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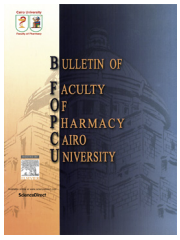

ORIGINAL ARTICLE

Cytotoxicity and antioxidant activity of new biologically active constituents from *Micromeria nervosa* grown in Egypt

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KEYWORDS

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Abstract Chromatographic fractionation of the acetone extract of *Micromeria nervosa*, resulted in the isolation and identification of new natural furanosesquiterpene alcohol; micromeriol (1), a known sterol; β-sitosterol (2), a new natural 5-β-cholestane type; nervosane (3), two known triterpenic acids; oleanolic acid (4), and ursolic acid (5). The cytotoxicity and the antioxidant activities of the acetone extract and the isolated pure compounds (1–5) were determined.

1. Introduction

Plants of genus *Micromeria* are well known due to a wide range of biological activities in folk medicine, such as antibacterial, heart disorders, headache, wounds, skin infections and the most usage of *Micromeria* species is in colds.[1–3]

Micromeria nervosa (Desf.) Benth. is a wild plant belonging to the family Lamiaceae, which are widespread in the Mediterranean region. The name of the genus was derived from Greek words *micro* and *meros* (meaning small and part) because they were found as dwarf fragrant of perennial herbs which usually grow in clefts of fissured rocky ground while *nervosa* with prominent nerves or veins. *Micromeria* genus was grown naturally in Egypt and in the eastern Mediterranean region.[4,5]

Previous research has established the genus *Micromeria* to be a rich source of polyphenolic compounds. Reviewing the current literature, nothing was reported concerning the chemical investigation of the studied plant excepting few reports concerning the occurrence of flavonoidal glycosides and volatile oil.

The present paper describes the isolation, structure elucidation and biological study of five compounds.

2. Experimental

2.1. Materials and methods

2.1.1. Plant material

The aerial parts of *M. nervosa* (2 kg) were collected from omrakham valley, Matrouh, Egypt. The plants were kindly identified by Dr. Nahaid El-Asinay, Assistant Professor of Plant Taxonomy, Faculty of Science, Cairo University. Corresponding specimens were deposited at the herbarium of the pharmacognosy department, Faculty of Pharmacy, Al-Azhar University (MV2005).

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2.1.2. General procedures

The ^{1}H and ^{13}C NMR, spectra were recorded on a Bruker NM 360 spectrometer operating at 500 for ^{1}H and 125 MHz, for ^{13}C NMR. All spectra were obtained in DMSO-d_6 and CD_3OD using TMS as internal standard, with the chemical shifts expressed in δ (ppm) and the coupling constants (*J*) in Hertz. ESI-MS analyses were measured on a TSQ700 triple quadrupole instrument (Finnigan, San Jose, CA, USA) by using the Finnigan electrospray atmospheric pressure chemical ionization source. A duo-UV lamp (λ 254/365 nm), Desaga, Heidelberg, Germany was used to find the location of the spots in TLC and column chromatography. UV-spectra were recorded by Hitachi 340 spectrophotometer, Japan. Melting point was obtained on a Stuart SMP3 apparatus. IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett–Packard Color Pro. Plotter. Column chromatography was carried out on various adsorbents including silica gel 40–60 μm (E. Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Thin layer chromatography (TLC) was performed on pre-coated silica gel F254 plates (E. Merck, Darmstadt, Germany). The pure compounds were visualized by spraying 1% vanillin-H_2SO_4, followed by heating at 100 °C for 5 min.

2.1.3. Extraction and isolation

The air-dried powdered aerial parts of the plant (2 kg) were subjected to extraction with acetone (82 g). The acetone extract was chromatographed on silica gel column, eluted with solvent systems of *n*-hexane, *n*-hexane: EtOAc (100:00–70:30), to give seven fractions (A-G). Fraction E (4 g) was rechromatographed on a silica gel flash column (Petroleum ether: $EtOAc^-$ 90:10–75:25) and Sepralyte C18 flash column (H_2O: $MeOH^-$ 60:40–85:15) to give three fractions of E1, E2 and E3. Fr. E1 (80 mg) was further subjected to silica gel column using Petroleum ether: EtOAc (85:15–80:20) to afford 56 mg of compound 1. Fr. E 2 (55 Mg) was subjected to silica gel column (petroleum ether: $EtOAc^-$ 90:10–75:25), silica gel flash column using *n*-hexane: EtOAc (90:10–70:30) and Sepralyte C18 flash column (H_2O:$MeOH^-$ 50:50–90:10) to afford compounds 2 (36 mg). Fr. E 3(50 mg) was subjected to silica gel column (Petroleum ether: $EtOAc^-$ 90:10–75:25), silica gel flash column using *n*-hexane: EtOAc (90:10–70:30) and Sepralyte C18 flash column (H_2O: $MeOH^-$ 70:30–90:10) to afford compounds 3 (50 mg). Fraction G (3.5 g) was rechromatographed on a silica gel flash column (Petroleum ether: $EtOAc^-$ 90:10–75:25) and Sepralyte C18 flash column (H_2O: $MeOH^-$ 60:40–85:15) to give two subfractions of G1 and G2. Fr. G1 (32 mg) was subjected to silica gel column (Petroleum ether: $EtOAc^-$ 90:10–75:25), silica gel flash column using *n*-hexane: EtOAc (90:10–70:30) and Sepralyte C18 flash column (H_2O: $MeOH^-$ 50:50–90:10) to afford compounds 4 (10 mg). Fr. G2 (62 mg) was subjected to silica gel column (Petroleum ether: $EtOAc^-$ 85:15–75:25), silica gel flash column using *n*-hexane: EtOAc (90:10–70:30) and Sepralyte C_{18} flash column (H_2O: $MeOH^-$ 50:50–90:10) to afford compounds 5 (35 mg). All separated compounds were purified by Sephadex LH-20 column eluted with MeOH.

2.1.4. Material used for biological study

2.1.4.1. Acetone extracts. Air-dried aerial parts of *M. nervosa* (Desf.) Benth. (20 g), were extracted separately at room temp with acetone (3 × 100 ml). The acetone soluble portion was evaporated under *vacuo* (130 mg).

2.1.4.2. Isolated compounds. 2 mg of each compound (1–5) was used in each method.

2.1.4.3. Well known antioxidant D,L-α-tocopherol and butylated hydroxytoluene (BHT) were used as reference standard. BHT has been added to foodstuffs but, because of toxicity issues, their use is being questioned.

2.1.5. In vitro assays antioxidant activity of M. nervosa acetone extract and isolated compounds

2.1.5.1. 2,2-diphenyl-1-picrylhydrazyl(DPPH) assay of antioxidant activity. DPPH assay was performed following the procedures of Hosny M. and Rosazza J. The lower the EC_{50}, the greater the antioxidant activity of the substance.[6]

2.1.5.2. Ferrous sulfate–H_2O_2 stimulated lipid peroxidation in rat tissue homogenate. The effect of acetone extract and isolated compounds on rat tissue (brain, heart and liver) homogenate induced by ferrous sulfate–H_2O_2 and lipid peroxidation was determined by malondialdehyde (MDA)-TBA adduct according to the method described by Hosny et al.[12,13]

2.1.5.3. Xanthine oxidase-induced generation of superoxide radical. The influence of acetone extract and isolated compounds was measured according to the method described by Luis Gongora et al.[14] IC_{50} values were calculated by linear regression analysis, and kinetic analysis of inhibition was determined only for those compounds with an IC_{50} lower than 50 μM (see Fig. 1).

2.1.5.4. Scavenging activities of superoxide radicals. Measurement of superoxide anion scavenging activity of *M. nervosa*

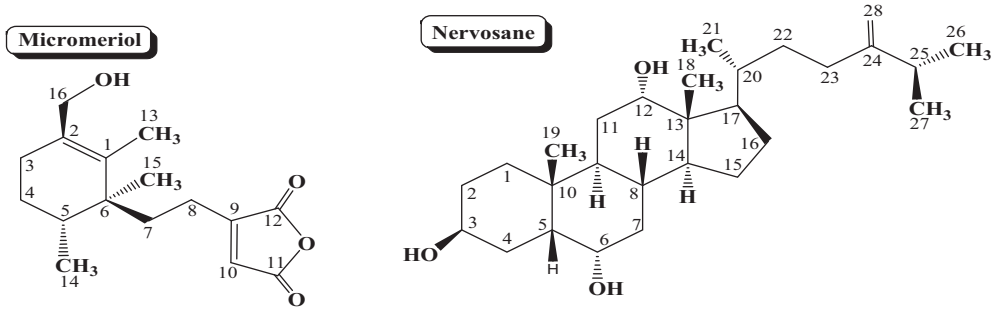

Figure 1 Structures of compounds 1 and 3.