



Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants

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ABSTRACT

Different parts of three plants (*Primula auriculata*, *Fumaria vaillantii* and *Falcaria vulgaris*) were extracted with three different solvents to yield 72 crude extracts. The phytochemical analysis (chemical screening, GC–MS) of three plants was investigated for their antioxidant and antibacterial activity using nine Gram-positive and Gram-negative bacteria. The principal antioxidant and antimicrobial components were determined using HPLC with UV detection. All extracts possessed antibacterial activity especially methanolic extracts from flowers of *P. auriculata*. The DPPH-radical scavenging assay exhibited high antioxidant activities in three plants (more than 80% at 50 µg). The *F. vulgaris* showed high content of carvacrol (29.8%) as main component. The contents of carvacrol and fumaric acid in the methanolic-water extracts were 1119 and 1966 mg/l respectively. Our results indicate that these plants would be able to promise sources of natural products with potential antibacterial and antioxidant activity.

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1. Introduction

Plant extracts and their components have been known to exhibit biological activities, especially antimicrobial (Iskan, Demirci, Kirimer, Kürkcüoğlu, & Baser, 2002), antifungal (Soković et al., 2009), antibacterial (Kanatt, Chander, & Sharma, 2008) and antioxidant activities (Seun-Ah, Sang-Kyung, Eun-Jung, Chang-Hyun, & In-Seon, 2010). The substances that can inhibit pathogens and have little toxicity to host cells could be considered candidates for developing new antimicrobial drugs (Bajpai, Pande, Tewari, & Prakash, 2005). These compounds find in various medicinal plant organs such as stems, roots, leaves, barks, flowers, fruits and seeds (Cutter, 2000). The most important of these medicinally compounds are alkaloids, tannins, flavonoids and phenolic compounds (Amal, Ashraf, & Hossam, 2009).

Antioxidants have great importance in terms of reducing oxidative stress which could cause damage to biological molecules (Bektas, Sokmen, Akpulat, & Sokmen, 2005). The protective action of medicinal plants has been attributed to the presence of antioxidants, especially poly phenolic compounds and antioxidant vitamins, (Soong & Barlow, 2004). Several studies have described the antioxidant properties of medicinal plants rich in phenolic

compounds (Nijveldt et al., 2001; Tsao Rong & Deng Zeyuan, 2004). In addition to antioxidant activity many phenolic compounds have been shown to exert their anticancer or anticarcinogenic/antimutagenic activity to a greater or lesser extent. Reactive oxygen species (ROS) are implicated in a wide range of human diseases. Hence, the study of antioxidant substances in foods and medicinal natural sources has gained increased interest. Thus, it is important to increase the antioxidant intake in the diet and search for natural antioxidant sources among plants used as food additives. In this study antimicrobial and antioxidant activities of three medicinal plants were evaluated. These plants belonging to three different families were collected in Hamedan province of Iran. *Primula auriculata* from Primulaceae family is one of the most important local medicinal plants in Hamedan province (locally named Tootia). *Primula* is a rosette plant with shortly rhizomatous, 20–50 cm height and oblong-lanceolate leaves. Flowers are bright-purple or violet that blooms mostly during the spring. This plant's inflorescences produce white powders that are the results of physiological activities of plants locally named Tootia. In traditional medicine these powders have been used to prevent eye's diseases (eye's blindness and anti-infection, cataract and trachoma) (Najafi, Kalvandi, & Safikhani, 2004). To the best of our knowledge, similar studies have not been carried out on the *P. auriculata*.

The genus *Fumaria* L. comprises 60 species, most of which grow around the Mediterranean region. In total, eight *Fumaria* species

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have been reported from Iran. Characteristics such as sepal size, upper petal shape and dry fruit shape are important in distinguishing these taxa. *Fumaria vaillantii* (Fumariaceae) is an annual plant that grows in a wide variety of areas of Iran. It has been reported to be used traditionally in the folk medicine for treatment of hepatobiliary disorders, dermatological diseases, dysfunction and gastrointestinal disorders and as a blood purifier (Ebrahimzadeh, Kheshavarzi, Sheidaii, & Ghadam, 2011).

Falcaria vulgaris (locally named ghazzyaghi/poghazeh) from Apiaceae family is consumed as a vegetable in Hamedan province also for healing of skin ulcer, stomach disorders including peptic ulcer, liver diseases and stones of kidney and bladder. Khazaei and Salehi reported that hydroalcoholic extract of *F. vulgaris* decreased the gastric ulcer in Rat (2006). The main aim of the present research was to carry out a phytochemical evaluation and possible antimicrobial and antioxidant activities of selected plants especially on those that are endemic and is using in Iranian traditional medicine.

2. Materials and methods

2.1. Plant materials

The different parts of *P. auriculata*, *F. vaillantii* and *F. vulgaris* were collected from Hamedan province in the west of Iran and identified by the Botanic laboratory of Bu-Ali Sina University in May, 2008.

2.2. Extraction of plant material

Plant materials (*F. vulgaris*, leaf; *P. auriculata*, leaf & inflorescence and *F. vaillantii* inflorescence) were dried in the shade at room temperature and ground in a mortar. Fifty grams of each plant were extracted with three solvents; distilled water, methanol and methanol/water mixture (60/40, v/v) by maceration for 48 h. The extracts were filtered using Whatman filter paper (No. 1), which were centrifuged at 12000 rpm for 10 min. The supernatant was again filtered using Whatman filter paper No. 1 under strict aseptic conditions. The collected filtrates were concentrated in vacuum at temperature below 40 °C using a rotary evaporator (Buchi, Switzerland). The residue obtained was stored in freezer at –20 °C until further test.

2.3. Determination of antimicrobial activity

2.3.1. Bacterial strain

The antibacterial activity of the extracts was tested individually on Gram-positive and Gram-negative bacterial strains isolated from human pathogenic bacteria cases. All bacterial strains were obtained from the medical diagnostic laboratory, Sina hospital in Hamedan, Iran. The two Gram-positive bacterial strains used were *Staphylococcus aureus* (PTCC-1112), *Bacillus cereus* (PTCC-1247), and seven Gram-negative bacterial strains used were, *Escherichia coli* (PTCC-1338), *Klebsiella pneumonia* (PTCC-1053), *Shigella flexneri* (PTCC-1234), *Enterobacter aerogenes* (PTCC-1221), *Pseudomonas aeruginosa* (PTCC-1430), *Serratia marcescens* (PTCC-1111), *Proteus vulgaris* (PTCC-1079). All test bacterial strains were purified by streaking and re-isolating three successive times on Muller Hinton Agar (MHA). Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing Muller Hinton broth and incubated overnight at 37 °C.

2.3.2. Antibacterial susceptibility assay

The antimicrobial activities of extracts were determined by two methods including disc diffusion test and micro-dilution assay

(Kim, Marshall, & Wie, 1995). The inoculum size of each test strain was standardized at 5×10^5 CFU/ml using McFarland Nephelometer according to the National Committee for Clinical Laboratory Standards (NCCLS) (Okeke, Iroegbu, Eze, Okoli, & Esimone, 2001). Suspension containing of bacteria spread on Muller Hinton Agar (MAH) medium. The sample solution of the material to be tested was prepared by dissolving definite amount of material in appropriate solvent to attain different concentrations (3.75, 7.5, 15, 30, 60, 120, 240 and 480 mg/ml). Sterile disc of 6 mm diameter (Schleicher and Schuell) were impregnated with 20 µl of the different concentrations of extract solution. The paper discs were dried and placed on the surface of the inoculated agar plates. Plates were kept for 1 h in refrigerator to enable prediffusion of the extracts into the agar. Then the inoculated plates with food-borne bacteria (food associated) were incubated at 37 °C for overnight to allow bacterial growth.

Tetracycline and gentamicin were used as positive control. Whereas negative controls were performed with paper discs loaded with 20 µl of solvents (methanol and water) and dried. The anti bacterial activities of the extracts were evaluated by measuring the inhibition zones.

2.3.3. Determination of MIC and MBC

The MIC of the organic extracts was determined by broth micro-dilution method described by Alade and Irobi (1993) with a few modifications. The extract was duplicate twofold serial diluted to 7.81–1000 µg/ml preparations dispensed (1.0 ml) into test tubes containing 1.0 ml of Muller Hinton broth. A bacterial cell suspension (prepared in the appropriate broth) of 100 µl, corresponding to 1×10^5 CFU/ml, was inoculated into the test tubes. The tubes were mixed and incubated appropriately as previously described. The gentamicin and tetracycline were used as positive controls. Controls for bacterial growth without extracts were also included on each tube. After incubation, the MIC of each extracts was determined. The MIC was defined as the minimum concentration of the extract that did not allow any visible growth or turbidity of the organism in broth. For determination of minimum bactericidal concentration (MBC), 0.1 ml of culture medium was a spirited from each micro broth assay tube showing no apparent growth and sub-cultured in fresh MHA. After incubation at 37 °C for 24 h, the least concentration showing no visible growth on sub-culture was taken as the MBC.

2.3.4. Gas chromatography analysis

The chemical composition of leaf oil from *F. vulgaris* was determined by GC–Mass using Hewlett–Packard series II gas chromatography equipped with HP-5 capillary columns (30 mm × 0.25 mm, 0.25 µm film thicknesses). Injector temperature was 280 °C, which can be programmed from 50 to 280 °C with the rate of 1 °C/min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. The identification of the constituents was achieved by the comparison of their retention indices and mass spectra with data generated under identical experimental conditions. Identification of compounds was based on comparison of their mass spectra and retention indices (RIs) with those recorded in the Wiley and NIST mass spectral databases, and authentic samples (compound available in our laboratories), additional library data of the GC–MS system and literature data (Adams, 2001). The retention indices were determined in relation to homologous *n*-alkanes series (C8–C24) under the same operating conditions. Components relative concentrations were obtained by peak area normalization.

2.4. Phytochemical screening of three plants

The screening of chemical constituents was carried out with the methanol extracts by using chemical methods. The different parts

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