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Online Publication Date: 01 January 2009

To cite this Article Gohari, Ahmad R., Saeidnia, Soodabeh, Gohari, Mahmood R., Moradi-Afrapoli, Fahimeh, Malmir, Maryam and Hadjiakhoondi, Abbass(2009)'Bioactive flavonoids from Satureja atropatana Bonge'.Natural Product Research,23:17,1609 — 1614

To link to this Article DOI: 10.1080/14786410902800707
URL: http://dx.doi.org/10.1080/14786410902800707

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Bioactive flavonoids from *Satureja atropatana* Bonge

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(Received 28 August 2008; final version received 3 December 2008)

From the ethyl acetate and methanol extract of the aerial parts of *Satureja atropatana* Bonge, which belongs to the Lamiaceae family, four flavonoids were isolated. Their structures were determined to be 5,6,30-trihydroxy-7,8,40-trimethoxyflavone (1), 5,6-dihydroxy-7,8,30,40-tetramethoxyflavone or 5-desmethoxynobiletin (2), 5,6,40-trihydroxy-7,8,30-trimethoxyflavone or thymonin (3) and luteolin (4) using 1H and 13C-NMR and MS spectra. Brine shrimp cytotoxicity effects of the crude extracts and isolated compounds were examined. Berberine hydrochloride (LC50 = 26 µg mL−1) was used as a positive control. Among them, compounds 2 (199 µg mL−1) and 3 (157 µg mL−1) were effective against *Artemia salina* larva.

**Keywords:** Lamiaceae; *Satureja atropatana*; thymonin; cytotoxicity; *Artemia salina*

1. Introduction

*Satureja* genus, which is generally called ‘Marzeh’ in Persian, belongs to the Lamiaceae family and comprises 13 species in Iran. Among them, *Satureja atropatana* and *S. khuzistanica* grow exclusively in Iran (Mozaffarian, 1996; Rechinger, 1982). Previous phytochemical investigations revealed the presence of phenolic acids (Pedersen, 2000; Zgorka & Glowniak, 2001), flavones (Ferreres, Barberan, & Tomas, 1987), anthocyanins (Saito & Harborne, 1992), sterols (Escudero, Lopez, Rabanal, & Valverde, 1985), diterpenes (Alvarenga, Gastmans, & Rodrigues, 2001; Labbe, Castillo, Fainia, Coll, & Connolly, 1994) and triterpenes (Connolly, & Hill, 1991; Gohari, Hadjiakhoondi, Sadat-Ebrahimi, Saeidnia, & Shafiee, 2005) in *Satureja* plants. *Satureja* species have been used in traditional medicine as antimicrobial, spasmylytic, cicatrizant and diuretic agents since antiquity (Gohari, Hadjiakhoondi, Shafiee, Sadat-Ebrahimi, & Mozaffarian, 2005).

A review of the literature shows that there is no report on the phytochemical investigation of *S. atropatana* Bonge. Recently, we reported the chemical composition of the essential oil of this plant, which contained 32 different compounds (Gohari et al., 2005). The main components of the oil of *S. atropatana* were thymol (62.1%), p-cymene...
(6.1%), spathulenol (5.2%) and \(\gamma\)-terpinene (3.3%). Here, we report the separation and identification of some flavonoids from the aerial parts of \textit{S. atropatana} which has not been previously reported, together with the cytotoxic evaluation of the main constituents against \textit{Artemia salina} larvae.

2. Results and discussion

Isolated compounds (Figure 1) from the ethyl acetate and MeOH extracts of \textit{S. atropatana} were identified as 5,6,3\textsuperscript{\prime}-trihydroxy-7,8,4\textsuperscript{\prime}-trimethoxyflavone (1), 5,6-dihydroxy-7,8,3\textsuperscript{\prime},4\textsuperscript{\prime}-tetramethoxyflavone or 5-desmethoxynobiletin (2), 5,6,4\textsuperscript{\prime}-trihydroxy-7,8,3\textsuperscript{\prime}-trimethoxyflavone or thymonin (3) and luteolin (4) by comparison of their NMR and MS spectral data with those reported in the literature (Agrawal, 1989; Artemios, Chrisi, & Ioannis, 1998; Gohari et al., 2003; Nguyen-Hai et al., 2004; Saeidnia, Yassa, Gohari, & Shafiee, 2005; Yamamura, Ozawa, Ohtani, Kasai, & Yamasaki, 1998). Table 1 shows the results of \(^{13}\)C-NMR for the isolated compounds 1–3. The \textit{Satureja} genus (e.g. \textit{S. obovata}, \textit{S. douglassii} and \textit{S. acinos}) is enriched with flavonoid methyl ethers such as chrisoeriol, diosmetin, genkwanin, cirsimartitin and xanthomicrol (Escudero, Lopes, Rabanal, & Valverde, 1985; Ferreres, Barberan, & Tomas, 1987; Sanchez de Rojas, Somoza, Ortega, & Villar, 1996). Compound 3, identified as thymonin, has been previously reported from \textit{S. obovata}, \textit{S. salzmannii} and \textit{S. spinosa} (Skoula, Grayer, & Kite, 2005). Although highly methoxylated flavonoids have been isolated from \textit{Satureja} species (Skoula et al., 2005), 5-desmethoxynobiletin (2) has not been previously reported from \textit{Satureja} plants and is found only in \textit{S. atropatana}. Brine shrimp cytotoxicity assay (BSA) of the main components of \textit{S. atropatana} is shown in Table 2. Results indicate that compound 3 (thymonin) is the most active (LC\textsubscript{50} = 157 \(\mu\)g mL\textsuperscript{-1}) flavon aglycon in this plant. Desmethoxynobiletin (2), which has been found only in \textit{S. atropatana}, is significantly effective (LC\textsubscript{50} = 199 \(\mu\)g mL\textsuperscript{-1}) against the larvae of \textit{Artemia salina}. Recently, the cytotoxic

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\text{Figure 1. The structures of isolated compounds from Satureja atropatana.}
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1 \(R_1=\text{OH}, R_2=\text{OCH}_3\)
2 \(R_1=\text{OCH}_3, R_2=\text{OCH}_3\)
3 \(R_1=\text{OCH}_3, R_2=\text{OH}\)
activity of the luteolin 7-O-glucoside was evaluated by using the BSA method, and
significant cytotoxicity (LC$_{50}$ = 85 µg mL$^{-1}$) was reported (Taskova, Mitova, Mikhova, &
Duddeck, 2003). It seems that methoxylation of the flavonoids can reduce the cytotoxic
activity against *A. salina*.

In conclusion, the *Satureja* genus is a main source of cytotoxic components. Some of
them belong to triterpenoids like ursolic and oleanolic acids (Gohari et al., 2005) and
others are flavonoid constituents such as thymonin and luteolin.

### 3. Experimental

#### 3.1. Plant material

Aerial parts of *S. atropatana* Bonge, at flowering stage, were collected from the
East-Azerbayjan province in the north-west of Iran in September 2004. A voucher

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<th>Table 1. $^{13}$C-NMR of the flavonoid components isolated from <em>Satureja atropatana</em>.</th>
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Note: $^{a}$Measured in CDCl$_3$; $^{b}$in DMSO-$d_6$.

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<th>Table 2. Results of the BSA of the main flavonoids isolated from <em>S. atropatana</em>.</th>
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specimen (79150 SBU) was deposited at the Herbarium of the Institute of Forests and Rangelands Research. The plant specimen was identified by Dr Vali-Allah Mozaffarian from the same institute.

3.2. Instruments and materials

$^1$H and $^{13}$C-NMR spectra were measured on a Brucker Avance TM 500 DRX (500 MHz for $^1$H and 125 MHz for $^{13}$C) spectrometer with tetramethylsilane as an internal standard, and chemical shifts were given in $\delta$ (ppm). MS data were recorded on an Agilent Technology (HP TM) instrument with a 5973 Network Mass Selective Detector (MS model). Silica gel 60F$_{254}$ pre-coated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde-H$_2$SO$_4$ reagent, followed by heating.

3.3. Isolation process

The flowered aerial parts of $S$. atropatana (1 kg) was cut into small pieces and then extracted with ethyl acetate and methanol at room temperature. The ethyl acetate extract (23 g) was subjected to silica gel column chromatography (CC) with hexane : CHCl$_3$ (8 : 2), CHCl$_3$ : AcOEt (2 : 8) and AcOEt as eluent to give six fractions (A–F). The fraction D (430 mg) was submitted to silica gel CC with CHCl$_3$ : AcOEt (6 : 4) to obtain five fractions D$_1$–D$_5$. The fraction D$_2$ (31 mg) was chromatographed on sephadex LH$_{20}$ with AcOEt : MeOH (3 : 1) to result in compound 1 (4 mg).

The MeOH extract (70 g) was successively subjected to silica gel column chromatography with hexane : AcOEt (8 : 2, 0 : 1) and MeOH as eluent to give nine fractions (M$_1$–M$_9$). Fraction M$_3$ (690 mg) was fractionated on sephadex LH$_{20}$ with MeOH to yield three parts (M$_{31}$–M$_{33}$). The fraction M$_{32}$ (61 mg) was chromatographed on sephadex LH$_{20}$ with AcOEt : MeOH (1 : 2) to result in M$_{321}$–M$_{324}$. Fraction M$_{322}$ was purified on sephadex LH$_{20}$ with MeOH to yield compound 2 (6 mg). Compound 3 (15 mg) and 4 (7 mg) were yielded from fraction M$_{324}$ using sephadex CC with MeOH.

3.4. Brine shrimp lethality assay

The method described by Mongelli, Martino, Coussio, & Ciccia (1996) was adopted to study the cytotoxic activity of the compounds. Water life brand brine shrimp ($Artemia salina$) eggs were purchased from the Shilat Center (Tehran). The eggs were hatched in a flask containing 300 mL artificial seawater made by dissolving distilled water. The flask was well aerated with the aid of an air pump, and kept in a water bath at 29–30°C. A bright light was left on. The nauplii hatched within 48 h. The extracts and pure compounds were dissolved in normal saline. Different concentrations were obtained by serial dilution. The solution of each concentration (500 µL) was transferred into clean 24 well plates via a pipette, and aerated seawater having 10–20 nauplii (500 µL) was added. A check count was performed, and the number of alive nauplii noted after 24 h. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 s of observation. The controls used were seawater and berberine hydrochloride ($LC_{50} = 26 \mu g mL^{-1}$). The lethality percentage was determined and the $LC_{50}$ calculated based on Probit Analysis with 95% confidence interval (Gohari et al., 2005).
Acknowledgement
This research has been supported by a Tehran University of Medical Sciences and Health Services grant (No. 4761).

References
