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Chemical constituents and NAD(P)H:quinone oxidoreductase 1 (NQO1) inducer activity of *Teucrium oliverianum* Ging. ex Benth.

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The aqueous methanolic extract of *Teucrium oliverianum* was partitioned with different solvent systems with increasing polarities. The phytochemical investigation of the $CHCl_3$ fraction led to the isolation of six compounds. The structures of the compounds were determined by NMR co-chromatographic TLC and found to be: 8-*O*-acetylharpagide 1, 12-*O*-methylteucrolin A 2, teucrolivin A 3, eupatorin 4, teucrolivin B 5 and 24 (S)-stigmasta-5, 22, 25-trin-3β-ol 6. The total extract and the isolated compounds were tested for their ability to induce the cytoprotective enzyme NAD (P)H:quinone oxidoreductase (NQO1). Among the purified compounds, the diterpenoid, teucrolivin B 5 was the only one which was able to induce NQO1 by more than 2-fold, whereas the flavonoid eupatorin 4 was the most potent inducer, increasing the NQO1 specific activity by 1.75-fold at a concentration of 25 µg/ml. Compounds 2, 3 and 6 were essentially inactive, indicating that compounds 1, 4 and 5 are the main contributors to the NQO1 inducer activity of the total extract.

Keywords: NQO1, Chemical constituents, *Teucrium oliverianum*, Chemoprotection, Labiatae
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Extracts of a wide range of medicinal plants are used as raw drugs, and they possess various medicinal properties. The different plant parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in small quantities by the local communities and folk healers for local use, many are harvested in larger quantities and traded in the market as the raw material for herbal industries[1]. Traditional medicine is used widely throughout Saudi Arabia either as preparations or herbal drugs. *Teucrium* species have been used for more than 2000 years as medicinal plants. They show anti-diabetic, anti-inflammatory, anti-ulcer, hypotensive, antispasmodic, anorexic, diuretic, diaphoretic, antiseptic and antipyretic activities[2,3]. They are also used in the treatment of stomachaches. Some of the *Teucrium* extracts exhibit activity towards the central nervous system[3-5].

The genus *Teucrium* (Family: Labiatae) is represented by approximately 300 species in worldwide. There are 4 species in Saudi Arabia. The plant is distributed mainly in Central and South-East Asia, Central and South America and around the Mediterranean. *T. oliverianum* is used in traditional Saudi medicine for the treatment of diabetes and is well known for its hypoglycemic activity[6,7]. The genus *Teucrium* is one of the richest sources of clerodane diterpenes. Most of the reported structures display a furan ring system in the side chain, with an oxygen-bearing C-12[8]. The structure of four C-3α-oxygenated neo-clerodane diterpenoids teucrolins A–D, together with the unusual C-10β-oxygenated diterpenoid teucrolin E has been described. Neo-Clerodane diterpenoids were isolated from the aerial parts of *T. oliverianum.* In the study of new bioactive secondary metabolites from plants, we carried out the phytochemical investigation and biological evaluation of several plants of Saudi Arabia[9,10]. The aim of the present study was to investigate the ability of the total extract and the isolated compounds from *Teucrium oliverinum* to induce the cytoprotective enzyme NAD(P)H:quinone oxidoreductase (NQO1).

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Methodology

Plant material

The aerial parts of *Teucrium oliverianum* (Labiatae) were collected from the Tanhat protected area in April 2012, Saudi Arabia. The plant was identified by the Plants Taxonomist at the Herbarium Unit. A reference voucher number 15930 was assigned to the plant sample and have been deposited at the Herbarium of the Pharacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Extraction and isolation

The plant samples were air-dried in shade and ground into fine powder. One and half kg from the air-dried powder was percolated in 80% methanol at room temperature three times (two days each). The mixture was filtered and the residues were re-percolated for three times, and the filtrates were combined. The total aqueous methanol extract was concentrated under reduced pressure at a temperature not exceeding 40 °C. to obtain residue with yields of 20 %. The residue was suspended in water and extracted successively with chloroform (4x500 mL) and ethyl acetate (4x500 mL) to give $CHCl_3$, EtOAc, and water-soluble portions. After solvent removal, the residues obtained were 70.3, 30.8 and 180 gm, respectively. A weight of 60 gm of the $CHCl_3$ extract was subjected to flash chromatography on silica gel (70–230 mesh) columns (85-2.3 cm i. d.) and eluted with pure *n*-hexane, a stepwise gradient *n*-hexane/chloroform, then chloroform/methanol mixtures (100%:0, in steps of 10% each). TLC of the collected fractions (50 mL each) was performed on silica gel layers (Silica gel 60 F^{254} on glass, Merck) eluted with solvent systems, *n*-hexane/ethyl acetate (1:1, 1:2, 1:4) and Haxane/Ethyl acetate/MeOH (1:1:0.5, 1:1:1) (v/v/v). Spots were analyzed under UV light and also sprayed with vanillin sulphuric acid and re-grouped on the basis of their TLC profiles. The fractions eluted with *n*-hexane–$CHCl_3$ (60:40) (0.5 gm) were further fractionated by repeated column chromatography on silica gel to yield compound 1 and 2. Further purification for the combined fractions eluted with *n*-exane/chloroform (50:50) (0.44 gm) was carried out by column chromatography on silica gel with *n*-exane/ EtOAc in steps of 10% each. The eluted fractions (11-23) were subjected to further purification on Sephadex LH-20 column using 90% methanol to afford compounds 3, 4 and 5. The fractions eluted with chloroform (100%) (0.1 gm) were further fractionated by repeated column chromatography on silica gel to yield compound 6.

^{1}H and ^{13}C NMR spectra in ($CDCl_3$) were recorded on Ultra Shield Plus 500 MHz (Bruker, Munich, Germany) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the residual solvent peak, the coupling constants (J) are reported in Hertz (Hz).

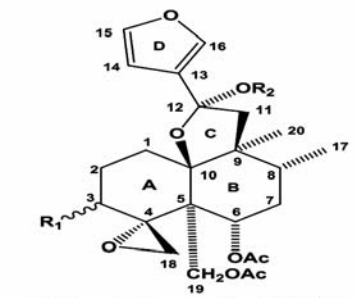
Biological assay

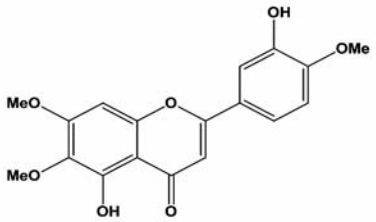
The NQO1 inducer activity of the extract and the isolated compounds was evaluated using a bioassay in murine Hepa1c1c7 cells as described previously[10,11]. The concentration that doubles the NQO1 specific activity (CD value) was used as a measure of inducer potency.

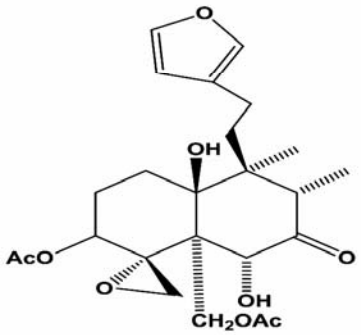
Results and discussion

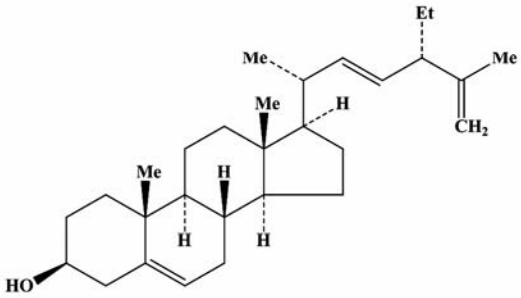
On the basis of the NMR data and direct co-chromatography[12], compounds 1-6 were identified as 8-*O*-acetylharpagide 1, 12-*O*-methylteucrolin A 2, teucrolivin A 3, eupatorin 4, teucrolivin B 5 and 24 (S)-stigmasta-5, 22, 25-trin-3β-ol 6. The structures of the isolated compounds from *Teucrium oliverianum* are presented in Fig. 1.

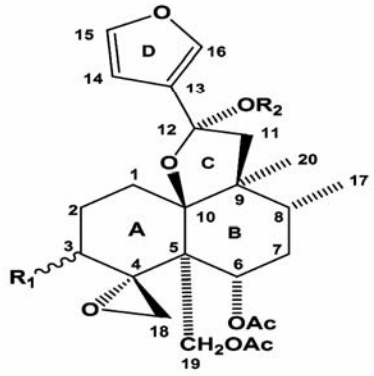
Evaluation of the NQO1 inducer activity showed that the extract was active with a CD value of 35 µg/ml and an induction magnitude of 3.3-fold at the highest concentration (100 µg/ml) tested (Table 1 and Fig. 2). The iridoid glycoside derivative, 8-*O*-acetylharpagide (compound 1) caused a 1.75-fold increase in the specific activity of NQO1 at a concentration of 100 µg/ml. This compound has been previously shown to have chemopreventive activity in animal models of pulmonary, cutaneous, and hepatic carcinogenesis[13,14]. The only purified compound which reached a CD value was the diterpenoid teucrolivin B (compound 5), at a concentration of 59 µgm/ml, and an induction magnitude of 2.7-fold at 100 µgm/ml, the highest concentration tested. Teucrolivin B has epoxy functionality, which is electrophilic and found in other NQO1 inducers[15]. Surprisingly however, the structurally related teucrolivin A (compound 3) showed a very weak activity, inducing NQO1 by 1.27-fold at a concentration of 100 µg/ml, whereas 12-O-methylteucrolin A (compound 2) was essentially inactive. These observations suggest that the presence of the electrophilic epoxide on these molecules is not sufficient to confer inducer activity.











R1 R2

α-OAc Me 12-O-methylteucrolin A (2)

=O H Teucrolivin A (3)

Fig. 1—Structures of the isolated compounds from *Teucrium oliverianum*

Table 1—NQO1 inducer activity of the total extract and the isolated compounds from *Teucrium oliverianum*

Compounds	CD (µM)	Induction magnitude (Fold)	Toxicity at 100 µg/ml
(Total extract) (TE)	35 µg/ml	3.31	No
8-*O*-acetylharpagide (Compound 1)		1.76	No
12-O-methylteucrolin A (Compound 2)		1.16 (at 33 µg/ml)	No
Teucrolivin A (Compound 3)		1.27	No
Eupatorin (Compound 4)		1.75 (at 25 µg/ml)	Not tested
Teucrolivin B (Compound 5)	59 µg/ml	2.71	No
24(S)-stigmasta-5,22,25-trin-3β-ol (Compound 6)		1.15	No

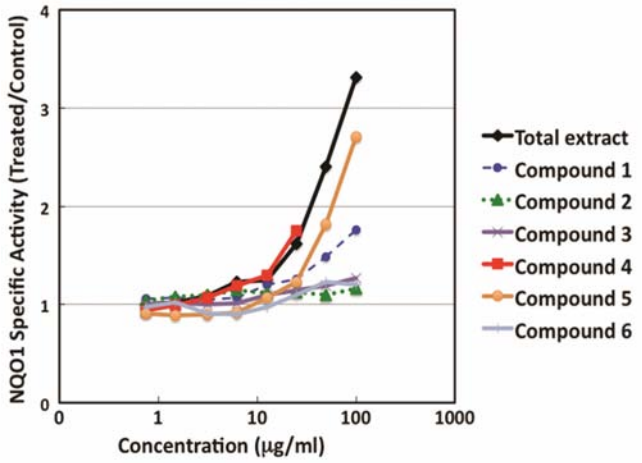


Fig. 2—Dose-response of NQO1 inducer activity of the total extract and the isolated compounds from *Teucrium oliverianum*

Although a CD value was not reached for the flavonoid eupatorin (compound 4), the dose-response curves suggested that eupatorin was the most potent among the purified compounds, increasing the NQO1 specific activity by 1.75-fold at a concentration of 25 µg/ml. However, eupatorin was insoluble at concentrations higher than 25 µg/ml, precluding testing at higher concentration. Notably, previous reports have documented cytotoxic activity for eupatorin[16] and its ability to inactivate the mitotic checkpoint, cause apoptosis and suppress cancer cell proliferation[17,18], some of which could be due to cytochrome P450 (CYP1) family-mediated metabolism[18]. Overall, compounds 2, 3 and 6 were essentially inactive; indicating that compounds 1, 4 and 5 are the main contributors to the NQO1 inducer activity of the total extract.

Conclusion

Evaluation of the NQO1 inducer activity showed that the *Teucrium oliverianum* extract was active with a CD value of 35 µg/ml and an induction magnitude of 3.3-fold at the highest concentration (100 µg/ml) tested. Based on the results obtained, the extract and the isolated compounds provide a basis for their potential use as herbal medicines.

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