6** (CH_2Cl_2: acetone (85:15), 32 mg). The fraction D yielded **7** (CH_2Cl_2:acetone (80:20), 8 mg), **3** (CH_2Cl_2:acetone (90:10), 73 mg), **4** (CH_2Cl_2:acetone (80:20),18 mg) and **8** (CH_2Cl_2: acetone (80:20), 22 mg).

2.4. Antiviral Activity Assays

2.4.1. Sample Preparation

All the samples were ~~first~~ dissolved in dimethylsulfoxide (DMSO) and tested at different concentrations according to Eagle's minimum essential medium (EMEM).

2.4.2. Cell Culture and Virus (Cell Viability Assay)

Vero cells are the normal African green monkey kidney epithelial cell lines which were grown and maintained in Eagle's minimum essential medium (EMEM) with Earle's saline, supplemented with an antibiotic-antimycotic mixture [penicillin (100 U/mL), streptomycin (100 µg/mL), amphotericin B (0.25 µg/mL)] and 10% fetal calf serum. The cells were kept in a humidified atmosphere containing 5% CO_2 at 37 ^oC. The Greer strain of human parainfluenza virus type 2 (HPIV-2) was used in all experiments and maintained in Vero cells. Approximately 10^5 plaque forming units (p.f.u.) of virus in a minimal medium were allowed to be absorbed by Vero cells with a gentle rocking of the tissue culture flask every 10 min within 90 min. The solution containing the virus was then removed, and a freshly prepared media, EMEM, containing 2% fetal calf serum, was added. The infected cells were allowed to grow until the cytopathic effect was completely obtained (usually in 48-72 h). The supernatant was removed, quickly frozen and stored at -80 ^oC until it was used. The infectivity was determined by plaque assay on Vero cell monolayers, and expressed as plaque assay on Vero cell monolayers as a plaque forming unit per milliliter (p.f.u./mL).

2.4.3. Cytotoxicity Assay

The cytotoxicity assays were performed according to the micro culture MTT method [44,45]. The cells were harvested (4.5-5.0 × 10^4 cells/well) and inoculated in 24 well microtiter plates. They were washed with phosphate buffered saline (PBS), and the cultured cells were then inoculated with and

without the samples to be investigated. The final concentration of DMSO did not exceed 0.2% (v/v), which is the concentration with no effect on cell replication. After 72 h incubation, the medium was aspirated. 150 µL of MTT solution (5 mg/mL, in PBS, pH 7.2) was then added to each well, and the plates were incubated for 4 h at 37 ºC, after which 800 µL of DMSO was added to each well of the plates, followed by gentle shaking for 15 min to solubilize the formazan dye. Absorbance was measured at 540 nm using a photometer, and the surviving compounds (or extract) were calculated.

2.4.4. Plaque Assay

Confluent monolayer cultures of Vero cells in a 60 mm plastic dish were washed twice with Dulbecco's phosphate buffer saline (PBS) and exposed to HPIV-2. After adsorption for 90 min at 37 ºC, the cells were washed twice with PBS, overlaid with EMEM, containing 0.9% agarose, and the acetone extract of *S. lycia* and its pure diterpenoids were prepared at four different concentrations. The cultures were incubated at 37 ºC for 3-4 days. At the end of this period, the cell monolayers were fixed in the formol-saline for 15 min and stained with amido black [45,46]. Concentration of each sample, which was required to inhibit the virus plaque number, was estimated.

2.4.5. Evaluation of Cytotoxic Activity Against Several Cancer Cell Lines

The compounds **1** - **3**, **7** and **8** were evaluated with cultured KB (human epidermoid carcinoma), P-388 (mouse leukemia), COL-2 (human colon cancer), hTERT RPE (human retinal pigmented epithelial cancer), LU1 (human lung cancer), LNCaP (hormone-dependent human prostate cancer) and A2780 (human ovarian cancer) cell lines [47-49].

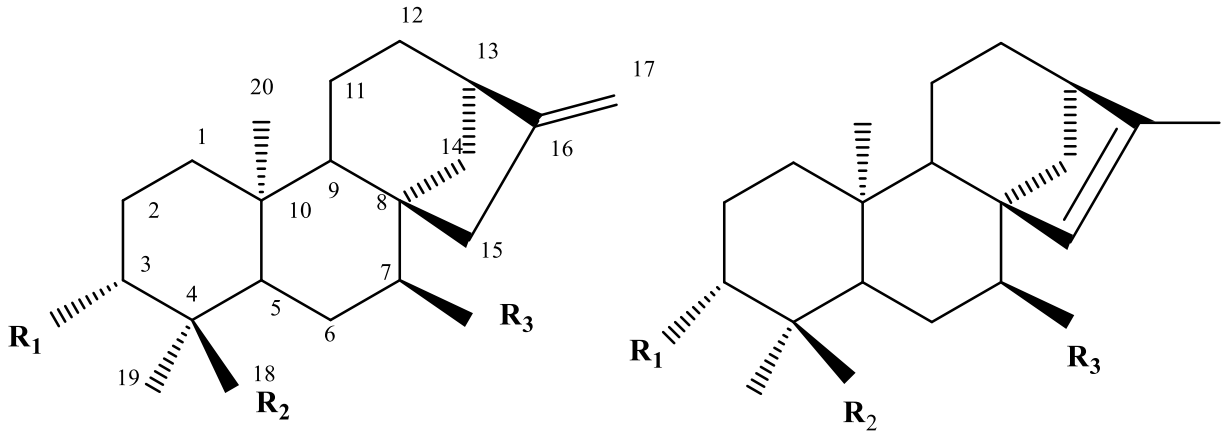
3. Results and Discussion

Fractionation and isolation studies from the acetone extract of *S. lycia* were performed on a silica gel CC (column chromatography) and purified by prep. TLC to afford 8 diterpenoids. Their structure elucidation was made based on 1D and 2D NMR and mass spectroscopic techniques which were identified as *ent*-kaurane diterpenoids; linearol (**1**), sidol (**2**), 7-*epi*-candicandiol (**3**), foliol (**4**) isolinearol (**5**), isosidol (**6**), siderol (**7**), and sideridiol (**8)** (Figure 1 and Table 1). Linearol (**1**) was obtained as the main compound of the acetone extract of *S. lycia* in a fairly high yield (0.33%, based on dried plant material weight). Some of the other Anatolian *Sideritis* species also yielded linearol in high yield, but, none of these species has afforded yet such high linearol percentage. These are *S. athoa* (0.1%) [22], *S. argyrea* (0.13%) [23] and *S. sipylea* (0.03%) [24], obtained in our previous studies. Thus, *S. lycia* should be considered as a rich resource of linearol. In terms of diterpenic compounds, *Sideritis* species are fairly rich compared to the most of the other genera of Lamiaceae family plants, such as *Salvia* species. The percentage of the other diterpenoids, isolated along with linearol from the same extract of *S. lycia*, are as follows: sidol (**2**) (0.035%), 7-*epi*-candicandiol (**3**) (0.048%), foliol (**4**) (0.012%), isolinearol (**5**) (0.016%), isosidol (**6**) (0.021%), siderol (**7**) (0.005%), and sideridiol (**8**) (0.014%). Linearol (**1**) [50,51], sidol (**2**) [52], 7-*epi*-candicandiol (**3**) [51], foliol (**4**) [51,53], isolinearol (**5**) [54], isosidol (**6**) [52,55], siderol (**7**) [56] and sideridiol (**8)** [55] were characterized by using 1D and 2D NMR techniques along with mass spectroscopy.

Concentrations of both inhibition of virus plaque formation (as ED_{50}) and reduced viability of Vero cells (as CD_{50}) of the acetone extract and the three diterpenoids of *S. lycia* were investigated. None of them was found to be cytotoxic up to 0.1 µg/mL, indicating that they could be promising antiviral agents, particularly isosidol and linearol. Furthermore, cytotoxicity of the isolated five *ent*-kaurane diterpenoids were screened against seven different cancer cell lines; only 7-*epi*-candicandiol showed meaningful results.

3.1. In vitro Antiviral Activity

Antiviral potency of the acetone extract was investigated at four concentrations, i.e. 0.1, 0.5, 1.0 and 10.0 µg/mL. Fifty percent effective dose required to inhibit virus plaque formation (Table 1).



	R_1	R_2	R_3		R_1	R_2	R_3
1	OH	CH_2OAc	OH	**5**	OH	CH_2OH	OH
2	OAc	CH_2OH	OH	**6**	OAc	CH_2OH	OH
3	H	CH_2OH	OH	**7**	H	OH	OAc
4	OH	CH_2OH	OH	**8**	OH	OH	OH

Figure 1. Structures of the isolated compounds (**1-8**)

The CD_{50} values of the tested compounds, linearol (**1**), sidol (**2**) and isosidol (**6**), and the acetone extract for the viability of Vero cells were determined as 29.32, 14.64, 27.27 and 2.91 μg/mL, respectively. Although acetone extract of *S. lycia* displayed the best CD_{50} and ED_{50} values, the most active compound was found to be isosidol (Table 2). Antiviral index (AI) values of linearol and isosidol were close to each other. However, sidol showed almost no plaque inhibition effect, thus, it was determined to be inactive.

Table 1. Antiviral potency of *ent*-kaurenes and acetone extract of *S. lycia* on HPIV-2 in Vero Cells

Compound	ED_{50} (μg/mL)[a]	CD_{50} (μg/mL)[b]	AI[c]
Linearol **(1)**	12.72 ± 2.80[e]	29.32 ± 3.32 [e]	2.31
Sidol **(2)**	NA[d]	27.27 ± 3.10	NA
Isosidol **(6)**	7.27 ± 1.59	14.64 ± 1.50	2.01
S. lycia (Acetone Extract)	1.13 ±0.25	2.91 ± 1.20	2.58

[a]Fifty –percent effective dose, or concentration required to inhibit virus plaque formation by 50%.
[b]The concentration of tested compounds/extract which reduced viability of Vero cells by 50%.
[c]Antiviral index (CD_{50}/ED_{50})
[d] NA: Not Active
[e]Values ED_{50} and CD_{50} are averages and standard deviations for three independent experiments

3.2. Cytotoxic Activities Against a Series Cancer Cell Lines

Linearol (**1**), sidol (**2**), 7-*epi*-candicandiol (**3**) siderol (**7**) and sideridiol (**8**) of *S. lycia* were evaluated for their cytotoxicity against KB, P-388, COL-2, hTERT RPE (human retinal pigmented epithelial cancer), LU1, LNCaP and A2780 cell lines (Table 2). While 7-*epi*-candicandiol (**7**) was found to be active in almost all the tested cell lines, including KB (13.3 μg/mL), COL-2 (11.8 μg/mL), LU1 (17.9 μg/mL), LNCaP (14.9 μg/mL) and A2780 (9.0 μg/mL) (Table 3), compound **2** (sidol) showed moderate activity against only A2780 at 15.6 μg/mL. The other diterpenoids did not display a satisfactory activity on the tested cancer cell lines (Table 2).

Table 2. Cytotoxic activity results of *ent*-kaurane diterpenoids (**1, 2-8**) against a panel of cancer cell lines[a]

Compound	KB	P-388	COL-2	hTERT RPE	LU 1	LNCaP	A2780
7-*Epi*candicandiol **(3)**	13.3	>20	11.8	NT	17.9	14.9	9.0
Sidol **(2)**	>20	>20	>20	>20	>20	>20	15.6
Siderol **(7)**	>20	>20	>20	>20	>20	>20	>20
Sideridiol **(8)**	>20	>20	>20	>20	>20	>20	>20
Linearol **(1)**	NT	NT	NT	NT	NT	NT	>20
Ellipticine (positive control)	0.02	0.3	0.04	0.3	0.1	0.8	-

[a]Compounds were initially tested at a concentration of 20 μg/mL, and followed by dose-response studies, as required to the yield ED_{50} values (μg/mL).

3.3. Insecticidal Activity

The use of synthetic insecticides in agriculture as pest control agents to increase the harvest and protect the stored products may involve serious health hazards for mammals. These insecticides are often associated with residuals that are hazardous for consumers and environment [56]. Particularly, the used chemicals in fighting these stored pests contain many dangers for human and animal health across the world [56-57]. The risk of developing insect resistance and the high cost-benefit ratio of synthetic pesticides channeled research towards alternative insecticides. The number of confirmed insect resistant species to synthetic pesticides has been continuing to rise [56,57]. Therefore, there is an urgent need to develop safer, environmentally friendly and efficient alternative to replace synthetic pesticides. As the natural products are well known to have a range of useful biological properties against insect pests, the acetone extract of *S. lycia* was tested against *Tetranychus urticae*, *Bemicia tabaci*, *Sitophilus granaries* and *Lasioderma serricorne* for the insecticidal activity profile. The P values' test was conducted for the statistical significance of dose and time against the above insects.

In the contact toxicity assay, *S. lycia* extract showed the highest toxicity *against Bemicia tabaci* with 63% (250 μL) and 81% (750 μL) mortalities at the end of 120 h. The extract also showed activity against *Tetranychus urticae* with 63% and *Sitophilus granarius* 47% (750 μL) at the end of 120 h. The lowest toxicity was observed against *Lasioderma serricorne* with 15% mortality (Figure 2). The toxicity of the acetone extract of *S. lycia* was significantly increased with time until up to 120 h (Figure 2).

Topical application of the *S.lycia* extract displayed a different toxicity compare with contact toxicity assay. The best insecticidal activity was observed against *Lasioderma serricorne* instead of *Bemicia tabaci*, with a mortality rate of 82% (2 μL) after 120 h. Toxicity of acetone extract was determined to have 68, 58 and 39% mortality against *S. granarius*, *Bemicia tabaci* and *Tetranychus urticae*, respectively (Figure 3).

The main compound linearol **(1)**, which is the 11.3% of the total acetone extract of *S. lycia*, was also tested against above organisms. By the contact toxicity assay, the mortality percentages of linearol **(1)** were determined as 80, 75, 70 and 40% (750 μL) against *Bemicia tabaci*, *Lasioderma serricorne*, *Tetranychus urticia* and *Sitophilus granarius*, respectively (Figure 3), which are matching with our previous result [29]. 7-*Epi*-candicandiol **(3)** from *S. lycia* was previously reported by our group, therefore, we do not report herein once more [36].

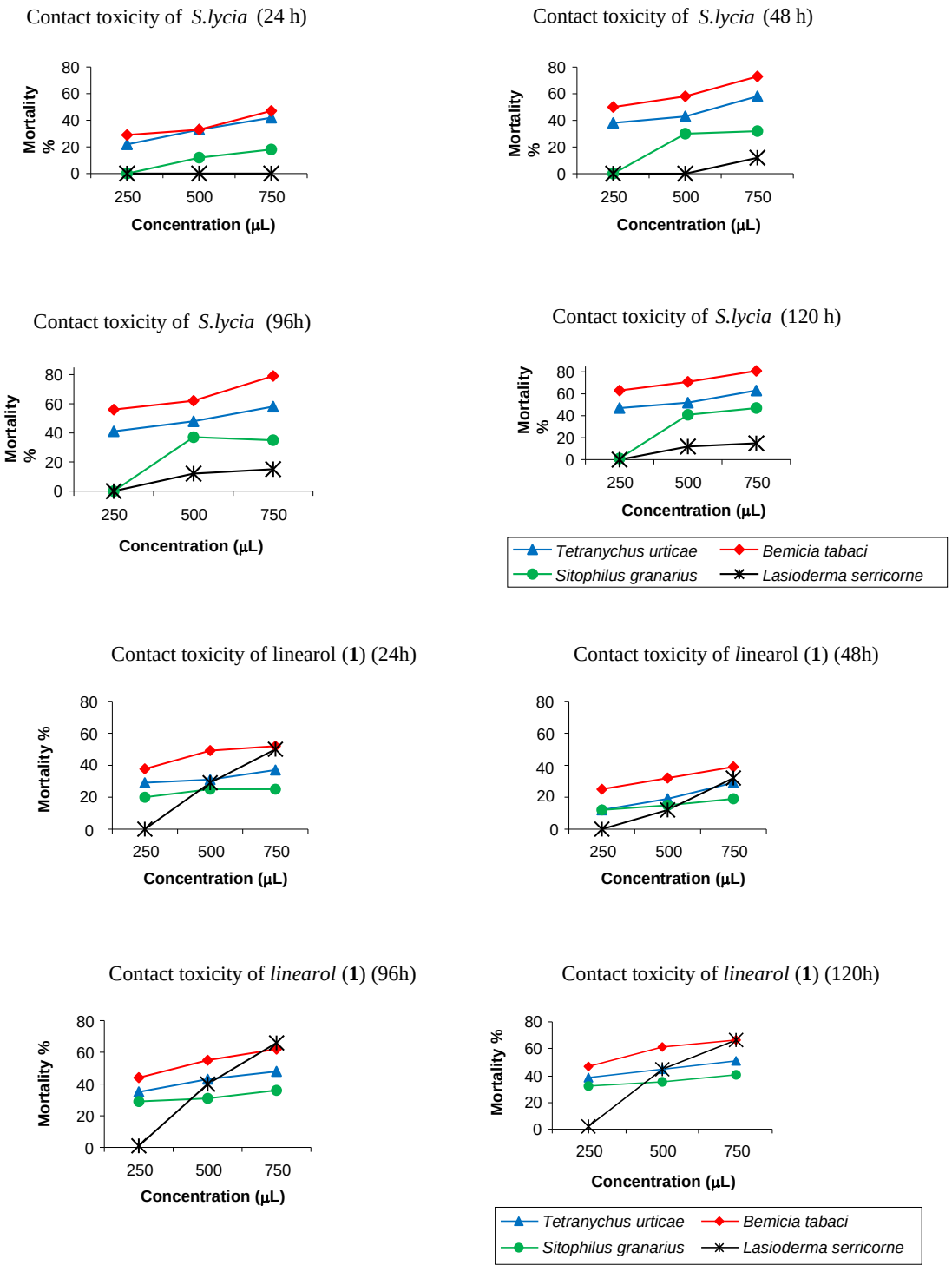


Figure 2. Percent mortality of insects for all doses and periods of the acetone extract of *S.lycia* and linearol (**1**) with contact toxicity assay

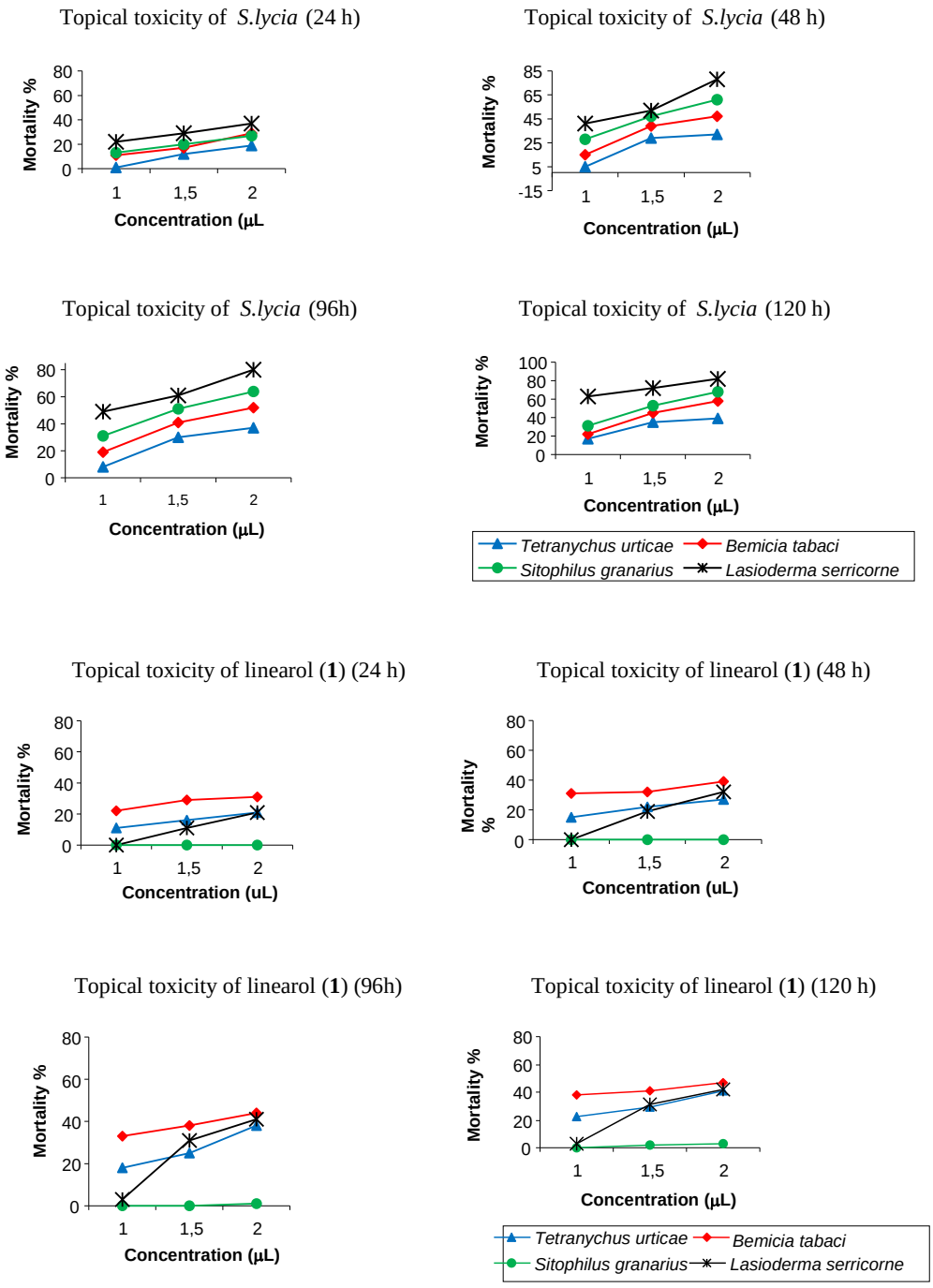


Figure 3. Percent mortality of insects for all doses and periods of the acetone extract of *S.lycia* and linearol (**1**) with topical application

Diterpenoids enriched acetone extract of *S. lycia* and main components of linearol **(1)** had a statistically significant effect on mortality of *Tetranychus urticae, Bemicia tabaci, Sitophilus granaries* and *Lasioderma serricorne* ($P < 0.01$) at 95% confidence level (Table S1). Based on these results, Linearol **(1)** should be concluded as one of the most active ingredients of *S. lycia. Sideritis* plants. containing Linearol and related kaurene diterpenoids ~~can~~ could be potential source to be used in sustainable pest management [29,36].

In order to determine whether there is statistically significant difference in toxicity between insecticidal activity and time, Statgraph software was used for the ANOVA test (Table S1 and Table S2), which indicated a significant difference at $P < 0.01$.

3.4. Structure Elucidation

The isolated compounds were identified to be known *ent*-kauranes linearol (**1**), sidol (**2**), 7-epi-candicandiol (**3**), foliol (**4**) isolinearol (**5**), isosidol (**6**), siderol (**7**) and sideridiol (**8**), using 1D and 2D-NMR and mass spectroscopic techniques as well as comparison with authentic samples. The main diterpenoids of *Sideritis* species have *ent*-kaurane skeleton possessing four rings. The characteristic signals in the ^{1}H NMR spectrum for *ent*-kaurane skeleton are due to the presence of maximum 4 methyl groups and, in some cases, two-three methyl groups, namely, C-18 or C-17 might be substituted with hydroxyl group. In some *ent*-kauranes, C-18 methyl group may have an acetate substituent, Me-17 might be converted into a methylene group (as seen in compounds **1, 2, 3** and **4**) or its isomer having a vinylic methyl group (as seen in compounds **5, 6, 7** and **8**). The most preferred location for the hydroxyl or acetyl groups in the *ent*-kauranes is C-7, followed by C-3. The most characteristic signal in all *ent*-kaurane diterpenoids is the appearance of C-13 proton signal, observed at about 2.3 ppm with a characteristic multiplicity. Generally, it appears like a broadened triplet rather than a multiplet due to the vicinity of two methylene pairs at C-12 and C-14 to C-13 atom. Although in some cases, some substituents may replace those C atoms, we only observed a few cases, such as in athonolone [20]. Since all the isolated diterpenoids had the known *ent*-kaurane structures in this study, their detailed structure elucidation is not given herein (see ^{3}C NMR data in Table 3).

*Linearol (**1**)* ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.82 (1H, br s, H-17a), 4.79 (1H, br s, H-17b), 4.07 (1H, d, J = 11.5 Hz, H-18a), 3.99 (1H, d, J = 11.5 Hz, H-18b), 3.61 (1H, t, J = 2.5 Hz, H-7α), 3.53 (1H, dd, J = 7.5 Hz and 9 Hz, H-3β) 2.72 (1H, m, H-13), 2.09 (3H, s, O-CO$\underline{C}H_3$), 1.05 (3H, s, Me-20), 0.77 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 3). EI-MS: (*m/z*) 362.2(14%) $[M]^+$ for ($C_{22}H_{34}O_4$), 344.2 (40%) $[M\text{-}H_2O]^+$, 326.2(98%) $[M\text{-}2H_20]^+$.

*7-Epi-candicandiol (**3**):* ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.80 (2H, brs, H_2-17), 3.66 (1H, t, J = 2.2 Hz, H-7), 3.47 (1H, d, J = 12 Hz, H-18a), 2.92 (1H, d, J = 12 Hz, H-18b), 2.69 (1H, m, H-13), 1.05 (3H, s, Me-20), 0.69 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI-MS: (*m/z*) 304.0 (3%) $[M]^+$ for ($C_{20}H_{32}O_2$), 286.2 (47%) $[M\text{-}H_2O]^+$, 271.0 (18%) $[M\text{-}H_2O\text{-}CH_3]^+$, 268.0 (99%) $[M\text{-}2H_2O]^+$.

*Sidol (**2**)*: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.90 (1H, dd, J = 5 and 11 Hz, H-3), 4.80-4.82 (2H, brs, H_2-17), 3.62 (1H, t, J = 3 Hz, H-7), 3.32 (1H, d, J = 12 Hz, H-18a), 2.97 (1H, d, J = 12 Hz, H-18b), 2.68 (1H, m, H-13), 2.08 (3H, s, O-CO$\underline{C}H_3$), 1.08 (3H, s, Me-20), 0.68 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI-MS: (*m/z*) 362.3 (4%) $[M]^+$ for ($C_{22}H_{34}O_4$), 332.3 (6%) $[M\text{-}H_2O]^+$, 326.3 (8%) $[M\text{-}H_2O]^+$, 314.2 $[M\text{-}H_2O\text{-}2CH_3]^+$(10%), 302.0 (4%) $[M\text{-}CH_3COOH]^+$.

*Foliol (**4**):* ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.84 (H-17a br s), 4.72 (H-17 br s), 3.67 (1H, dd, J = 6.0 and 11Hz, H-3), 3.47 (1H, d, J = 12 Hz, H-18a), 3.63 (H-7, t, J = 2.5 Hz), 3.40 (1H, d, J = 12.0 Hz, H-18b), 2.69 (H-13, m), 0.78 (H-19, s), 1.06 (H-20, s). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI-MS: (*m/z*) 320.3 (3%) $[M]^+$ for ($C_{20}H_{32}O_3$), 302.3 (49%) $[M\text{-}H_2O]^+$, 294.3 (50%) $[M\text{-}2H_2O]^+$, 272.3 (98%) $[M\text{-}H_2O\text{-}CH_2OH]^+$.

Table 3. ^{13}C-NMR data of the isolated *ent*-kaurenes (**1-8)** from *S. lycia*

Position	1	2	3	4	5	6	7	8
1	38.2	38.4	39.7	39.0	38.4	38.3	44.5	42.0
2	27.6	23.4	17.9	28.1	26.4	23.4	18.3	18.4
3	72.2	74.5	38.3	74.0	72.3	74.6	35.2	35.2
4	38.8	43.6	37.7	39.3	41.9	38.7	36.9	37.1
5	38.0	37.4	37.1	42.1	37.8	37.0	44.5	44.6
6	26.4	26.5	26.6	21.6	26.7	26.0	23.5	25.0
7	76.8	76.9	77.1	76.5	74.9	74.9	78.3	75.4
8	48.2	50.2	48.2	48.7	53.1	53.2	51.8	51.8
9	50.2	50.2	50.4	50.6	43.9	44.1	44.8	44.8
10	38.2	38.1	39.6	39.0	39.1	41.7	39.2	39.2
11	17.8	17.4	17.9	18.2	18.4	18.5	17.9	18.0
12	33.5	33.6	33.5	33.9	24.8	24.9	24.8	26.3
13	43.8	44.1	43.7	44.2	44.6	44.7	39.8	44.2
14	38.1	39.1	34.9	38.8	42.0	42.0	39.8	44.2
15	44.6	44.8	45.2	46.1	130.3	130.0	129.8	129.7
16	155.0	153.9	155.1	155.9	143.9	143.8	143.8	146.1
17	103.6	103.6	103.4	103.4	15.4	15.4	15.4	15.5
18	66.0	64.1	70.5	68.7	66.0	64.1	71.3	71.1
19	11.9	12.8	17.9	12.8	11.8	12.7	17.4	17.7
20	17.8	15.4	17.8	18.2	18.0	18.0	17.8	17.7
O-CO**C**H$_3$	21.2	21.2			21.2	21.2	21.4	
O-**C**OCH$_3$	171.8	170.8			171.9	171.8	170.8	

*Isolinearol (**5**)* ^{1}H NMR: (600 MHz, $CDCl_3$): 5.45 (1H, s, H-15), 4.01 (1H, d, *J* = 11.0 Hz, H-18a), 3.92 (1H, d, *J* = 11.0, H-18b), 3.58 (1H, t, *J* = 2.5 Hz, H-7), 3.46 (1H, dd, *J* = 7.0 and 11.0 Hz, H-3), 2.32 (1H, m, H-13), 2.04 (3H, s, (O-CO$\underline{C}$H$_3$), 1.68 (3H, d, *J* = 1.0 Hz, Me-17), 1.01 (3H, s, Me-20, s), 0.71 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI- MS: (*m/z*) 362.3 (35%) $[M]^+$ for ($C_{22}H_{34}O_4$), 326.3 (8%) $[M\text{-}2H_20]^+$.

*Isosidol (**6**):* ^{1}H NMR: (600 MHz, $CDCl_3$): 5.50 (1H, s, H-15), 4.87 (1H, dd, *J* = 7.0 and 11.0 Hz, H-3), 3.58 (1H, t, *J* = 2.5 Hz, H-7), 3.26 (1H, d, *J* = 11.0, H18a), 2.93 (1H, d, *J* = 11.0, H18b), 2.31 (1H, m, H-13), 1.67 (3H, d, *J* = 0.5 Hz, H-17, s), 2.01 (3H, s, (O-CO$\underline{C}$H$_3$), 1.02 (3H, s, Me-20), 0.68 (3H,s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI-MS: (*m/z*) 362.3 (26%) $[M]^+$ for ($C_{22}H_{34}O_4$), 314.2 (7%) $[M\text{-}H_2O\text{-}2CH_3]^+$, 300.2 (14%) $[M\text{-}CH_3COOH]^+$.

*Siderol (**7**)*:^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.25 (1H, s, H-15), 4.60 (1H, t, *J* = 2.5 Hz, H-7), 2.98 (1H, d, *J* = 11.5 Hz, H-18a), 3.31 (1H, d, *J* = 11.5 Hz,H-18b), 2.37 (1H, m, H-13), 2.05 (3H, s, O-CO$\underline{C}$H$_3$), 1.69 (3H, d, *J* = 0.5 Hz, Me-17), 1.11 (3H, s, Me-20), 0.67 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI- MS: (*m/z*) 346.0 (32%) $[M]^+$ for ($C_{22}H_{34}O_3$), 315.0 (3%) $[M\text{-}CH_2OH]^+$, 303.9 (68%) $[M\text{-}44]^+$, 287.0 (76%) $[M\text{-}OCOCH_3]^+$, 268.1 (76%) $[M\text{-}CH_3COOH\text{-}H_2O]^+$.

*Sideridiol (**8**)*: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.45 (1H, brs, H-15), 3.61 (1H, t, *J* = 2.5 Hz, H-7), 3.47 (1H, d, *J* = 11.5 Hz, H-18a), 2.95 (1H, d, *J* = 11.5 Hz, H-18b), 2.36 (1H, m, H-13), 1.73 (3H, d, *J* = 1.0 Hz, Me-17), 1.05 (3H, s, Me-20), 0.68 (3H, s, Me-19). ^{13}C NMR (150 MHz, $CDCl_3$) (see Table 1). EI-MS: (*m/z*) 304.2.0 (89%) $[M]^+$ for ($C_{20}H_{32}O_2$), 286.2 (58%) $[M\text{-}H_2O]^+$, 273.2 (53%) $[M\text{-}CH_2OH]^+$, 268.2 (7%) $[M\text{-}2H_2O]^+$.

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