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Studies on the antioxidant and antimicrobial activity and flavonoid derivatives from the fruit of *Trigonosciadium brachytaenium* (Boiss.) Alava

Malihe Akhavan[a], Sajjad Jahangiri[b], Ali Shafaghat[c,*]

[a] Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran
[b] Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[c] Department of Chemistry, Khalkhal Branch, Islamic Azad University, Khalkhal, Iran

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ABSTRACT

The objective of this study was to evaluate the potential use of the fruits of *Trigonosciadium brachytaenium* in the pharmaceutical and food industries. Flavonoids are present in food and medicinal plants and are thus consumed by humans. They are often found in plants as glycosides. Their biological activities have an impact on human health so that they serve as target molecules to develop new drugs. From the methanolic extract of the fruit of *T. brachytaenium* (Boiss.) Alava. (Umbelliferae), two flavonoid derivatives namely 5-hydroxy-3′-methoxy-4′-ethoxyflavone-7–*O*-[(4‴-acetyl) rhamnosyl (1 → 2) rhamnoside)], a luteolin derivative (**1**) and 5-hydroxy-4′-methoxy-8-ethoxyflavone-7-*O*-[(2‴-acetyl) rhamnosyl (1 → 2) rhamnoside)], an apigenin derivative (**2**) have been isolated by column chromatography (CC) and preparative TLC (PTLC). Their structures were elucidated by UV, ^{1}H and ^{13}C NMR, HMBC, NOE, EI-MS and IR spectra. The antioxidant activity of methanol extract was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that the methanol extract from the fruits possess considerable antioxidant activity (IC_{50} = 47 μg/mL). This study reveals that the methanolic extract of this plant is attractive sources of flavonoids, especially the essential ones, as well as of effective natural antioxidants. The antimicrobial activity of the methanol extract of the fruit was determined against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The bioassay showed that the extract exhibited good antimicrobial activity. These flavonoid compounds were isolated for the first time from *T. brachytaenium*.

1. Introduction

Flavonoids are ubiquitous polyphenolic metabolites in plants that have diverse beneficial biochemical and antioxidant effects (Dajas et al., 2005). Their dietary intake is quite high, compared to other dietary antioxidants like vitamins C and E. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been principally reported to have antioxidant activities (Jovanovic et al., 1994). It is reported that phenolics may prevent lipid peroxidation via hydrogen atom donation from the hydroxyl group(s) attached to the benzene ring (Sawa et al., 1999). However, the connection between the structure of phenolics and their antioxidant activity is still being actively investigated. In recent years, there has been an increased interest in phenolic compounds derived from fruits and vegetables for their possible health benefits. The anticarcinogenic, antimutagenic, and cardioprotective effects of phenolic compounds are reported to be generally associated with their antioxidant properties of eliminating free radicals and alleviating lipid peroxidation (Potter, 2005). Our previous report on the methanolic extract of *Tanacetum parthenium* from North-West Iran showed that its flavonoids were flavonol, kaempferol, fisetin and naringenin (Shafaghat and Salimi, 2008). The genus *Trigonosciadium* is represented in Iranian flora by three species, among which *Trigonosciadium brachytaenium* is endemic (Mozaffarian, 2007). In the course of phytochemical studies of the North-West medicinal plants from Iran, in particular *Trigonosciadium* species, the Iranian *T. brachytaenium* (Umbelliferae) (GOLPARAK in Persian) was

* Corresponding author. Tel.: +98 04524251221.
E-mail address: shafaghata@yahoo.com (A. Shafaghat).

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investigated. No phytochemical studies on *T. brachytaenium* have been reported. To the best of our knowledge, this is the first report on the flavonoids from the fruit and the antioxidant and antibacterial activities of *T. brachytaenium* (Boiss.) Alava. from Iran.

2. Materials and methods

2.1. Plant material

The fruits of *T. brachytaenium* were collected in June 2011 from Khalkhal area (Ardabil province) in northwest of Iran at an altitude of 1950 m. A voucher specimen (No: 028) has been deposited at the Herbarium of the Agriculture Research Centre (A.R.C.) Ardabil, Iran.

2.2. Chemicals and methods

The IR spectra were determined on a Bruker Tensor 27 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer. Column chromatography was performed over silica gel (70–230 mesh, Merck,) using petroleum ether, AcOEt, methanol gradients as elution solvents. UV spectra were recorded on a Perkin-Elmer Lambda 12 spectrophotometer and The HREIMS was measured on a Bruker Micro TOF-QII instrument. Specific rotations were measured at room temperature in methanol on a Perkin Elmer TM, Model 341 Polarimeter with a 10 mm flow tube (Fig. 1).

2.3. Extraction and isolation

Dried and finely powdered *T. brachytaenium* fruits (300 g) were exhaustively macerated with MeOH to yield 41 g of crude extract after evaporation of the solvent *in vacuo*. The concentrated total extract was extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH, respectively. A part of the EtOAc portion (3 g) was subjected to silica gel column chromatography (70–230 mesh, Merck), eluted with an equivalent petroleum ether, EtOAc, methanol stepwise gradients to obtain 23 fractions (15 mL each). Fractions 6–11(90 mL) after solvent evaporation were in turn chromatographed over silica gel with $CHCl_3$:MeOH mixtures (100:0; 95:5; 90:10; 85:15; 80:20; . . .; 5:95; 0:100) to provide 12 subfractions. Subfraction 8 after solvent evaporation (145 mg) was rechromatographed on silica gel into 13 fractions (13× 15 mL.) using 8.5:1.5, $CHCl_3$:MeOH as the eluents. The combined fractions 5–10(22 mg) were further purified on a preparative TLC (solvent; $CHCl_3$:MeOH; 50:50) to give compound **1** (17 mg). A portion of the EtOAc (0.31 g of fractions 15–21(100 mL) after removal of solvent) was chromatographed over a small column (15 cm × 1.5 cm) with EtOAc:MeOH (8:2) as eluent. A total of 11 fractions were collected. The combined fractions 8–11(60 mL) (48 mg) according to TLC analysis were further purified by preparative TLC (solvent; $CHCl_3$:MeOH; 40:60) to give compound **2** (23 mg). The flavonoids were readily identified as 5-hydroxy-3′-methoxy-4′-ethoxyflavone-7-*O*-[(4‴-acetyl)rhamnosyl (1 → 2)rhamnoside)] (luteolin derivative), and 5-hydroxy-4′-methoxy-8-ethoxyflavone-7-*O*-[(2‴-acetyl)rhamnosyl (1 → 2)rhamnoside)] (apigenin derivative) by comparing their physical and spectroscopic data with those reported in the literature (Markham, 1982; Markham et al., 1978; Mabry et al., 1970; Reddy et al., 2003; Kiplimo et al., 2011; Hyun et al., 2010; Feng et al., 2007).

2.3.1. Compound (**1**)

5-Hydroxy-3′-methoxy-4′-ethoxyflavone-7-*O*-[(4‴-acetyl) rhamnosyl (1 → 2) rhamnoside)] yellow amorphous solid, mp 189–193 °C, $[\alpha]_D^{20}$ −23.1(c 0.45, MeOH).

IR: 3355 (O—H), 2928, 1672(C═O), 1609, 1496, 1439 cm^{-1}. UV λ_{max} (CH_3OH): 337, 292 and 269 nm. ^{1}H NMR and ^{13}C NMR (CD_3OD): see Table 1. HMBC: Fig. 2. NOE: Fig. 3. HRFABMS [M+H]$^+$: 663.1782; (calc. 663.1767). Molecular formula: $C_{32}H_{38}O_{15}$.

2.3.2. Compound (**2**)

5-Hydroxy-4′-methoxy-8-ethoxyflavone-7-*O*-[(2‴-acetyl) rhamnosyl (1 → 2) rhamnoside)]. Yellow amorphous solid, mp 185–188 °C. $[\alpha]_D^{20}$ −21.7(c 0.45, MeOH). IR: 3351 (O—H), 2928, 1685(C═O), 1612, 1486, 1435 cm^{-1}. UV λ_{max} (CH_3OH): 328, 294 and 269 nm. ^{1}H NMR and ^{13}C NMR (CD_3OD): see Table 1. HMBC: Fig. 2. NOE: Fig. 3. HRFABMS [M+H]$^+$: 663.1779; (calc. 663.1775). Molecular formula: $C_{32}H_{38}O_{15}$.

2.4. Antioxidant activity tests

The DPPH assay was carried out according to the modified method (Cheung et al., 2003). Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of extracts in different concentrations (0.1–1.6 mg/mL) and kept in the dark for 10 min. The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of ethanol. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as IC_{50} values (μg extract/mL) for comparison. IC_{50} value of each sample defined as the concentration of sample required for the 50% decrease in absorbance of the blank was calculated.

2.5. Antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the extract was evaluated by the disc diffusion method (DDM) using Mueller–Hinton agar for bacteria and Sabouraud Dextrose agar for fungi (Baron and Finegold, 1990). Discs containing 30 μL of the methanol extract were used and growth inhibition zones were measured after 24 h and 48 h of incubation at 37 °C and 24 °C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria and nystatin for fungi were used as positive controls. The microorganisms used were: *Bacillus subtilis* ATCC 9372, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 3583, *Pseudomonas aeruginosa* ATCC 27852, *Escherichia coli* ATCC 25922, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 5027 and *Saccharomyces cerevisiae* ATCC 9763.

3. Results and discussion

The results obtained in the NMR analyses of the methanol extract of *T. brachytaenium* fruits are listed in Table 1. The ^{1}H NMR spectrum of compound **1** displayed the characteristic signals of the luteolin nucleus. Compound **1** was obtained in the form of yellow amorphous solid, mp 189–193 °C, $[\alpha]_D^{20}$ −23.1(c 0.45, MeOH). The molecular formula was established as $C_{32}H_{38}O_{15}$ by HRFABMS [M+H]$^+$ at *m*/*z* 663.1782. The identification of the compounds was supported by comparison of the spectra data obtained of this work with published data of related compounds (luteolin and apigenin) (Fathy et al., 2002; Kang et al., 2000; Singh et al., 1999; Demole and Enggist, 1974). Its UV absorptions in methanol were at λ_{max} 337, 292 and 269 nm. Its IR absorptions showed the presence of a hydroxyl (3355 cm^{-1}), a conjugated carbonyl (1672), and aromatic rings (1609, 1496 and 1439 cm^{-1}). The combination of ^{1}H, ^{13}C NMR, HMBC and NOESY correlation spectral data of **1** indicated the presence of the methyl groups of the two sugar moieties at δ_H 3.41–3.76 (Table 1) and at [δ_H 0.98 (3H, d, *J* = 6.3 Hz, Me-6″) and (δ_H 1.10(3H, d, *J* = 6.2 Hz, Me-6‴)].

In this compound one methyl group was observed in the spectrum which was assigned to the Ar-O-methyl [δ_H 3.92 (3H, s)], with the corresponded δ_C 55.2 and an ethyl group which assignment to Ar-O-ethyl [δ_H 4.13 (2H, q, *J* = 7), δ_H 1.25 (3H, t, *J* = 7)], with the