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***Original Article***

# Bioassay-guided isolation in *Salvia abrotanoides* Karel. stem based on its anti-fungal and anti-trichomonas activity

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## *Abstract*

**Background and purpose** *Salvia abrotanoides* is considered a medicinal plant and has a broad distribution in Iran. In Iran's traditional medicine, it is also used to treat leishmaniasis, malaria, atherosclerosis, cardiovascular disease, and as a disinfectant. This research aimed to determine the anti-*Candida* component from *S. abratonoides* and anti-*Trichomonas* natural compounds from the stems of this plant.
**Experimental approach:** The plant shoots were collected, dried, and after removing the leaves, grounded. Dried plant material was extracted in a maceration tank, concentrated by a Rotavap, degreased, and fractionated by normal column chromatography. Based on anti-fungal screening against *Candida* species, Fr. 4, with more anti-fungal activity, was selected for phytochemical analysis, by different chromatographic methods on the silica gel column and Sephadex LH-20. Isolated compounds were elucidated by NMR analysis, mass spectrum, and ultraviolet spectroscopy. Anti-fungal effects were investigated using the fungal suspension, incubation, and parasite-counting methods on purified compounds. Antibacterial effects were assessed using the Broth dilution test and reported according to the MIC parameter.
**Findings/Results:** Two diterpenoid compounds named carnosol (compound 1), 11-hydroxy-12-methoxy-20-norabiata-8, 11, 13-trien (compound 2), and a flavonoid: 6,7-dimethoxy-5, 4'-dihydroxyflavone (compound 3) were isolated and identified. Compound 1 had selective anti-fungal effects against *C. albicans*, *C. glabrata*, and *C. parapsilosis*, but weak toxicity against *Trichomonas vaginalis* with $IC_{50}$ of 675.8 µg/mL, less than metronidazole with an $IC_{50}$ of 13.2 µg/mL.
**Conclusion and implications:** Carnosol as the main component was assayed against *Candida*, *Aspergillus*, *Rhizopus*, and *Trichomanas* species. The results confirmed its effect on *Candida* compared to standard drugs.

***Keywords:*** *Candida;* Carnosol; *Salvia*; *Trichomonas*.

## INTRODUCTION

The tendency to use medicinal plants extensively can be due to fewer side effects, better patient acceptance, lower price of medicinal plants, and compatibility with the normal physiological function of the human body. The genus *Salvia* is one of the Lamiaceae plants with high medicinal values (1). Based on a new molecular and taxonomical report, *S. abrotanoides* Karel. (*Perovskia abrotanoides* Kar.) and *S. yangii* B.T. Drew (*P. atriplicifolia* Benth.) were considered instead of the previous names (2). *S. abrotanoides*, is a perennial shrub mainly growing in rocky areas from central, Northeastern, and Southeastern parts of Iran, Northern Pakistan, and Northwestern India (3).

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Different activities have been reported for *Perovskia*, including anti-leishmania, antioxidant (4), anticancer, and antidiabetic activity (5). Previous reports revealed the presence of four main classes of secondary compounds, including flavonoids, phenolic acids, anthocyanins, and terpenoids in these plants (6). Ghaffari *et al*. reported the primary and secondary metabolites derived from the isoprenoid pathway in the *Perovskia* species with different light treatments (1). Morteza-Semnani reported essential oil constituents. Its major components were camphor (34.1%), 1,8-cineole (18.0%), beta-caryophyllene (8.2%), and alpha-humulene (6.5%) (7). In a study directed by Tabefam *et al*. on screening of aerial parts of *P. abrotanoides*, for anti-parasitic activity against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani*, with a different class of compounds including isopimarane, abietane, and icetexane type diterpenoids were isolated with $IC_{50}$ values varied in the range of 0.8 to 230 μM (8). Karimzadeh and coworkers reported 28 diterpenoids mostly from abietane derivatives, including 12 new analogues, with activity against J774A.1 macrophages and a new sesquiterpenoid named Pervkanol with antiparasite activity against *T. brucei* rhodesiense, *P. falciparum*, and *L. donovani* from the roots of the plant (9,10). Sairafianpour *et al*. reported antileishmanial and antiplasmodial activity (*P. falciparum*) of newly isolated tanshinones named cryptotanshinone, 1-hydroxycryptotanshinone, and 1-oxomiltirone in the range of 5-50 μM (11). In traditional medicine, its crushed roots in sesame oil are used to treat cutaneous leishmania wounds as a poultice (11). Previous research mostly has been focused on the root and leaves of this species and this is the first report on the stem. So, the aims of this research were to determine the anti-*Candida* component from *S. abratonoides* as well as anti-*Trichomonas* natural compounds from the stems of this plant, based on bioassay screening.

## MATERIALS AND METHODS

### *Plant material*

Stems of *S. abrotanoides* (Kar.) were collected at flowering in July 2017 at an altitude of 2100 meters in Kesheh, Natanz, Iran. After drying in shade at 25 ℃, the stem was separated from the flowering part. Botanical identification was performed by Dr. Hojjatollah Saeidi (Isfahan University of Science, Iran) using Flora Iranica (Rechinger, 1963). A voucher (Alt. 11015) was stored in the botanical complex of Isfahan Natural Resources and Animal Sciences Research Center, Isfahan, Iran. This sample was also adapted and approved with the herbarium number: Sam-3538, available in the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

### *Extraction and isolation*

*S. abrotanoides* stems (1 kg) were soaked in acetone (6 L) as the solvent for three days by two times in a maceration tank. After maceration, the resulting extract was concentrated in a rotary apparatus at a pressure of 75 mBar. The gummy extract (150 g) was suspended in methanol-water (70:30) and passed through a reverse silica gel through a vacuum liquid chromatography system used to remove chlorophyll and fats. The filtered extract was concentrated by a rotary (52 g) and fractionated on a silica gel column with a dimension of 7 × 40 cm, using hexane: ethyl acetate mobile phase in a gradient system (90:10, Fr. 1; 80:20, Fr. 2; 70:30, Fr. 3; 60:40, Fr. 4; each 2 L) (12).

Frs. 1 to 4 were concentrated and sent for screening against *Candida* species (Table 1) (13-15).

**Table 1.** The minimum inhibitory concentration of fractions 1-4 against different *Candida* species.

| Species | Minimum inhibitory concentration (μg/mL) | | | | |
|---|---|---|---|---|---|
| | **Fr. 1** | **Fr. 2** | **Fr. 3** | **Fr. 4** | **Fluconazole** |
| ***C. albicans*** | 8 | 16 | 16 | 8 | 4 |
| ***C. glabrata*** | 16 | 16 | 16 | 8 | 8 |
| ***C. krusei*** | > 64 | > 64 | > 64 | > 64 | > 64 |
| ***C. parapsilosis*** | 16 | 16 | 16 | 8 | 2 |

Based on antifungal assays shown in Table 1, Fr. 4 with more anti-*Candida* effects was selected for the next steps in phytochemical analysis. It was dissolved in a minimum amount of chloroform:methanol (90:10) and placed at room temperature to be gradually evaporated to form crystals. After 24 h, the crystals were separated and polished on a small Sephadex LH-20 column with a dimension of 30 × 1 cm and a mobile phase of methanol to yield its major component as compound 1 (110 mg) in the pure state. The supernatant containing minor components was concentrated and submitted on a long Sephadex LH-20 column (80 × 2 cm, MeOH) and afforded fractions Fr. 4-1 to Fr. 4-4. After checking the thin-layer chromatography (TLC) profile, Fr. 4-2 was selected for purification by re-chromatographing on another Sephadex LH-20 column (30 × 1 cm, MeOH; Fr. 4-2-1 to Fr. 4-2-3). Fr. 4-2-2 was obtained in a pure state and labeled as compound 3 (15 mg). Fr. 4-2-3 was purified as compound 2 (4 mg) on a preparative 20 × 20 cm silica gel TLC plate with a thickness of 0.5 mm developed in a TLC tank saturated with chloroform: methanol (98:2) as mobile phase. Compound 1, as the main component isolated from Fr. 4 was sent for biological assay to check its anti-*Candida* and anti-*Trichomonas* activities. Isolated compounds were elucidated by NMR analysis, mass spectrum, and ultraviolet spectroscopy.

***Anti-fungal susceptibility testing***

Anti-fungal susceptibility testing has been performed twice. Once for screening the fractions Fr. 1 to 4 and selection of the most active fraction for the next steps in phytochemical analysis. The second time for evaluation and confirming the effect of major component isolated from selected fraction Fr. 4. Briefly, it was done according to the guidelines from the clinical laboratory standard institute (CLSI), against different *Candida*, *Aspergillus* species, and *Rhizopus oryzae* (13-15).

The anti-*Candida* agents were diluted in the standard RPMI-1640 medium (Sigma Chemical Co. Germany) buffered to pH 7.0 with 0.165 M-morpholine propanesulfonic acid (Sigma, Germany) with L-glutamine without bicarbonate to yield two times their concentrations and dispensed into 96-well microdilution trays with final concentrations of 0.063 to 64 µg/mL for fluconazole (Pfizer, Groton, CT, USA) and compounds. All stock solutions were prepared in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the test wells was 1%. Briefly, homogeneous conidial suspensions were measured spectrophotometrically at the 530 nm wavelengths to a percent transmission in the range of 75-77. Therefore, the final densities of the stock inoculum suspensions of the isolates tested ranged from 2.5-5 × $10^3$ colony-forming units/mL, as determined by quantitative colony counts on Sabouraud glucose agar (Difco, Germany) and incubated at 35 °C and examined visually after 24 h to determine minimum inhibitory concentration (MIC) values.

*Aspergillus* stock cultures were inoculated on potato dextrose agar (Difco, Germany) and grown under standardized conditions (35 °C) to induce adequate sporulation. Inoculum suspensions were prepared under biosafety laboratory regulations by slightly scraping the surface of mature colonies with a loop on the sterile saline solution with Tween™ 40 (0.05%). Large possible aggregates were allowed to settle for several minutes; then, the homogeneous conidial suspensions were transferred to sterile tubes and the supernatants were adjusted spectrophotometrically at 530 nm wavelengths to an optical density that ranged from 0.09 to 0.13 nm. Therefore, the final size of the stock inoculum suspensions of the isolates tested ranged from 0.4 × $10^4$ to 3.1 × $10^4$ CFU/mL as performed by quantitative colony count on Sabouraud glucose agar (Difco, Germany) to determine the viable number of colonies forming units per milliliter. The inoculum suspensions including mostly non-germinated conidia were diluted 1:50 in RPMI 1640 medium. Microdilution plates were incubated at 35 °C and examined visually for MICs and minimum effective concentration (MEC) determinations. The MIC endpoints were determined with the aid of a reading mirror and were defined as the lowest concentration of drug that prevents any recognizable growth (*i.e.* exerts 50% inhibition for fluconazole and samples) compared with the growth of a drug-free control. *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) reference strains were included as quality controls (16).

***Anti trochomonal assay***

*Trichomonas vaginalis* was obtained from the Parasitology Laboratory of Isfahan University of Medical Sciences. The parasite *T. vaginalis* was cultured *in vitro* at 37 °C in TYIS33. The cells of *T. vaginalis* were collected at the logarithmic growth phase, and a number of $1 \times 10^4$ cells/mL was used for antimicrobial evaluation (17). To evaluate antitrichomonal activity, different sample concentrations and metronidazole as the positive control with the final concentrations of 0.75, 7.5, 75, 375, and 750 $\mu$g/mL were dissolved in distilled sterile water and added to the microtubes. Distilled sterile water was used as the negative control, respectively. Approximately $10^4$ cells of *T. vaginalis* were added to each TYIS33 medium containing the prepared concentrations of the sample. The media were incubated at 37 °C for 72 h. In each sample, live *T. vaginalis* cells were counted each time using a hemocytometer slide. The active parasites and those with motile flagella were considered live cells. The growth-inhibitory percentage (GI%) was calculated and reported using the following equation:

$$GI\% = a - b/a \times 100$$

where, the letter "a" represents the average number of live parasites in the positive control tube, and "b" represents the average number of live parasites in a test tube.

***Statistical analysis***

The statistical analysis was performed using SAS software Ver. 8. For this data one way ANOVA was done followed by a Tukey posthoc test. Data were presented as mean ± SEM.

## RESULTS

***Determination of compounds***

The results of studies on the anti-*Candida* activity of fractions 1-4 against different *Candida species* compared with the standard drug fluconazole are presented in Table 1. Fractions 1 to 4 showed toxicity against *C. albicans*, *C. glabrata*, and *C. parapsilosis* with MIC values in the range of 8-16 μg/mL. Based on anti-fungal screening against *Candida* species, Fr. 4, with more anti-*Candida* activity, was selected for phytochemical analysis by different chromatographic methods and yielded one abietane, one 20-norabietane diterpenoid (compounds 1 and 2), and one 6-methoxyflavone derivative (compound 3).

Compound 1: (0.011% w/w), white color. $^1$H NMR data (DMSO-$d_6$, 400 MHZ) $\delta_H$ 2.44 (m, H-1a), 2.65 (br d, 14.8, H-1b), 1.52 (br d, 14.0-H-2a), 1.71 ( m-H-2b), 1.23 (br dd, 13.3, 13.3, H-3a), 1.44 (br d, 13.3, H-3b), 1.60 (ddd, 10.8, 5.7, H-5), 1.75 (m, H-6a), 2.08 (ddd, 14.1, 4.9, 4.0, H-6b), 5.46 (m, H-7), 6.69 (br s, H-14), 3.22 (sept, 6.8, H-15), 1.12 (d, 7.0, H-16), 1.12 (d, 7.0, H-17), 0.81 (s, H-18), 0.79 (s, H-19). $^{13}$C NMR data (DMSO-$d_6$, 100 MHZ) $\delta_C$ 9.2 (C-1), 19.0 (C-2), 41.0 (C-3), 34.6 (C-4), 45.4 (C-5), 29.7 (C-6), 77.4 (C-7), 132.0 (C-8), 122.4 (C-9), 48.3 (C-10), 143.5 (C-11), 143.7 (C-1), 134.7 (C-13), 111.7 (C-14), 26.7 (C-15), 23.1 (C-16), 23.2 (C-17), 31.8 (C-18), 19.9 (C-19), 175.9 (C-20). Pos electrospray ionization (ESI) mass: 331 $[M+H]^+$ (Fig. 1).

Compound 2: (0.0004% w/w), white color. $^1$H NMR data (DMSO-$d_6$, 400 MHZ) $\delta_H$ 0.93 (m, H-1a), 3.56 (m, H-1b), 1.44-1.62 (H-2), 1.27 (m, H-3a), 1.49 (m, H-3b), 1.38 (m, H-5), 1.01-1.09 (overlapped, H-6a), 1.78 (m, H-6b), 2.76 (m, H-7), 2.68 (m, H-10), 6.41 (m, H-14), 3.16 (sept, 7.0, H-15), 1.18 (d, 7.0, H-16), 1.20 (d, 7.0, H-17), 0.94 (s, H-18), 1.0 (s, H-19), 3.64 (s, MeO). $^{13}$C NMR data (DMSO-$d_6$, 100 MHZ) $\delta_C$ 34.6 (C-1),23.6 (C-2), 41.8 (C-3), 54.11 (C-5), 23.7 (C-6), 32.4 (C-7), 39.3(C-10), 116.9 (C-14), 26.3 (C-15), 24.1 (C-16), 23.7 (C-17), 21.83 (C-18), 33.31 (C-19), 61.6 (MeO). Electron ionization mass: 302 (M), 300 (M-2H), 285 (300-Me), 271 (M-MeO), 257 (300-isopropyl chain), 244, 229, 217 (Fig. 1).

Compound 3: (0.0015% w/w), pale color. UV (MeOH. nm) $\lambda_{max}$: 276.2 (1.236), 334.7 (1.713). $^1$H NMR data (DMSO-$d_6$, 400 MHZ) $\delta_H$ 3.74 (3H, s, 6-MeO), 3.93 (3H, s, 7-MeO), 6.86 (1H, s, H-8), 6.93 (1H, s, H-3), 6.94 (2H, d, *J* = 6.8 Hz, H-3', H-5'), 7.98 (2H, d, *J* = 6.8 Hz, H-2', H-6'), 10.41 (1H, s, 4'-OH), 12.93 (1H, s, 5-OH). $^{13}$C NMR data (DMSO-$d_6$, 100 MHZ) $\delta_C$ 182.20 (C-4), 164.08 (C-2), 161.28 (C-4'), 158.59 (C-7), 152.60 (C-5), 151.99 (C-9), 131.80 (C-6), 128.53 (C-2', C-6'), 120.99 (C-1'), 115.97 (C-3', C-5'), 105.00 (C-10), 102.58 (C-3), 91.56 (C-8), 60.03 (7-OMe), 56.42 (6-OMe). Pos ESI-Mass: 315 $(M+H]^+$ (Fig. 1).

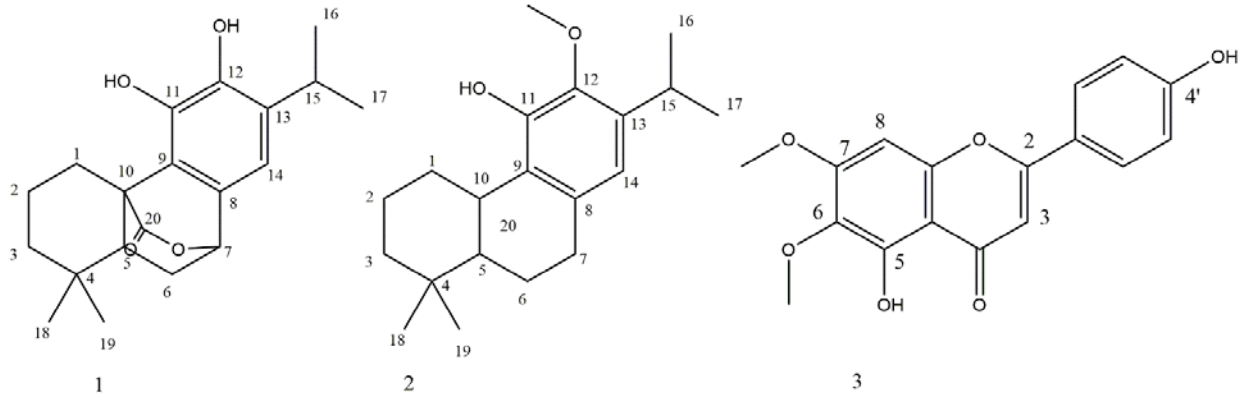

**Fig. 1.** Isolated compounds from the stems of *Perovskia abrotanoides.*

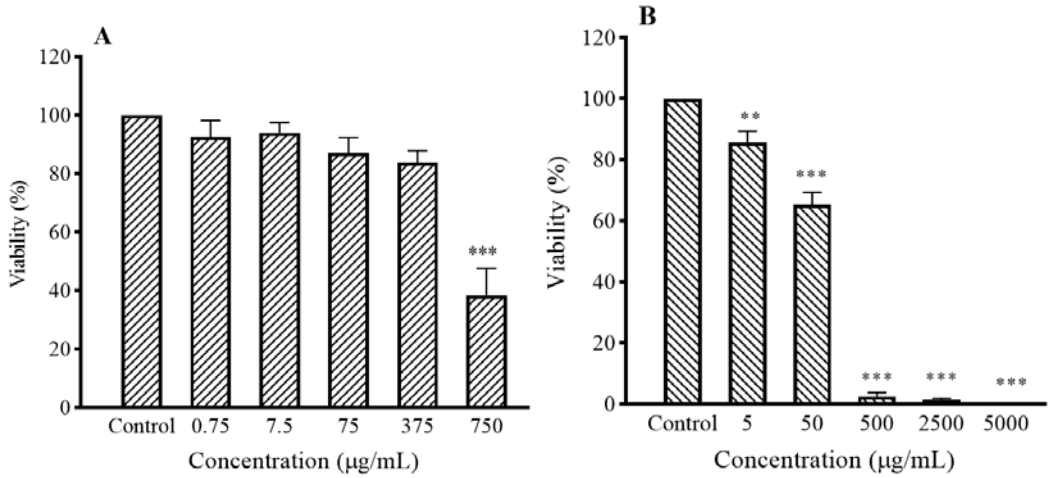


**Fig. 2.** Evaluation of the effects of (A) carnosol and (B) metronidazole against the viability of *Trichomonas vaginalis* parasite. Data are presented as mean ± SEM. ***P* < 0.01 and ****P* < 0.001 indicate statistically significant differences compared to the control group.

**Table 2.** Minimum inhibitory concentration (MIC) of compounds 1 and 2 against *Candida* species

| Collection name | Species | Minimum inhibitory concentration (µg/mL) | | |
|---|---|---|---|---|
| | | Fluconazole | Compound 1 | Compound 2 |
| ATCC 10231 | *C. albicans* | 4 | 1.3 | 16 |
| ATCC 15545 | *C. glabrata* | 8 | 2.6 | 16 |
| ATCC 6258 | *C. krusei* | > 42 | > 42 | > 42 |
| ATCC 22019 | *C. parapsilosis* | 2 | 0.6 | 16 |
| FDC 229 | *C. auris* | > 42 | > 42 | > 42 |

### *Anti-fungal susceptibility testing*

The results of studies on the effects of compounds 1 and 2 based on the MIC against different *C. species* compared with the standard drug fluconazole are presented in Table 2. Carnosol as the main compound isolated from the Fr. 4 showed selective anti-*Candida* activity against *C. albicans*, *C. glabrata*, and *C. parapsilosis* in the range of MIC of 0.6 to 2.6 µg/mL comparable with fluconazole in the range of MIC of 2-8 µg/mL. It showed weak activity against *A. tubingencis*, *A. flavus*, *A. terreus*, *A. niger*, and *R. oryzae*, with MIC > 64 µg/mL.

### *Anti-trichomonal assay*

Regarding the effects of anti-*Trichomonas* activity, as shown in Fig. 2, carnosol at lower concentrations had no significant effects against *T. vaginalis* parasite, and at 750 µg/mL has shown significant effects. According to the $IC_{50}$ parameter, its inhibitory effect equals 675.8 µg/mL, far less than metronidazole as a positive control with an $IC_{50}$ value of 13.2 µg/mL.

## DISCUSSION

In compound 1, based on hydrogen and carbon data in NMR and distortionless enhancement by polarization transfer spectra, the core of twenty carbons includes four methyl groups in the δC 23.1, 23.2, 31.8, and 19.9, four simple methylene groups at δC 29.2, 19.0, 41.0 and 29.7, four methine groups (one of them is attached to oxygen with δC 77.4, one olefin with δC 111.7, and two simple at $\delta_C$ 45.6 and 26.7) and seven non-hydrogen bonded carbons (two simple quaternary groups at $\delta_C$ 48.3 and 36.3) three simple olefin groups at $\delta_C$ 0.132, 122.4, and 134.7 and two oxygen-bound olefin groups at $\delta_C$ 143.5 and 143.7 ppm). In the $^1$H NMR spectrum, there are two doublet methyl groups ($\delta_H$ 1.2, d, *J* = 7.0 Hz, Me-16, Me-17), two singlet methyl groups ($\delta_H$ 0.81, s, Me-18/0.79, s, Me-19), four simple methylene at $\delta_H$ 2.44, m/2.65, br d, *J* = 14.8, H-1a, b; 1.52, br d, *J* = 14.0 / 1.71 Hz, m, H-2a, b; 1.23, br dd, *J* = 13.3, 13.3 / 1.44 Hz, br d, *J* = 13.3 Hz, H-3a, b; 1.75, m / 2.08, ddd, *J* = 14.1, 4.9, 4.0 Hz, H-6a, b, as well as two simple methine groups, one at $\delta_H$ 1.60, bdd, *J* = 10.8 and 5.7 Hz for H-5, and another at $\delta_H$ 3.22, sept, *J* = 6.8 Hz for H-15, two methine groups in the downfield, an oxygen-bound group at $\delta_H$ 5.46, m for H-7, and an olefin group at $\delta_H$ 6.69 br s related to H-14. In addition, δH: 8.11 and 8.45 belong to the two free hydroxyl groups. In the heteronuclear single quantum coherence (HSQC) correlation analysis, the corresponding hydrogens of the carbon were determined. Finally, according to the information obtained from magnetic resonance spectroscopy of carbon and hydrogen nuclei, as well as the ESI mass [M + Na] of 353.1 corresponded to the molecular formula of $C_{20}H_{26}O_5$ + $Na^+$, the structure of compound 1 was defined as carnosol according to the literature (18,19).

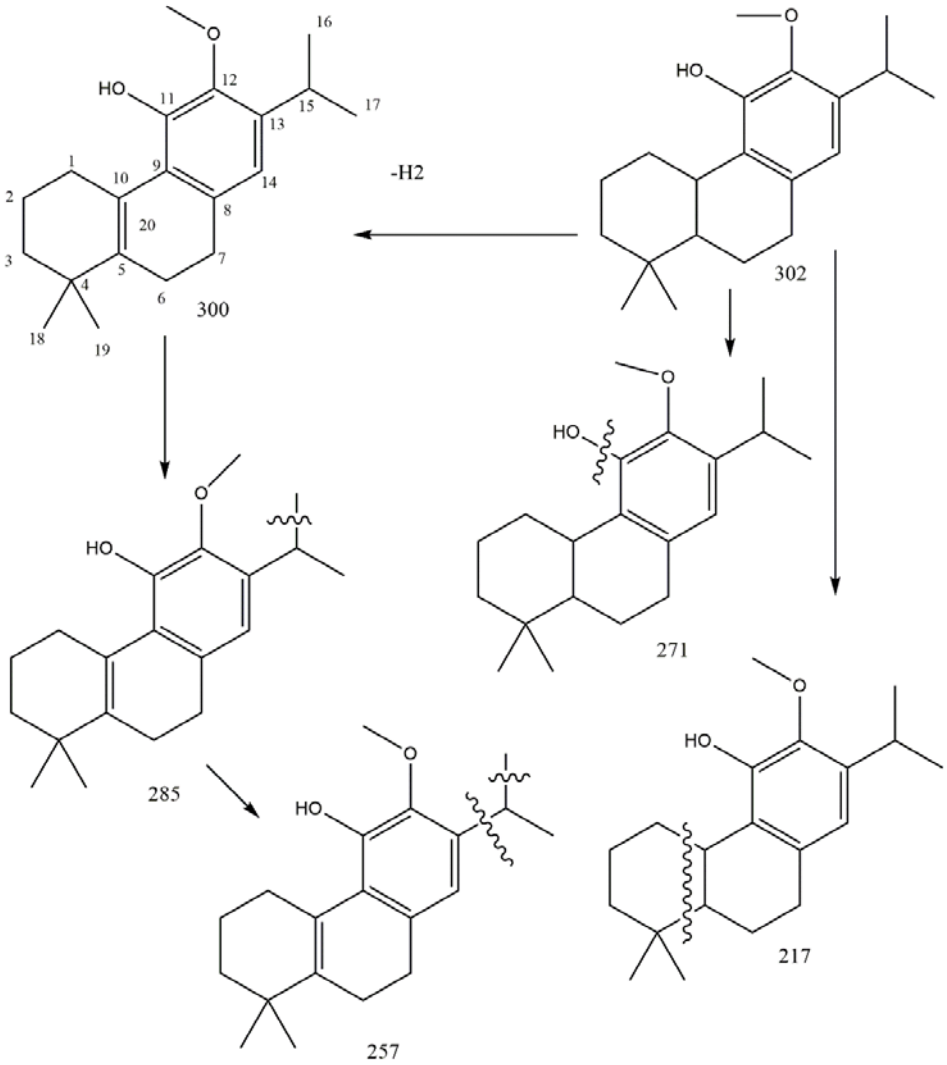


**Fig. 3.** Electron ionization mass fragmentation profile of compound 2.

In compound 2, two groups of doublet methyls at $\delta_H$ 1.20, 3H, d, *J* = 7.0 and 1.18 Hz, 3H, d, *J* = 7.0 Hz, corresponding to Me-16 and Me-17, two groups of singlet methyls at $\delta_H$ 0.94, s / 1.0, s related to Me-18 and Me-19, five simple methylene groups at $\delta_H$ 3.56, m / 0.96, m related to H-1a, b; $\delta_H$ 1.44- 1.62 related to H-2a, b; $\delta_H$ 1.27, m / 1.49, m related to H-3a, b; $\delta_H$ 1.78, m related to H-6; $\delta_H$ 2.76, m related to H-7, three simple methine groups at $\delta_H$ 1.35, m related to H-5, at $\delta_H$ 2.68 (m) related to H-10 and at $\delta_H$ 3.16 (sept, 7.0) For H-15, a methine group is seen in the downfield attached related to olefin group at $\delta_H$ 6.41 (s) for H-14. In the EI fragmented profile mass spectrum, m/z 302 related to molecular weight as well as 300 (M-2H), 285 (300-Me), 271 (M-MeO), 257 (300-isopropyl), and 217 [M- ($CH_2$-$CH_2$-$CH_2$-C ($CH_3$) $CH_3$)] were seen (Fig. 3). Finally, according to the $^1$H NMR and $^{13}$C NMR derived from the HSQC spectrum and electron ionization mass spectrum, the structure was identified as 11-hydroxy-12-methoxy-20-norabieta-8,11,13-trien (8).

Compound 3, responded positively to the natural product and ferric chloride reagents and had the UV pattern of flavonoids. It showed NMR signals of $\delta_C$ 60.03 ($\delta_H$ 3.93,3H, s), 56.42 ($\delta_H$ 3.74, 3H, s), related to two methoxy groups, $\delta_C$ 128.53 ($\delta_H$ 7.98, 2H, d, *J* = 6.8 Hz, H-2', H-6'), and 120.99 ($\delta_H$ 6.94, 2H, d, *J* = 6.8 Hz, H-3', H-5') related to AA', and BB' system in ring B, $\delta_C$ 102.88 ($\delta_H$ 6.93, 1H, s), and 91.56 ($\delta_H$ 6.86, 1H, s) related to C-3, and C-8, and two deshielded phenolic signals at $\delta_H$ 12.93 (1H, s), 10.41 (1OZH, s) related to 5-OH, and 3'-OH. The UV $\lambda_{max}$ was 276 nm for band II and 334 nm for the band I. After adding the aluminum chloride reagent, the band I shifted equivalent to +19 nm from 334 to 354 nm, and band II equivalent to +22 nm from 276 to 298 nm, indicative of a 6-methoxy apigenin derivative (20,21). The addition of NaOMe didn't shift band II because of the lack of ionization at C-7. It suggested that remained methoxy group was located at C-7 (20). Based on $^1$H, and $^{13}$C NMR spectra, and UV spectroscopic data, compound 3 was identified as 6,7-dimethoxy-5,4'-dihydroxyflavone (cirsimaritin) in agreement with literature data (22). It is the first report of cirsimaritin in this plant. This data is in agreement with previous reports of this flavone in other *Salvia* species including *S. nubicola*, *S. plebeia*, and *S. palaestina* (23-25).

Abietane diterpenoids including compounds 1 and 2 are first reported in the stems without leaves and flowers in a bioassay guide study against *Candida* species and are in agreement with previous reports of these compounds by Hamburger *et al*. in the aerial parts including leaves, and flowers in a bioassay guide study against *Leishmania* and *Trypanosoma* species, and Moridi *et al*. in the roots of the plant with immunosuppressive activities against *E. coli* lipopolysaccharide activated macrophages (8,10). The results of this study against the fungal species *Candida*, *Aspergillus*, and *Rhizopus* compared with fluconazole showed that carnosol selectively has anti-fungal effects against *C. albicans*, *C. glabrata*, and *C. parapsilosis* species. While, regarding the anti-trichomonal results shown in Fig. 3, carnosol showed weak inhibitory activity compared to metronidazole as a standard drug.

Carnosol is a natural compound that is produced due to the oxidative degradation of carnosic acid and is found in plants belonging to the family Lamiaceae such as sage, rosemary, lavender, and oregano (26-29). It is a well-known natural product that has also been identified in other studies in the literature as an anticancer, antibacterial, and antioxidant agent in both laboratory and animal models (30,31). It exhibits anti-inflammatory activity and prevents the activation of various inflammatory signaling pathways, such as the NF-κB pathways and mitogen-activated protein kinase (32). In a screening study by Tabefam *et al*. in 2018 to find new natural anti-parasitic products from Iranian plants, carnosol showed potent inhibitory activity against *Trypanosoma brucei* and *L. donovani* (8).

## CONCLUSION

In this research, the study was performed on anti-*Candida* and anti-*Aspergillus* activity on *S. abrotanoides* and its fractions using a bioassay guide method. Accordingly, the most effective fraction was selected and analyzed to

yield three compounds that were responsible for such activities. Carnosol as the main component was assayed against *Candida*, *Aspergillus*, *Rhizopus*, and *Trichomanas* species. The results confirmed its effect on *Candida* as compared to standard drugs. However, in comparison with metronidazole, the effect of this compound was not considerable. Finally, this compound and Fr. 4 were introduced for further pharmaceutical research against *C.* species Viz. *C. albicans, C. glabrata, and C. parapsilosis* studies.

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***Conflict of interest statement***

All authors declared no conflict of interest in this study.

***Authors' contributions***

All authors contributed to the design of the study, data interpretation, and manuscript editing. Sh. Gharibi performed the experiments, analyzed the data, and wrote the initial draft of the article. M. Ghanadian designed the research, provided the materials, and edited the manuscript. A. Matkowski guided the experiment and data analysis and edited the manuscript. H. Fakhim, R. Jahanshahi Afshar, and H.A. Yousefi performed the bioassay analyses; M. Khodadadi contributed to the interpretation and edition of the final version of the manuscript. The finalized manuscript was confirmed by all authors.

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