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Identification and biological activity of the volatile compounds of *Glycyrrhiza triphylla* Fisch. & C.A.Mey

Abolfazl Shakeri [a], Javad Akhtari [b, c], Vahid Soheili [d], Seyedeh Faezeh Taghizadeh [e], Amirhossein Sahebkar [f], Rezvan Shaddel [g], Javad Asili [h, *]

[a] Student Research Committee, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Immunogenetic Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[c] Department of Nanobiomedicine, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[d] Department of Drug Control, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Department of Horticultural Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[f] Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[g] Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[h] Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Chemical composition and biological (antimicrobial, antioxidant and cytotoxic) activities of essential oils (EO) obtained from the aerial parts of *Glycyrrhiza triphylla* Fisch. & C.A.Mey (*G. triphylla*) were evaluated in the present study. The EO was isolated and analyzed using gas chromatography-mass spectrometry (GC-MS). Fifty-five compounds representing 99.3% of the total oil composition were identified. Major components of the oil were β-caryophyllene (25.4%), limonene (16.7%), β-myrcene (16.0%) and α-humulene (4.4%). The oil composition was dominated by the presence of sesquiterpene hydrocarbons comprising 43.6% of the total oil. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil were determined against eight bacterial strains and one fungus. The EO showed a good antibacterial activity against both Gram-positive and Gram-negative bacteria. The most susceptible strain was *Micrococcus luteus* (MIC = 2.7 μg/mL, MBC = 43.6 μg/mL). The antioxidant potential of the EO was examined using DPPH and β-carotene/linoleic acid (BCB) assays. The oil was considerably active in the DPPH assay (IC_{50} = 100.40 ± 0.03 μg/mL). Moreover, *in vitro* cytotoxic activity was assessed against six cancer cell lines using MTT assay. The EO showed no significant cytotoxic activity. In light of the present findings, *G. triphylla* oil may deserves to be further investigated for its potential therapeutic effects and also as a natural preservative in food industry.

1. Introduction

Essential oils (EOs) are a rich source of biologically active phytochemicals [1,2] and widely used for their ethno-medicinal, culinary and cosmetic effects. Some important biological activities of EOs are antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and anticancer properties [3]. The composition of EOs is usually predominated by the presence of terpenoids. Similar to other important natural products (e.g. phenolics, flavonoids and coumarins), terpenoids are secondary metabolites with various biological activities [4]. The structure of terpenoids comprises a hydrophobic skeleton to which hydrophilic functional groups (in oxygenated terpenoids) are bound. The presence of both hydrophobic and hydrophilic moieties confers oxygenated terpenoids the capacity to pass through biological membranes of microbial and mammalian cells, thereby reaching cytosolic environment where interactions with molecular targets could occur [5]. Due to increased market demand, EOs are gaining more interest as natural antimicrobial and antioxidant agents used in food preservation. Notably, some EOs are recognized as safe substances by the Food and Drug Administration (FDA) [6]. Although pharmacological properties of EOs have been shown in a variety of studies [7], their use for therapeutic approaches is still limited owing to the need for comprehensive assessment of safety profile particularly at high doses that are often required for clinical applications. Therefore,

* Corresponding author.
E-mail address: asilij@mums.ac.ir (J. Asili).

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investigation of the cytotoxic activity of EOs can be helpful for a better assessment of EO safety. The *Glycyrrhiza* genus belongs to Leguminosae and comprises about 20 species [8]. It is widely distributed all over the world. *Glycyrrhiza* species are reputed in the traditional medicine for the treatment of coughs, colds and painful swellings and also diuretic and choleretic effects [9]. *Glycyrrhiza triphylla* Fisch. & C.A.Mey is a perennial plant that grows to a height of 30–60 cm, with numerous stems branching from the base. The plant has yellowish-reddish corolla in May with green leaves 10–20 mm long and 5–10 mm wide [10]. Phytochemical analysis of *Glycyrrhiza* spp. has revealed the presence of bioactive phytochemicals such as triterpene saponins, flavonoids and isoflavonoids and over 400 compounds have been isolated from the genus [11]. According to our knowledge, there has been one previous report on the chemical composition of the EO of *Meristotropis xanthioides* Vassilcz. (a synonym of *G. triphylla*). However, the biological activity of this plant has not been the subject of any previously published study. Therefore, the present study aimed to investigate the chemical composition, antimicrobial, antioxidant and cytotoxic properties of the EO obtained from the aerial parts of *G. triphylla*.

2. Materials and methods

2.1. Plant material

The aerial parts of *G. triphylla* were collected during its flowering stage from Quchan, Khorasan-Razavi province, Iran, in July 2014. The plant was identified by Mrs. Souzani and a voucher specimen (no. 12622) was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

2.2. Isolation of essential oil

EO was isolated using hydro-distillation of air-dried material with a Clevenger-type apparatus. Following a period of about 3 h, the pale yellow oil was obtained with a yield of 0.5% v/w. The oil was dried over anhydrous sodium sulphate and stored at 4 °C in the dark until further testing.

2.3. GC–MS analysis

GC-MS analyses were performed using an Agilent 5975 apparatus with a HP-5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses) interfaced with a quadruple mass detector and a computer equipped with Wiley 7n.L library. Other analytical settings were: oven temperature: 50 °C (5 min), 50 °C–250 °C (3 °C/min), 250 °C (10 min); injector temperature 250 °C; injection volume: 0.1 μL; split ratio: 1:50; carrier gas: helium at 1.1 mL/min; ionization potential: 70 eV; ionization current: 150 μA; and mass range: 35–465. The linear retention indices (RI) were calculated for all components using retention times (RT) of a homologous series of n-alkanes (C6-C21) that were injected in conditions equal to the sample one. Identification of the components of the EO was based on retention indices relative to n-alkanes and computer matching with the Wiley 7n.L library as well as comparisons of the fragmentation pattern of the mass spectra with the data published in the literature [12].

2.4. Determination of antimicrobial activity

2.4.1. Bacterial strains and culture media

Eight microorganisms including *Escherichia coli* (ATTC 10536, American Type Culture Collection), *Salmonella typhi* (PTCC 1609, Persian Type Culture Collection) and *Pseudomonas aeruginosa* (ATCC 15442) as Gram-negative bacteria as well as Bacillus cereus (PTCC 1247), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Micrococcus luteus* (ATCC 9341) and *Listeria monocytogenes* (PTCC 1165) as Gram-positive bacteria were employed (IROST, IRAN). For antibacterial screening tests, 24 h growth culture at 37 °C on soybean casein digest agar (SCDA) was used and adjusted to 106 CFU/mL with sterile normal saline (0.9%).

2.4.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The EO was dissolved in Mueller-Hinton broth (MHB) using dimethyl sulfoxide (DMSO) as a co-solvent. In order to achieve the first concentration (10% v/v), 650 μL of EO was added to 325 μL DMSO and the volume was adjusted to 6.5 mL with MHB. Other concentrations were prepared according to the two-fold serial dilution method, reaching a final concentration of 0.153%. 200 μL of each concentration was inoculated in a cell culture plate (96 well) and 20 μL of bacterial suspension was added (106 CFU/mL). The test is repeated for each bacterium. In an attempt to demonstrate the sterility of media, MHB by itself was used as negative control. Each inoculum was used as the positive control. After incubation of the plates (24 h, 37 °C), the bacterial growth was assessed using 20 μL of 2,3,5-triphenyltetrazolium chloride (TTC) (5 mg/mL) as a colorimetric indicator. Afterwards, the plates were incubated one more time at 37 °C for 1 h. MIC was defined as the lowest EO concentration that inhibited the color change to red. MBC (the lowest concentration without any bacterial growth) was also determined by incubation of 20 μL of the wells without any color change, on the surface of plates containing Mueller-Hinton agar (24 h, 37 °C) [13,14].

2.4.3. Anti-fungal activity

To evaluate the anti-fungal activity of the EO, *Candida albicans* (ATCC 10231) was used as the test strain. The test was performed with a 48 h growth culture at 25 °C on SCDA and adjusted to 10^6 CFU/mL with sterile normal saline (0.9%). The EO concentrations were prepared according to the above-mentioned method except that the final volume of the sample was adjusted to 1 mL with sabouraud dextrose broth (SDB). Other concentrations were prepared according to the two-fold serial dilution method. Thereafter, 200 μL of cell suspension (10^6 CFU/mL) was inoculated in each tube. The MIC was the first concentration with no visible microbial growth and minimum fungicidal concentration (MFC) was determined in the same way as for the bacteria [13,15].

2.5. Antioxidant activity

Numerous methods can be utilized for the evaluation of antioxidant potential of a given compound. In this study the antioxidant activity of EO was evaluated using two assays: one in aqueous system (DPPH) and another in lipid system (BCB).

2.5.1. DPPH radical scavenging

The free radical scavenging activity of EO was measured by DPPH [16]. 2.5 mL of the EO at different concentrations (12.5–2000 μg/mL) was added to 1.0 mL of a DPPH methanolic solution. The mixture produced was shaken vigorously and then left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 518 nm. The scavenging activity of EO was evaluated according to the formula:

$$AA\% = 100 - [(Abs\ sample - Abs\ blank)/Abs\ control] \times 100$$

Methanol (1.0 mL) plus EO (2.5 mL) was used as a blank. DPPH