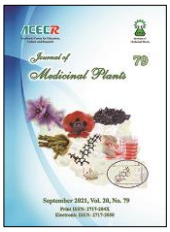
J. Med. Plants 2021; 20(79): 24-36

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Rearranged abietane diterpenoids from roots of *Teucrium hircanicum* L.

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ARTICLE INFO

Keywords:
Teucrium hircanicum
Phytochemical profiling
Diterpenoids
Antioxidant activity
Villosin A

ABSTRACT

Background: Medicinal plants play a significant role in preventing and treating diseases due to their traditional uses. **Objective:** The aim of the study was to isolate, purify and identify phytochemicals from the *n*-hexane extract of *Teucrium hircanicum* L. roots and evaluate the antioxidant activity of the extract and its purified compounds. **Methods:** The *n*-hexane extract (2.27 g) was extracted from the roots of this plant. The extract was fractionated by column chromatography with normal phase by eluting with *n*-hexane-EtOAc and following with EtOAc-Methanol. Fractions with similar phytochemical fingerprints combined to produce 23 main fractions. Final purification was carried out by preparative reversed-phase HPLC-UV. The structures of isolated were secured by different spectroscopic methods such as 1D, 2D NMR, and mass spectroscopy methods and comparing of these data with literature reported values. The antioxidant activity of the *n*-hexane extract of *T. hircanicum* roots and its purified diterpenoids was evaluated in DPPH assay and radical scavenging activity was calculated. **Results:** Extraction and isolation methods were used to purify three rearranged abietane-type diterpenoids villosin A (**1**), teuvincenone B (**2**) and 5, 8, 11, 13, 15-abietapentaen-7-one (**3**), a phenethyl ester namely 4-hydroxyphenethyl pentacosanoate (**4**) and one sterol namely 22-dehydroclerosterol (**5**). **Conclusion:** The *n*-hexane extract from roots of *T. hircanicum* and three rearranged abietane type diterpenoids were showed good antioxidant activities ranged from 3.5-4.3 µg/ml compared with the reference value (BHT = 16.5 µg/ml).

1. Introduction

Herbal products have been essential for the maintenance of human health from time immemorial. The biological activity of plant extracts depends on the type of secondary metabolism in the plant. As a result, these

Abbreviations: DPPH, 2,2-Diphenyl-1-picrylhydrazyl; BHT, Butylated Hydroxytoluene; CC, Column Chromatography; RSA, Radical Scavenging Activity; HMBC, Heteronuclear Multiple Bond Correlation; HSQC, Heteronuclear Single Quantum Coherence

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doi: 10.52547/jmp.20.79.24
Received 25 March 2021; Received in revised form 7 June 2021; Accepted 27 June 2021

biological effects are specific to some species or groups of plants [1-3]. The impact of medicinal plants on the body is mostly based on one or a combination of several chemicals. The effects of the drug are caused by the synergy of several secondary metabolites, which act at one or more target locations associated with a physiological process [1]. The essential oils, extracts and purified phytochemicals are essential causes of biological activity such as antibacterial, antimalarial, antipyretic, analgesic, anti-inflammatory, antiviral, anticancer, antifungal and cytotoxic activity [4, 5]. To date, there have been numerous studies on traditional herbal remedies for various diseases that have led to the development of alternative drugs and treatment strategies [6].

Lamiaceae are a large plant family with 236 genera and 6900 to 7200 species. The plants belonging to Lamiaceae are considered an excellent source for discovering new natural products with bioactive capacities [7, 8]. Most of the plants in the Lamiaceae family are used as a perfumery or source of bioactive phytochemicals [9]. A lot of them are cultivated as ornamentals such as *Ajuga, Coleus,* and *Salvia*. Where others are grown as herbal medicines and spices for cooking such as sage (*Salvia*), thyme (*Thymus*), mint (*Mentha*), oregano or marjoram (*Origanum*), rosemary (*Rosmarinus*), lavender (*Lavandula*), and basil (*Ocimum*) are grown [10].

The genus *Teucrium* belongs to the Lamiaceae family, of which about 434 species currently recognized in Southeast Asia, Central and South America, the Mediterranean and the Middle East [11-14]. Plants in this genus are perennial and sometimes shrubby. In Iran, 19 species of the genus have been recorded, mostly in the Iran-Turan region. They are widely distributed in most parts of Iran. *T. persicum* is existing only at highlands in the southern regions of Iran. *T. polium* and *T. orientale* species commonly used in folk medicine are distributed in steppe, arid and semi-arid region. One of the most well-known species of the genus is *T. polium*. In traditional medicine, *T. polium* has been used for a variety of disease disorders such as gastrointestinal disorders, inflammations, diabetes and rheumatism [16, 17]. It has been reported other biological effects such as antioxidants [18], antinociceptive [19], lipid-lowering [20], severe hyperglycemia [21], improved memory [22], blood pressure-lowering [23], and prevention of hypertension [24]. Neo-clerodane diterpenoids are accepted as chemotaxonomic markers for *Teucrium* species, a natural source of many of these compounds. The neo-clerodane diterpenoids were purified in the aerial parts of the *Teucrium* species, but the rearranged abietane diterpenes were obtained from the roots [14]. The species of this genus are used as diuretics, diaphoretic, carminatives, astringents, stimulants and tonics, antiseptics, antipyretics and anti-inflammatory drugs in popular medicine. The species of this genus are used as diuretics, diaphoretic, carminatives, astringents, stimulants and tonics, antiseptics, antipyretics and anti-inflammatory drugs in popular medicine [14].

Teucrium hyrcanicum L. an indigenous plant that grows in the northern part of Iran [15]. This study aimed to isolate, purify and identify phytochemical compounds from *n*-hexane extract from *T. hircanicum* and evaluate the antioxidant activity of *n*-hexane extract and its purified phytochemical.

2. Materials and Methods

2.1. Chemical and reagents

The silica gel 60, TLC plates, solvents and chemical reagents used in the current study were purchased from Merck Chemical Company and Sigma-Aldrich.

[DOR: 20.1001.1.2717204.2021.20.79.3.8]
[DOI: 10.52547/jmp.20.79.24]

2.2. Plant material

Teucrium hircanicum L. was collected in the Hyrcanian forests (Babol, Mazandaran) of northern Iran (south of the city of Babol) in September 2016. The plant material was identified by Dr. Sonboli and voucher specimens (MPH-2434) deposited at Herbarium of Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

2.3. Extraction and isolation

After the collection of *T. hircanicum*, the roots were separated from their aerial parts. Roots of *T. hyrcanicum* have been thoroughly dried in a place away from direct sunlight for five days. The plant's dried roots were ground with the laboratory grinder. The powder of the roots (470 g) was extracted by maceration in 3 L of *n*-hexane and repeated 5 times. The *n*-hexane extract obtained concentrated with a 40 °C vacuum rotary evaporator resulted in 2.27 g of gummy extract, the extract stored in the refrigerator for the next step. For the separation of the extract, a glass column of 3.5 cm in diameter and 50 cm long was used. The extract dissolved in dichloromethane has been associated with 10 g of silica gel 60 (0.063-0.200 mm). The resulting mixture was dried by rotary evaporation until a fine powder was obtained. In this study, 100 g of silica gel 60 (0.063 to 0.200 mm) as stationary phase was weighed and packed into the column. The *n*-hexane extract powdered with silica gel 60 was charged on silica gel in the column chromatography. The extract was fractionated to 104 parts by gradient elution with *n*-hexane-EtOAc and in the following EtOAc-Methanol by increasing the polarity of the mobile phase. The volume of collected fractions was 100 ml. All fractions were controlled by TLC under UV 254 nm and anisaldehyde reagent. The fractions with similar phytochemicals fingerprints combined and then 23 major fractions were obtained.

Compound (**1**), a light yellow powder (5 mg), was isolated from column chromatography (CC) using 6 % EtOAc and 94 % *n*-hexane and was purified from E-fraction by precipitation. Compound (**2**) (8 mg), a yellow powder, and compound (**3**) (7 mg), an orange powder, were extracted from column chromatography in 4% EtOAc and 96 % *n*-hexane and were purified from D fraction by precipitating and preparative HPLC. Compound (**4**) isolated a colorless powder (21 mg), was extracted from column chromatography in 7 % EtOAc and 93 % *n*-hexane, and was purified from F fraction by precipitating. Compound **5**, (84 mg) isolated as a colorless powder in 8 % EtOAc and 92 % *n*-hexane from CC, and additional purified from fraction G by precipitation.

2.4. Antioxidant activity

Investigation of antioxidant activity of *T. hircanicum* root *n*-hexane extract and rearranged abietane type diterpenoids its purified compounds. The antioxidant activity of the *n*-hexane extract of *T. hircanicum* roots and its purified diterpenoid compounds was evaluated by reducing the color reaction between DPPH solution and sample extracts. For this purpose, we used the method described in previous literature [25]. Briefly, the optical absorption rate of the samples and butylated hydroxytoluene (BHT) reference standard recorded in 5 different concentrations 0.1, 0.2, 0.4, 0.8 and 1.6 ppm and in 4 replicates of each concentration after a shake hour in a dark environment by using an ELISA device at a wavelength of 517 nm. A decrease in the DPPH solution absorbance indicates an increase in RSA %.

$$RSA\ \% = [1 - (S - SB) / C] \times 100$$

[DOR: 20.1001.1.2717204.2021.20.79.3.8]
[DOI: 10.52547/jmp.20.79.24]

In this formula, RSA %, S, SB and C, respectively, are the percentage of radical scavenging activity DPPH, the amount of sample adsorption (methanol + sample + DPPH), the amount of standard adsorption (methanol + sample) and the amount of control adsorption (methanol + DPPH). The IC_{50} value is obtained by plotting different values of RSA % according to different concentrations of the sample and calculating the regression line equation.

3. Results

The *n*-hexane extract was obtained from the shade-dried powdered *T. hircanicum* roots. The extract was separated using column chromatography over silica gel and preparative reversed-phase HPLC-UV afforded three rearranged abietane-type diterpenoids and one sterol and phenethyl ester. The structures of compounds were secured by different spectroscopic methods such as 1D, 2D NMR, and mass spectroscopy methods and comparing of these data with literature reported values. The identified compounds were villosin A (**1**) [26], teuvincenone B (**2**) [27], 5, 8, 11, 13, 15-abietapentaen-7-one (**3**) [28] and 4-hydroxyphenethyl pentacosanoate (**4**) and 22-dehydroclerosterol (**5**) (Fig. 1).

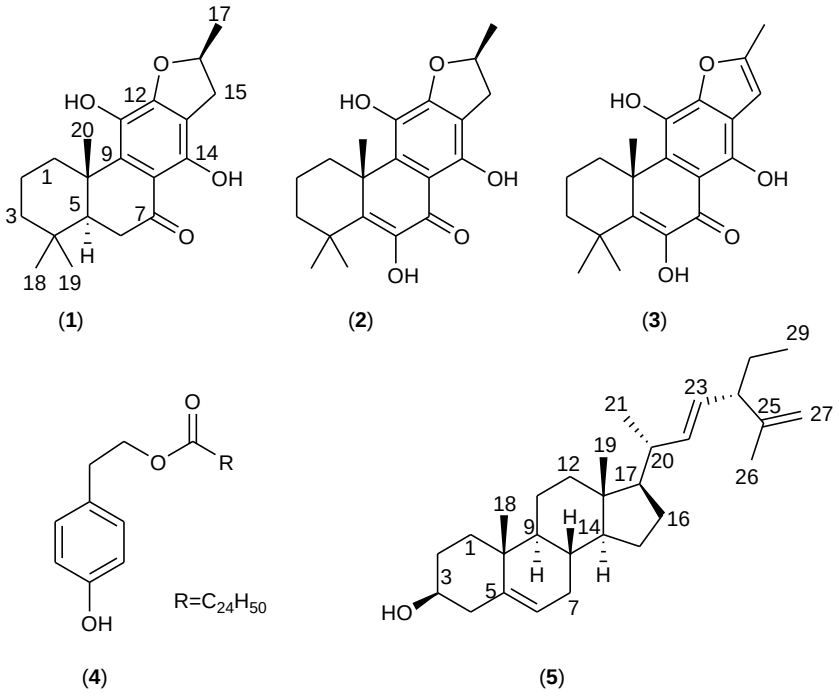

Fig. 1. Structures of purified compounds from *n*-hexane extract roots of *T. hircanicum* L. **1**: Villosin A, **2**: Teuvincenone B, **3**: 5, 8, 11, 13, 15-Abietapentaen-7-one, **4**: 4-Hydroxyphenethyl pentacosanoate and **5**: 22-Dehydroclerosterol.

Compound (**5**), isolated as a colorless powder in 8 % EtOAc and 92 % *n*-hexane from CC, and additional purified from G fraction by precipitating. In the H-H-COSY spectrum we can see that H-4 (δ 2.30, CH_2) is related to H-3 (δ 3.52, (td), CH) and weakly to H-6 (δ 5.36, (m), CH). In the HMBC spectrum, shows the relationship of H-4 with C-2 (δ 31.60), C-10 (δ 36.53), C-3 (δ 71.68), C-5 (δ 140.53) and C-6 (δ 121.35). Five methyl signals there are at 1.03 (s, 3H), 0.72 (s, 3H), 1.04 (d, 3H), 1.66 (s, 3H), 0.86 (3H) in the upfield region. In the ^{1}H-NMR

spectrum, Me-18 signal appear at chemical shift 1.03 ppm. In the HMBC spectrum, shows the relationship of this signal with 37.36 (C-1), 140.53 (C-5), 50.30 (C-9) and 36.53 (C-10). Me-19 (0.72, s) signal has relationship with 39.80 (C-12), 42.50 (C-13), 56.92 (C-14) and 56.12 (C-17) in the HMBC spectrum. Me-21 (1.04, d) signal has relationship with 56.12 (C-17), 40.13 (C-20) and 137.09 (C-22) in the HMBC spectrum. Me-26 (1.66, s) signal has relationship with 51.78 (C-24), 148.18 (C-25) and 109.46 (C-27) in the HMBC spectrum. Me-26 (0.86, dd) signal has relationship with 51.78 (C-24), and 25.60 (C-28) in the HMBC spectrum.

In the H, H-COSY spectrum, H-17 (δ 1.19, CH) signal have cross-peaks with H-16 (δ 1.30 and 1.73, CH_2) and H-20 (δ 2.07, CH), and H-16 signals form cross-peak with H-15 (δ 1.43 and 1.52, CH_2) signals, and so H-15 signals have a cross peak with H-14 (1.04, CH) signal. The signal of H-1 (δ 3.52, td, CH) with H-2 (δ 1.52 and 1.85, CH_2) and H-4 (δ 2.30, CH_2) signals form cross-peaks. The signals of H-12 (δ 2.02, CH_2) and H-11 (δ 1.10 and 1.60, CH_2) have cross-peaks. And so H-11 signals have a relationship with H-9 (δ 0.97, CH). The H-6 (δ 5.36, CH) and H-7 (δ 2.00 and 1.54 CH_2) have cross-peaks in the H-H COSY spectrum. And in the HMBC spectrum H-6 has a relationship with C-4 (δ 42.40), C-5 (δ 140.53), C-7 (δ 32.07), C-8 (δ 32.24) and C-10 (δ 36.53). The sterol structure of 22-dehydroclerosterol (**5**) was confirmed by comparing experimental and literature data.

Compounds teuvincenone B (**2**), a yellow powder, and compound 5,8,11,13,15-abietapentaen-7-one (**3)**, an orange powder, were extracted from column chromatography in 4 % EtOAc and 96 % *n*-hexane and were purified from D fraction by precipitating and preparative HPLC. Compound villosin A (**1**), a light-yellow powder, was extracted from column chromatography in 6 % EtOAc and 94 % *n*-hexane, and was purified from E fraction by precipitating. ^{1}H-NMR and ^{13}C-NMR data of these three compounds were given in tables 1 and 2. Other spectra of compound (**1**) are shown in figures 2 to 5.

Table 1. ^{1}H-NMR data (δH (*J* in Hz) of compounds **1**-**3** (500 MHz, $CDCl_3$)

No	1	2	3
1	1.43 (m, 1H)	1.87 (m, 1H)	1.93 (m, 1H)
	3.29 (dtd, *J* = 13.5, 3.5, 1.5 Hz, 1H)	3.66 (m, 1H)	3.73 (m, 1H)
2	1.60 (m, 1H)	1.75 (m, 1H)	1.67 (m, 1H)
	1.76 (dt, *J* = 13.9, 3.5 Hz, 1H)	1.89 (dt, *J* = 12.3, 3.6 Hz, 1H)	1.88 (m, 1H)
3	1.30 (m, 1H)	1.43 (tdt, *J* = 12.9, 6.0, 2.9 Hz, 1H)	1.40 (m, 1H)
	1.50 (m, 1H)	2.10 (dt, *J* = 15.9, 3.3 Hz, 1H)	2.04 (m, 1H)
5	1.81 (dd, *J* = 11.5, 5.8 Hz, 1H)		
6	2.62 (m, 2H)		
15	3.37 (dd, *J* = 15.3, 9.0 Hz, 1H)	3.34 (ddd, *J* = 15.4, 8.9, 2.8 Hz, 1H)	6.71 (q, *J* = 1.5 Hz, H)
	2.86 (dd, *J* = 15.3, 7.3 Hz, 1H)	2.80 (ddd, *J* = 15.2, 7.8, 2.8 Hz, 1H)	
16	5.12 (m, 1H)	4.94 (m, 1H)	
17	1.52 (d, *J* = 6.3 Hz, 3H)	1.28 1.28 (dd, *J* = 6.4, 2.9 Hz, 3H)	2.25 (d, *J* = 2.5 Hz, 3H)

[DOR: 20.1001.1.2717204.2021.20.79.3.8]
[DOI: 10.52547/jmp.20.79.24]

Table 1. ^{1}H-NMR data (δH (*J* in Hz) of compounds **1**-**3** (500 MHz, $CDCl_3$) (Continued)

No	1	2	3
18	0.96 (s, 3H)	1.66 (d, *J* = 3.2 Hz, 3H)	1.68 (d, *J* = 2.7 Hz, 3H)
19	0.99 (s, 3H)	1.62 (d, *J* = 2.8 Hz, 3H)	1.65 (d, *J* = 2.8 Hz, 3H)
20	1.39 (s, 3H)	1.94 (d, *J* = 2.7 Hz, 3H)	1.98 (d, *J* = 2.8 Hz, 3H)
OH-6		9.13 (s, 1H)	9.29 (s, 1H)
OH-11	4.73 (s, 1H)	4.76 (s, 1H)	4.76 (s, 1H)
OH-14	13.43 (s, 1H)	13.37 (s, 1H)	13.77 (s, 1H)

Table 2. ^{13}C-NMR data of compounds **1**-**3** (125 MHz, $CDCl_3$).

No	1	2	3
1	36.1	29.7	30.1
2	17.5	17.7	17.2
3	41.9	36.1	36.7
4	33.7	27.5	36.3
5	50.0	144.2	145.5
6	35.2	143.5	141.6
7	204.3	182.5	184.1
8	110.5	107.7	107.8
9	140.0	141.2	133.5
10	40.7	41.81	41.6
11	130.9	130.7	130.9
12	155.0	156.5	156.0
13	110.2	111.1	117.1
14	155.7	153.3	150.2
15	34.2	33.9	101.1
16	83.2	81.7	154.6
17	21.9	21.5	13.1
18	33.1	27.9	27.7
19	21.5	27.3	28.0
20	18.2	27.3	27.3

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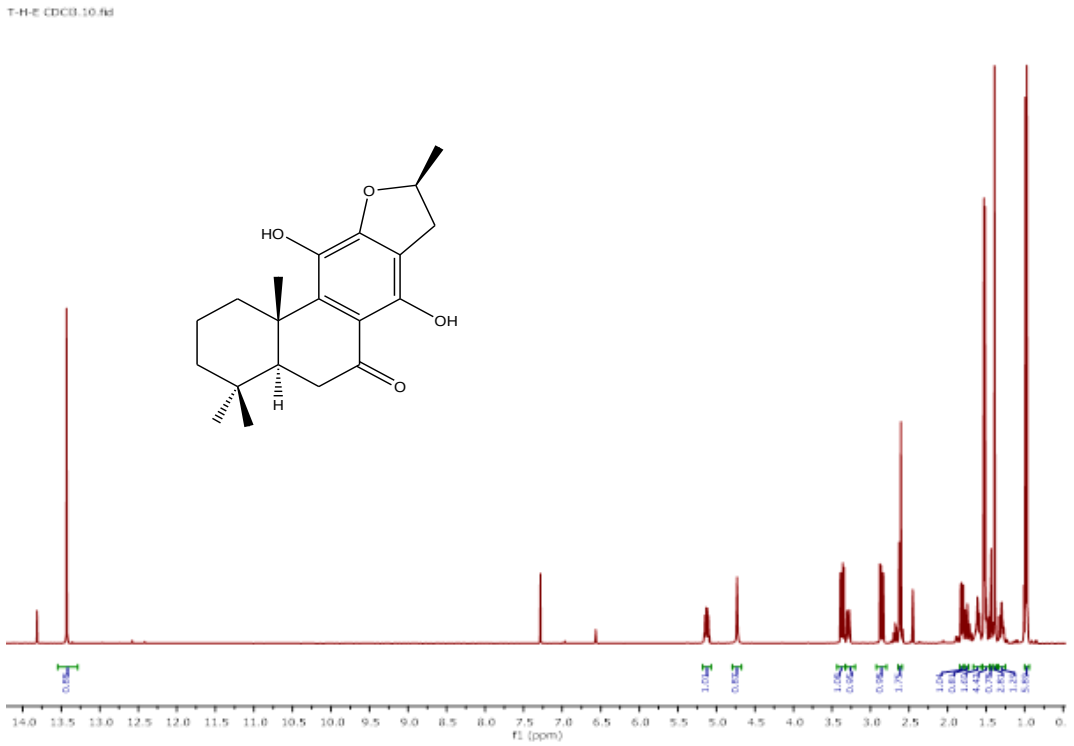

Fig. 2. ^{1}H-NMR spectrum of villosin A (**1**).

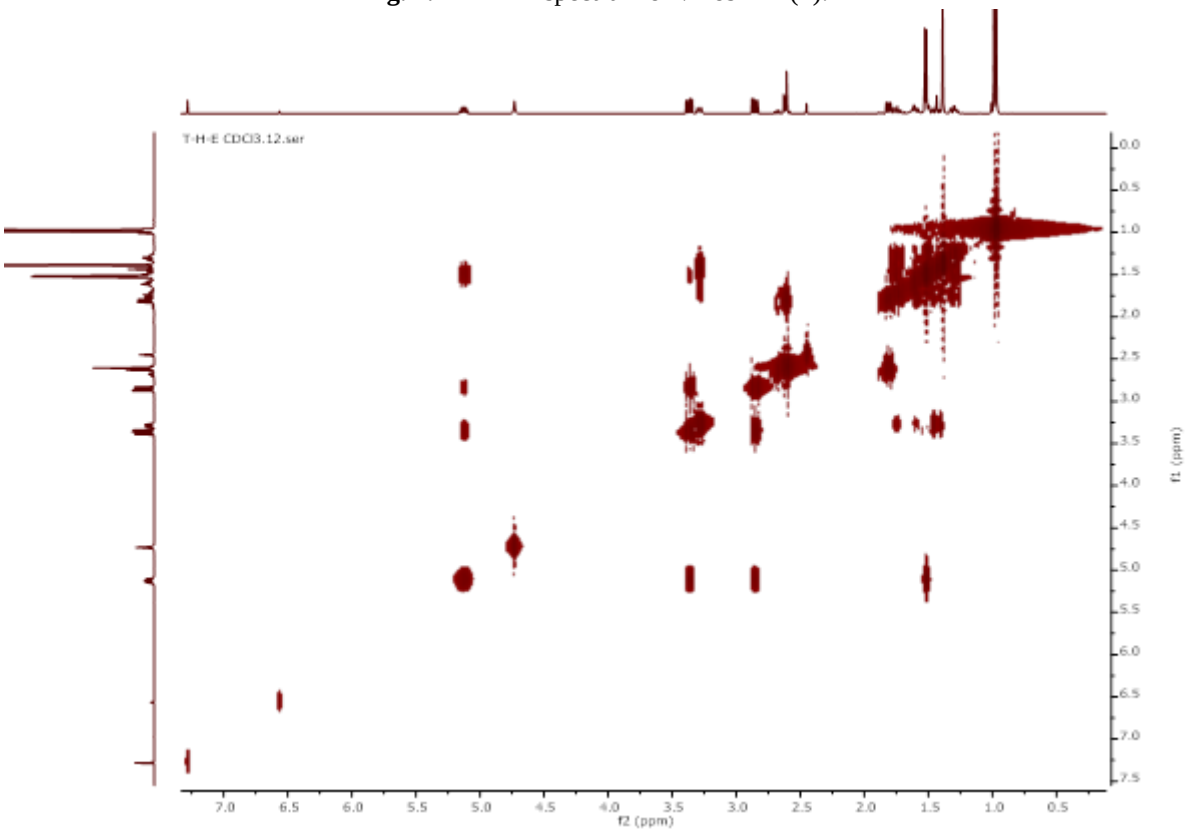

Fig. 3. H, H-cosy spectrum of villosin A (**1**).

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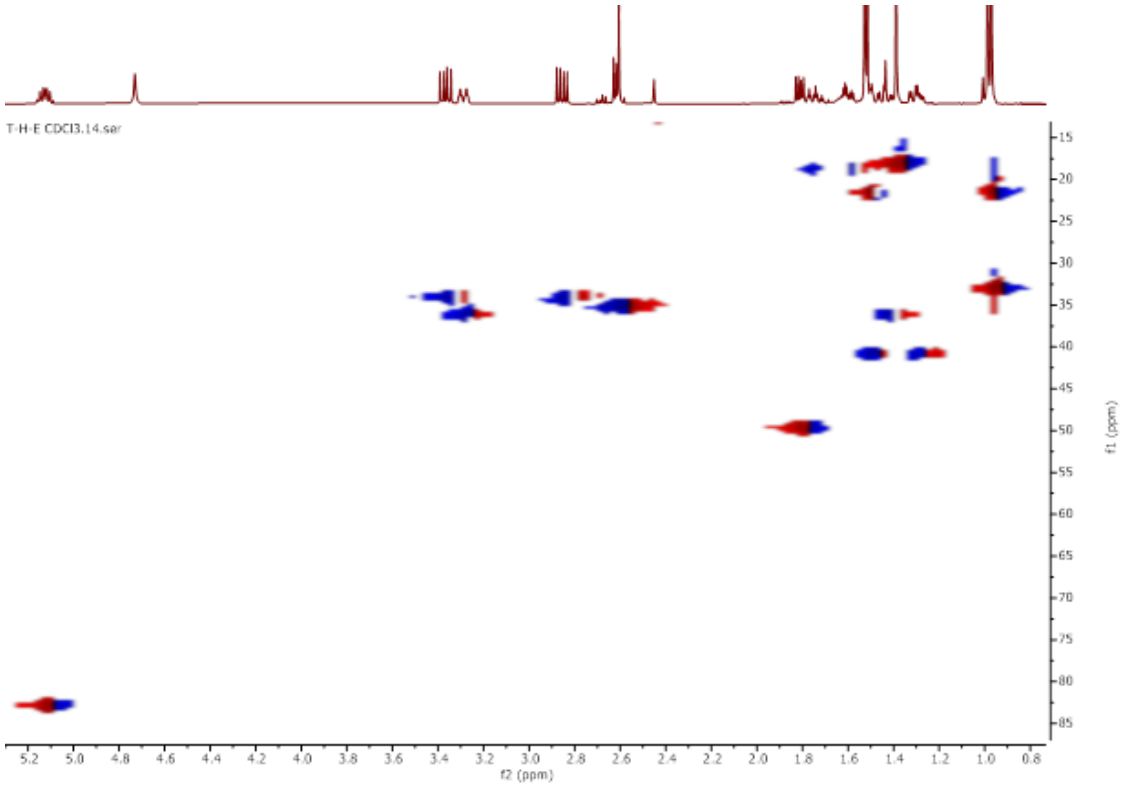

Fig. 4. HSQC spectrum of villosin A (**1**).

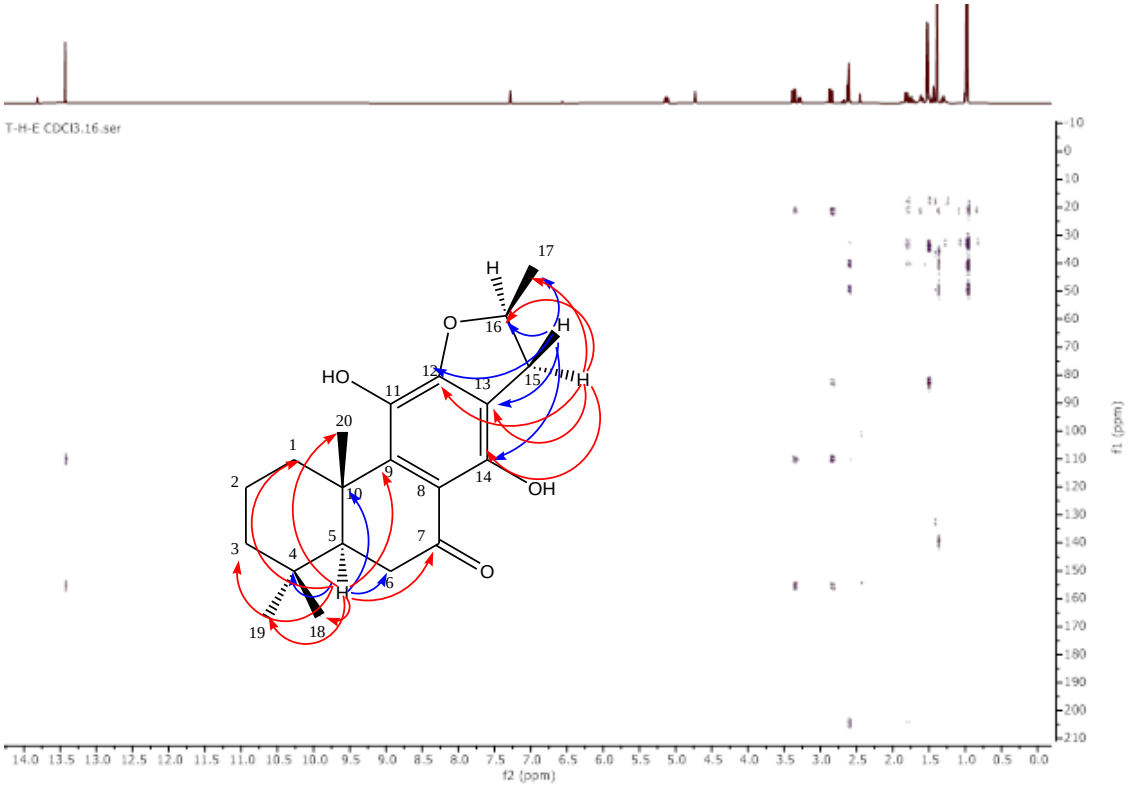

Fig. 5. HMBC spectrum of villosin A (**1**).

Finally, the antioxidant capacity of *n*-hexane extract and three rearranged abietane-like diterpenoids from *T. hircanicum* roots were evaluated by a DPPH scanning assay. Scanning activities for DPPH free radicals of *n*-hexane extract and pure compounds are shown in Table 3. The antioxidant activity was determined with the *in vitro* DPPH reagent and expressed as the required concentration of extracts or purified compounds for 50 % radical scanning (IC_{50}).

Table 3. DPPH radical scavenging capacity of crude *n*-hexane extract and purified compounds

Sample	IC_{50} µg/ml ± SD
n-Hexane extract	83.7 ± 0.1
Villosin A (**1**)	4.3 ± 0.1
Teuvincenone B (**2**)	4.0 ± 0.1
5,8,11,13,15-Abietapentaen-7-one (**3**)	3.5 ± 0.1
BHT	16.5 ± 0.2

4. Discussion

The compounds purified by the phytochemical study of *T. hircanicum* L. are reported for the first time from this plant. The rearranged abietane diterpenes were reported from the roots of genus *Teucrium* [14]. Also, in this study, three rearranged abietane-type diterpenoids were obtained from the roots of this plant. Previously, these compounds were purified and identified from the roots of other species of the genus *Teucrium* [26-28]. Villosin A was isolated for first time from the acetone extract of the *T. divaricatum* subsp. *villosum* roots [26]. Teuvincenone B was reported from the acetone extract of *T. polium* subsp. *vincentinum* roots [27]. And compound 5, 8, 11, 13, 15-abietapentaen-7-one was first time isolated and characterized from EtOAc extract of the *T. polium* roots [28]. The biosynthetic pathway for 4-hydroxyphenethyl pentacosanoate (**4**) and 22-dehydroclerosterol (**5**) are present in many plants, and for this reason, these compounds are popoular in many different plants.

When these antioxidant activities were compared with the reference value (IC_{50} of BHT = 16.5 µg/ml), the DPPH inhibitory activities of rearranged abietane type diterpenoids were high (IC_{50} = 3.5-4.3 µg/ml). The *n*-hexane extract's radical scanning activity (IC_{50} = 83.7 µg/ml) was very small relative to the positive control. These results show that rearranged abietane-type diterpenoids have high antioxidant strength and the ability to recover free radicals.

In this study, the results of the analysis of antioxidants are presented in the form of IC_{50} µg/ml (Table 3). These results show that the adsorption rate of free radicals of three rearranged abietane-type diterpenoids is much better than BHT. However, *n*-hexane extract has less antioxidant activity than BHT. The antioxidant activity of these three rearranging abietane-like diterpenoids is aligned with their structure. Compound 5, 8, 11, 13, 15-abietapentaen-7-one (**3**) has the most potent antioxidant activity in inhibiting free radicals. This compound has more conjugated double bonds than the other two abietane-type diterpenoids. In addition, teuvincenon B, in addition to having double binding than the compound vilosin A, has an additional hydroxyl group at the carbon 6 position. Numerous studies on the antioxidant activity of rearranged abietane diterpenes and rearranged abietane-like diterpenoids have shown that this class of compounds has significant free radical scanning activity [29-36].

5. Conclusion

Phytochemical investigations on genus *Teucrium* showed that some species include

[DOR: 20.1001.1.2717204.2021.20.79.3.8]
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various metabolites such as rearranged neo-clerodane or abietane diterpenes, sesquiterpenes, triterpenes, steroids, flavonoids and aromatic compounds. These metabolites are responsible for different pharmacological effects. This investigation assessed the phytochemical study and antioxidant activity on *n*-hexane extract of *T. hircanicum* roots from the Hyrcanian forests, North Iran. In total, five compounds were reported from the roots of this plant. The roots of *T. hircanicum were* found to be a rich source of rearranged abietane diterpenoids. The biological evolution of *n*-hexane extract and isolated compounds indicated the high antioxidant potential. The beneficial effects of antioxidant compounds are known for human health. Natural bio-antioxidants (biologically active compounds with antioxidant potential) and their synthetic analogs have various applications. They are used as essential drugs, antibiotics, agrochemical substitutes, and food preservatives. Today, many drugs are artificially modified natural substances [37].

The Phytochemical investigation of *T. hircanicum* often focuses on essential oils [38, 39]. However, our study on the *n*-hexane extract of *T. hircanicum* roots shows that rearranged abietane diterpenoids are responsible for their antioxidant activity in this extract. Ultimately, *n*-hexane extract of *T. hircanicum* roots can be introduced as a new antioxidant source.

Author contributions

GV: Experimental part, writing the manuscript, SNE: Supervision, experimental validation in phytochemical part, developing draft of the paper; MG: Analysing HPLC data, developing draft MMT: supervision extraction of plant material and creating the draft of MS HR: Assistance on recording NMR spectra and preparative HPLC. AS: Collection and identification plant materials.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

Golestan University financially supported this work.

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How to cite this article: Valizadeh Gh, Nejad Ebrahimi S, Gholami M, Mazaheritehrani M, Rezadoost H, Sonboli A. Rearranged abietane diterpenoids from roots of *Teucrium hircanicum* L. ***Journal of Medicinal Plants*** 2021; 20(79): 24-36.
doi: 10.52547/jmp.20.79.24

فصلنامه گیاهان دارویی ۱۴۰۰؛ ۲۰(۷۹): ۲۴-۳۶

فصلنامه گیاهان دارویی

Journal homepage: www.jmp.ir

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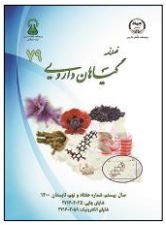

مقاله تحقیقاتی

دی‌ترپنوئیدهای آبیتانی نوآرایی شده حاصل از ریشه گیاه مریم نخودی خزری

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اطلاعات مقاله

گل‌واژگان:
مریم نخودی خزری
پروفایل فیتوشیمیایی
دی‌ترپنوئیدها
فعالیت آنتی‌اکسیدانی
ویلوسین آ

چکیده

مقدمه: گیاهان دارویی به دلیل استفاده‌های سنتی از آنها در پیشگیری و درمان بیماری‌ها نقش بسزایی دارند. **هدف:** هدف از این مطالعه جداسازی، خالص سازی و شناسایی مواد شیمیایی از عصاره هگزان ریشه گیاه مریم نخودی خزری و ارزیابی فعالیت آنتی‌اکسیدانی این عصاره و اجزای خالص شده آن بود. **روش بررسی:** عصاره هگزانی (۲/۲۷ گرم) از ریشه های این گیاه استخراج شد. عصاره با کروماتوگرافی ستونی با فاز نرمال بوسیله شستشو با هگزان- اتیل استات و سپس با اتیل استات- متانول فراکسیونه گردید، فراکسیون‌ها با اثر انگشت فیتوشیمیایی مشابه با هم ترکیب شده و ۲۳ فراکسیون اصلی بدست آمد. خالص‌سازی نهایی به روش HPLC-UV تهیه‌ای فاز معکوس صورت پذیرفت. ساختارهای ترکیبات جدا شده با روش‌های مختلف طیف‌سنجی مانند رزونانس مغناطیسی هسته‌ای یک بعدی و دو بعدی و طیف سنجی جرمی و مقایسه این داده ها با مقادیر گزارش شده شناسایی شدند. فعالیت آنتی‌اکسیدانی عصاره هگزانی ریشه مریم نخودی خزری و دی‌ترپنوئیدهای خالص از آن با آزمون DPPH مورد ارزیابی قرار گرفت و فعالیت مهار رادیکال آزاد آن محاسبه شد. **نتایج:** روش‌های استخراج و جداسازی منجر به خالص‌سازی سه دی‌ترپنوئید از نوع آبیتان نوآرایی شده ویلوسین آ (**۱**)، تووینسنون بی (**۲**)، ۱۵،۱۳،۱۱،۸،۵- آبیتا پنتائن-۷- اُن (**۳**)، یک فنتیل استر به نام ۴- هیدروکسی فنتیل پنتاکوزانوات (**۴**) و یک استرول به نام ۲۲- دهیدروکلروسترول (**۵**) شد. **نتیجه‌گیری:** عصاره هگزانی ریشه مریم نخودی خزری و سه دی‌ترپنوئید آبیتانی نوآرایی شده آن فعالیت آنتی‌اکسیدانی بالایی در بازه ۳/۵ تا ۴/۳ میکروگرم در میلی‌لیتر در مقایسه با مقدار مرجع (۱۶/۵ میکروگرم در میلی‌لیتر برای BHT) از خود نشان دادند.

مخففها: DPPH، ۲،۲- دی فنیل پیکریل هیدرازیل؛ BHT، دی‌بوتیل هیدروکسی تولوئن ؛ CC، کروماتوگرافی ستونی؛ RSA، فعالیت روبش رادیکال؛ HMBC، طیف‌سنجی همبستگی چند پیوندی ناجور هسته‌ای؛ HSQC، طیف‌سنجی همبستگی تک کوانتومی هسته‌ای

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تاریخ دریافت: ۴ فروردین ۱۴۰۰؛ **تاریخ دریافت اصلاحات:** ۶ تیر ۱۴۰۰؛ **تاریخ پذیرش:** ۱۲ تیر ۱۴۰۰

doi: 10.52547/jmp.20.79.24