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Flavonoid and Diterpenoid Components from *Teucrium orientale* subsp. *orientale* and their Radical Scavenging Activity

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Abstract: Being a member of the Lamiaceae family, *Teucrium orientale* (named Maryam Nokhodi in Iran) grows in Iran, Turkey, Iraq, Caucasus, Syria, Lebanon, Afghanistan, Pakistan, Turkmenistan, Central Asia, Europe, and North Africa. Among the pharmacological effects, *Teucrium orientale* is confirmed to have antidiabetic, antihemorrhoidal, antidysmenorrhea, antioxidant, antipyretic, antibacterial, anticandidal, and pesticide effects. The genus *Teucrium* comprises many bioactive compounds, including flavonoids, monoterpenes, sesquiterpenes, diterpenoids, phenolic acids, and fatty acid esters. In this work, in order to investigate the phytochemistry of *Teucrium orientale*, the aerial parts of the plant were extracted by maceration. The column chromatography technique was used to fractionate the acetonic extract. Purified compounds were obtained by smaller chromatography columns and techniques, including preparative thin layer chromatography, precipitation, and recrystallization. Chemical structures of compounds were identified by NMR and FT-IR spectroscopy and also elemental analysis. The compounds were elucidated as sclareol, quercetin, and luteolin. Furthermore, the antioxidant activity of the isolated compounds was evaluated.

Keywords: *Teucrium*; Diterpenoid; Flavonoid; Pharmacognosy; Antioxidant

1. Introduction

Being a member of the Lamiaceae family, the genus *Teucrium* comprises approximately 300 species worldwide [1] and 12 species distributed in Iran, which include *T. macrum* and *T. persicum* exclusively grow in Iran [2]. Other species are as follows: *T. orientale, T. oliverianum, T. procerum, T. parviflorum, T. hircanicum, T. scordium, T. melissoides, T. chamaedrys, T. polium,* and *T. stocksianum,* which grow in Iran, Turkey, Iraq, the Caucasus, Syria, Lebanon, Afghanistan, Pakistan, Turkmenistan, Central Asia, Europe, and North Africa.

In herbal medicine, *Teucrium* species are utilized to cure many pathological conditions such as diabetes, wounds, fever, insomnia, neurological disorders, abdominal cramps, gastrointestinal disorders, cold, hypertension, diarrhea, stomachache, inflammation, and rheumatism [3].

The pharmacological experiments approve many of the effects mentioned above. Moreover, the genus is confirmed to have antioxidant, anti-inflammatory, DNA protective,

antinociceptive, cytotoxic, insulinotropic and antidiabetic, hepatoprotective, antiulcerogenic, antidiarrheal, anti hemorrhoid, antimicrobial, antiparasitic, and antifeedant properties [4-8].

The genus *Teucrium* is rich in flavonoids, sesquiterpenes, abietane, clerodane, and neoclerodane diterpenoids, phenolic acids, and fatty acids [9-12]. Also, essential oils with monoterpenes and sesquiterpenes as main components are volatile components of *Teucrium* species [13-15].

Teucrium orientale, locally named "Maryam Nokhodi" in Iran, is traditionally used as an antidiabetic, antipyretic, and anti hemorrhoidal agent. Furthermore, it is believed that this plant is effective in reducing menstrual pain. It is also known to be beneficial in healing wounds and skin injuries [16].

Phytochemical investigations have shown that *Teucrium orientale* comprises many bioactive compounds, including flavonoids, iridoids, neoclerodane diterpenoids, and phenolic acids [17], as well as containing monoterpenes and sesquiterpenes as the major components of the essential oil [17, 18]. This study aimed to purify and characterize the bioactive constituents of acetone extract of *T. orientale subsp. orientale* and evaluate their antioxidant ability.

2. Materials and Methods

2.1. Chemicals.

Being purchased from Merck (Germany), the reagents were used without further purification. Organic solvents were purchased as follows: Acetone, ethyl acetate, pyridine, and sulfuric acid from Carlo Erba. Chloroform, dichloromethane ethanol, hexane, and methanol from Dae-Jung.

2.2. Plant material.

Arial parts of *T. orientale* were collected in summer 2015 at the flowering stage from Ghasemlou Valley, Urmia, West Azerbaijan province, Iran. The taxonomist made botanical identification of Urmia Faculty of Pharmacy. A herbarium specimen (HUPS-2) has been deposited in the Herbarium of the Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran.

2.3. Extraction and isolation.

Air-dried aerial parts of *T. orientale* (700 g) were macerated for 72 h by acetone and then the extract was filtered. The same procedure was carried out in triplicate for the residue. The acetone extract was concentrated to dryness using a rotary evaporator. The methanolic extract was obtained from the residue through the procedure mentioned above.

The column chromatography technique was used to fractionate the acetone extract. Twenty-nine grams of dried extract were fractionated by a one-meter high column, filled with 300 g silica gel 60 (0.063-0.200 mm) as the stationary phase and 500 mL hexane as the mobile phase. Gradient elution was performed with ascending ratios of ethyl acetate in hexane. Samples were collected every 200 mL or after the color change of outlet solution and were concentrated by rotary evaporator. TLC analyses were performed for each concentrated sample, and identical samples were combined according to TLC results. Finally, 19 fractions (F1-F19) were obtained.

F16 was dissolved in 15 mL methanol and 15 mL chloroform. After precipitation, the precipitate was washed with methanol and turned to white. The sample was white sediment named compound 1 (9 mg).

To purify the plant's biochemical compounds, F4, F14 and a combination of F7 and F8 were selected due to their quantity and TLC results and were purified by smaller chromatography columns and techniques, including preparative thin layer chromatography (Preparative TLC or plates) and recrystallization.

Three fractions (F14-1, F14-2, and F14-3) obtained from column chromatography of main fraction F14, were combined and undergone multiple recrystallizations for further purification. A light yellow solid was obtained and named compound 2 (17 mg).

Fraction F14-18, also obtained from column chromatography of main fraction F14, contained some yellowish sediment. The sediment was washed with acetone to remove impurities. The yellow sediment was named compound 3 (24 mg).

2.4. NMR experiments.

To obtain ^{1}H and ^{13}C NMR spectra, Bruker Avance 400 MHz spectrometer (operated at 400 MHz and 100 MHz, respectively) was employed. The deuterated solvents were $CDCl_3$ and DMSO-d_6 and the internal standard was TMS in these experiments.

2.5. FT-IR analysis.

IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer, and KBr pellets were used as a carrier for the samples.

2.6. Melting point.

Measurement of the melting points was carried out in open glass capillaries and taken by the electrothermal melting point apparatus.

2.7. Elemental analysis.

Costech elemental analyzer was utilized to perform the elemental analysis for C, H, and N atoms.

2.8. Antioxidant activity assessment.

Purified compounds were dissolved in methanol to make various concentrations (7.8-500 µg/mL). A concentration of 80 µg/mL of DPPH (the substance used to evaluate the antioxidant activity) was prepared by dissolving 8 mg of DPPH in methanol. One mL of diluted solutions were separately added to a 1 mL solution of DPPH and left for 30 min due to the reaction to be completed. Also, the same steps were repeated for the reference compound, rutin. The UV absorbance of samples was recorded at 517 nm. The assays were made three times, and the average absorption was calculated for each concentration [19].

3. Results and Discussion

Three natural compounds were purified from the acetonic extract of the aerial parts of *T. orientale* (Figure 1). Their chemical structure was elucidated as sclareol (1), quercetin (2),

and luteolin (3) based on spectroscopic analysis (NMR and FT-IR) and comparison with those reported in the literature.

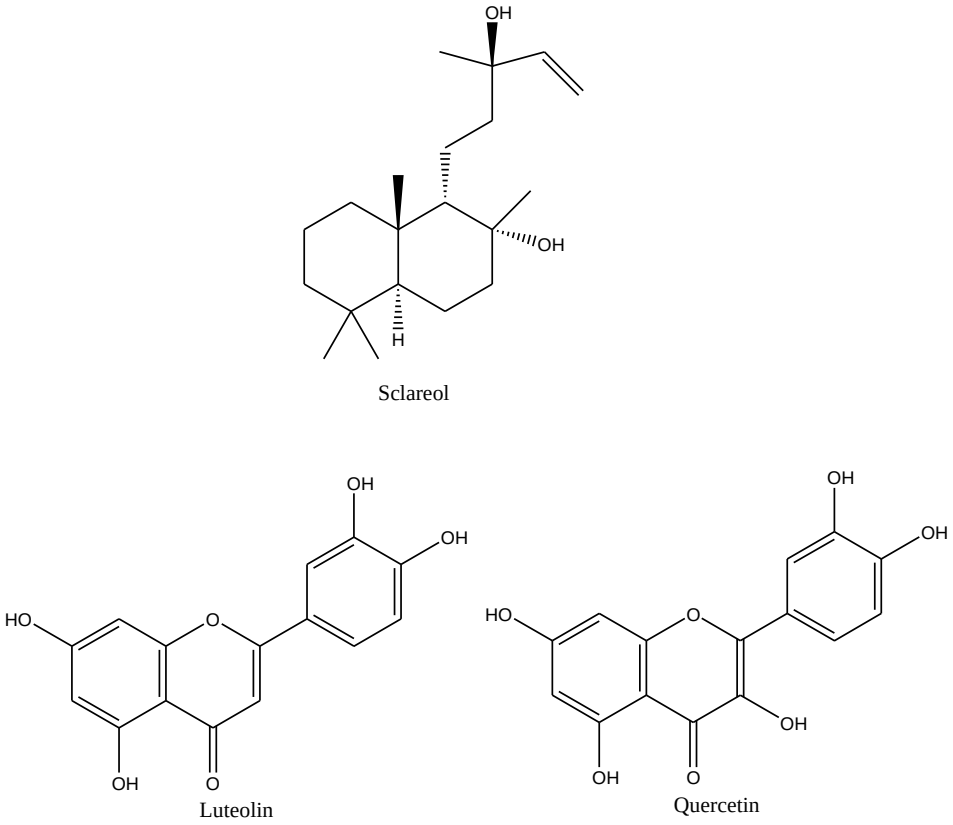

Figure 1. Chemical structure of isolated compounds from *T. orientale*.

Compound 1 (sclareol) was obtained as a white powder and is reported from the genus *Teucrium*'s extracts for the first time (Figure 2). It was isolated from the *Salvia* species (Lamiaceae family) before. Sclareol belongs to labdane diterpenoids with molecular formula $C_{20}H_{36}O_2$. It was found to have antiproliferative activity against cancerous cells and also anticholinesterases effect [20].

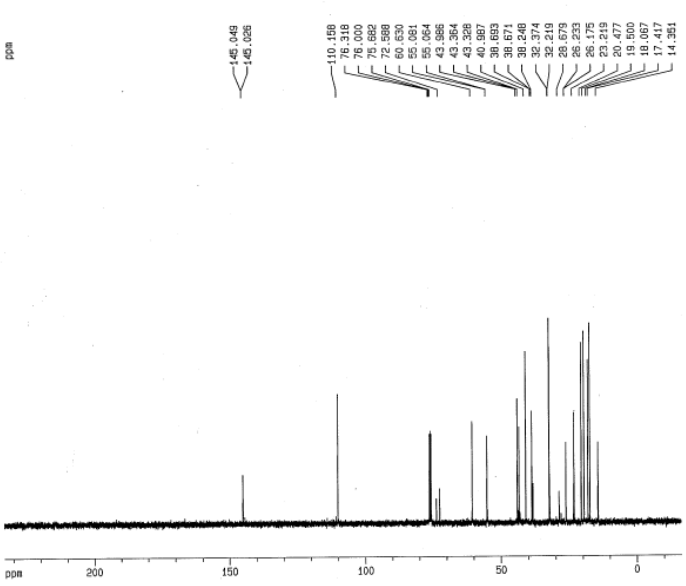

Figure 2. ^{13}C-NMR of sclareol in $CDCl_3$ at 100 MHz.

Compound 2 (quercetin) was obtained as a yellowish powder (Figure 3). Quercetin belongs to flavonols from the large class of natural product flavonoids. It is a common flavonoid compound in medicinal plants, vegetables, wine, and fruits [21]. Quercetin has been reported from different *Teucrium* species. It is consumed as a dietary supplement. Moreover, quercetin has great pharmacological properties, including antioxidant, anti-inflammatory, and protein kinase inhibitory activity [22].

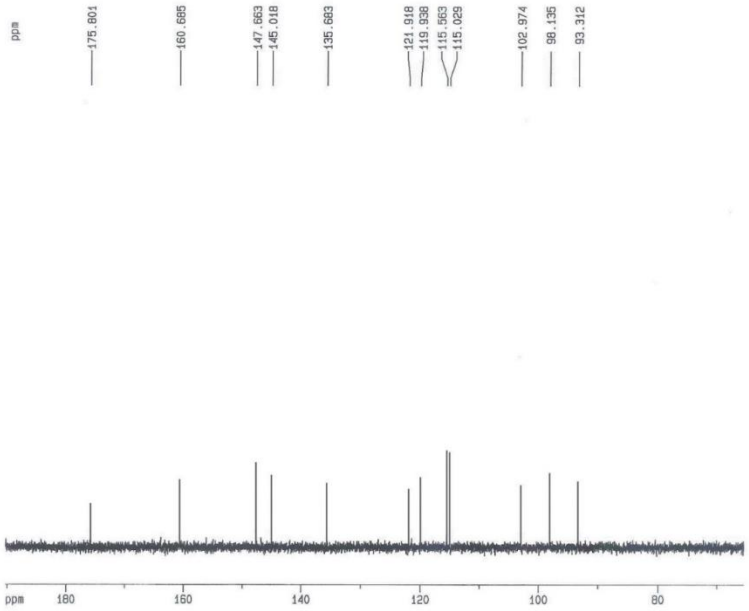

Figure 3. ^{13}C-NMR of quercetin in DMSO-d6 at 100 MHz.

Compound 3 (luteolin) was purified as an amorphous yellowish solid (Figure 4). Luteolin is a flavone naturally occurring in medicinal foods such as rosemary, sage, peppermint, olive oil, and carrot. It was also previously reported from the genus *Teucrium*. Luteolin has important biological activities, including anticancer, anti-inflammatory, antimicrobial, and anti-Alzheimer's disease [23].

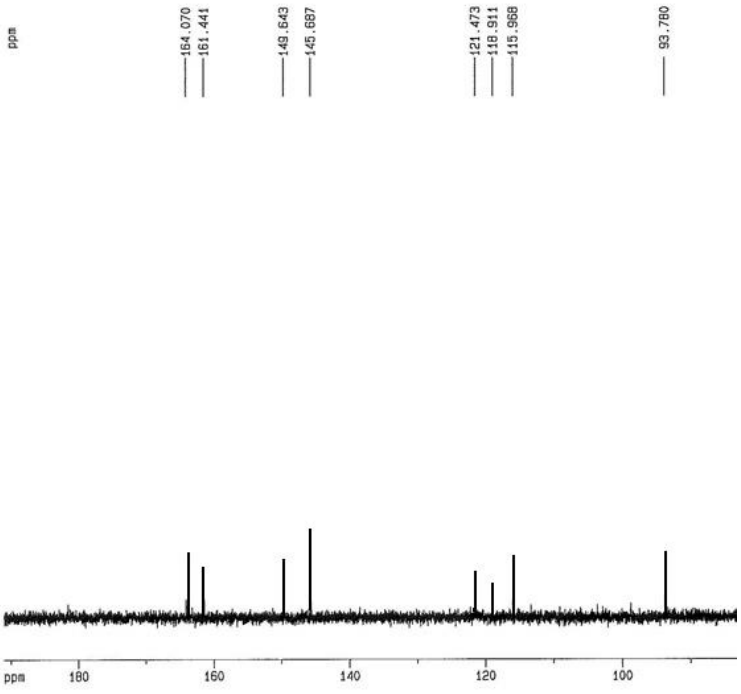

Figure 4. ^{13}C-NMR of luteolin in DMSO-d_6 at 100 MHz.

In this study, the DPPH radical scavenging ability of the isolated compounds was evaluated. In this direction, different concentrations of the samples were used for the measurement of radical scavenging activity. Rutin was used as a standard antioxidant agent. As could be seen from Table 1, quercetin with IC_{50} value of 17.7 ± 1.2 µg/mL showed the strongest antiradical activity followed by luteolin (18.6 ± 1.9 µg/mL). Sclareol exerted a mild antioxidant activity.

Table 1. Antioxidant activity of isolated compounds from *T. orientale*.

Compound	IC_{50} (µg/mL)
Sclareol	129.3 ± 11.8
Quercetin	17.7 ± 1.2
Luteolin	18.6 ± 1.9
Rutin (standard)	22.1 ± 1.6

4. Conclusions

Teucrium orientale (Lamiaceae) was phytochemically investigated. Chromatographic techniques were employed for the separation of bioactive components. Diterpenoid and flavonoid metabolites were purified from acetone extract of the plant. NMR, FT-IR, and elemental analysis were used for the structure elucidation of pure compounds. Sclareol, quercetin, and luteolin showed moderate to high radical scavenging activity. Results indicate that *T. orientale* could be considered as an important source of biologically active natural products.

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Conflicts of Interest

The authors declare no conflict of interest.

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