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Isolation and Identification of Cinnamic Acid Derivatives from the Aerial Parts of *Seidlitzia Rosmarinus* Ehrenb. Ex Boiss

Behzad Zolfaghari[a], Moloud Mazaheri[b], Masoud Sadeghi Dinani[a*]

[a]Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences,Isfahan, Iran.
[b]Faculty of Pharmacy, Isfahan University of Medical Sciences,Isfahan, Iran.

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Seidlitzia rosmarinus Ehrenb .ex Boiss. is a woody plant belongs to the Chenopodiaceae family with a vast geographical distribution in Asian countries. As a famous plant in Iran, it is called "Oshnan" in Persian and has been used as a natural soap for many centuries. Despite of vast spread and importance, *S. rosmarinus* has not been studied phytochemicaly, and this study was conducted to identify the main constituents of the aerial parts of the plant. Phytochemical investigation of *S. rosmarinus* resulted to the isolation of two cinnamic acid derivatives and a benzaldehyde derivative as the main phenolic compounds of aerial parts of the plant. Using comprehensive spectroscopic methods, including 1D and 2D NMR and MS, chemical structure of the isolated compounds were determined as N-*cis*-feruloyltyramine (**1**), N-*cis*-caffeoyltyramine (**2**) and *p*-hydroxyacetophenone (Piceol) (**3**), respectively. To the best of our knowledge, isolation and identification of these compounds from *S. rosmarinus* are reported for the first time in this study.

*Corresponding Author: **Masoud Sadeghi Dinani, E-mail:** *m_sadeghi@pharm.mui.ac.ir*
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Introduction

Chenopodiaceae is a widespread plant family, comprised of 102 genus and 1400 plant species that mostly can find in salty soil [1]. Most plants in this family have high salt-tolerance and graded as halophyte plants [2, 3].

Seidlitziarosmarinus which is called "Oshnan" in Persian belongs to the Chenopodiaceae family, widely found in the Middle East and Central Asia [4], and is one of the famous plants in the central parts of Iran. It is a perennial woody plant grown in dry and salty deserts, mostly along the banks of salt marshes and in soils with high saline water absorption. The plant has an important role in soil preservation and maintenance and the leaves, stems and seeds of the plant are used as a fodder for livestock [2].

Beyond to its industrial applications in dyeing, making soaps and pottery applications [2], traditionally, dried leaves of the plant are grounded and used for washing cloths and dishes due to their detergent like and foam making properties. There are also some reports about the medicinal properties of *S. rosmarinus*, especially in the treatment of dermatologic diseases. It has been shown that the ash remained after burning the leaves and stems of the plant has antiseptic and antibacterial efficacy and it has been used for the treatment of some kinds of acnes and also to cure Leishmaniasis [5]. *S. rosmarinus* has also been tried as an herbal medicine for the treatment and reduction of clinical symptoms in BPH [6].

In spite of its importance and specific medicinal uses, there is not any comprehensive phytochemical study about the *S. rosmarinus*, and the main secondary metabolites of the plant have not been identified properly. So as a part of our research program on phytochemical investigation of important medicinal plant species, especially saponin and flavonoid rich plants, phytochemical investigation of S. *rosmarinus* has been conducted and this paper reports the isolation and identification of some of the main phenolic compounds, especially cinnamic acid derivatives, from the chloroform-methanolic extract of the plant.

Materials and methods

General experimental procedures

Medium pressure liquid chromatography (MPLC) was performed by a Buchi Gradient System C-605 apparatus using glass columns of LiChroprep® RP-18 (25-40μm) and C-660 Buchi fraction collector. TLC performed on SiO_2 plates with $BuOH:H_2O:CH_3COOH$ (60:25:15 v/v/v) (BAW) as a mobile phase and cerium sulfate in 2N H_2SO_4 and natural product (NP) as reagents for visualizing the spots. HPLC was performed by Waters 515 apparatus equipped with a refractive index detector (Waters 2414) and UV detector (Waters 2487), using semipreparative C18 column (Novapak® 7.8 x 300 mm) and analytical C18 column (Novapak® 3.9 x 300 mm) in isocratic mode.

H and C NMR spectra recorded by Bruker 400MHz (H at 400 MHz and C at 100 MHz) spectrometer, using solvent signal for calibration (CD_3OD: δ_H=3.31, δ_C=49.0). Distortionless enhancement by polarization transfer (DEPT) experiments was used to determine the multiplicities of C NMR resonances and 2D heteronuclear multiple bond correlation (HMBC), optimized for $2–3J_{CH}$ of 8 Hz, was used for determination of two and three bond heteronuclear $^1H–^{13}C$ connectivities. ESIMS spectra were prepared by Shimadzu LCMS 2010 EV, using methanol as the solvent.

Plant material

The aerial parts of *S. rosmarinus* were collected from Yazd province on June 2013. The plant was identified by Mrs Khatamsaz and a voucher specimen (No.3532) deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences.

Extraction and Isolation

The air-dried aerial parts of S. *rosmarinus* were finely powdered by means of a mill and the powder (2000g) was extracted at room temperature in a four step extraction method with increasing solvent polarity using the solvents; hexane, chloroform, chloroform-methanol (9:1) and methanol. Extraction was done using

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maceration method, performing each step four times with 6 L of solvent under occasional stirring. The chloroform-methanol (9:1) extract of the sample was concentrated under vacuum, yielding a crude dried extract (15 g) which was fractionated by MPLC on a RP-18 column (36x460 mm) using a linear gradient solvent system of H_2O to CH_3OH. Fractions were analyzed by TLC (SiO_2, BAW, reagents: cerium sulfate in 2N H_2SO_4 and natural product (NP)) and similar fractions were mixed together. Final fractions were subjected to HNMR spectroscopy and based on TLC and preliminary NMR analysis; three fractions (CM-8, CM-11 and CM-14) were considered to be rich in phenolic compounds and selected for purification of the constituents by HPLC.

The first fraction (CM-8) was subjected to purification by HPLC using a semi preparative C18 column (Novapak® 7.8*300 mm) and H_2O– CH_3OH (60:40) mobile phase in isocratic mode, resulted the compound (**1**) (53 mg, tR = 18 min).

The second fraction (CM-11) was purified by HPLC using a semi preparative C18 column and H_2O– CH_3OH (60:40) isocratic mobile phase, resulted the compound (**2**) (80 mg, tR = 16 min) while the purification of third fraction (CM-14) performed by same HPLC column and H_2O–CH_3OH (65:35) isocratic mobile phase, yielded the pure compound (**3**) (53 mg, tR = 9 min).

Results

Based on TLC and preliminary NMR screening, three fractions of the chloroform-methanol extract of the plant showed signals typical of phenolic compounds which were selected for further purification, resulted the isolation and identification of 2 cinnamic acid derivatives (**1** and **2**) and a benzaldehyde derivative (**3**). The chemical structure of isolated compounds was determined using spectroscopic methods and also by comparison of the NMR spectral data with those reported in the literature.

Characterization of compounds (1) and (2)

Compound (**1**) showed a pseudomolecular ion peak at m/z 312 [M-H] in the negative-ion ESIMS, indicated the presence of a nitrogen atom and suggested the molecular formula as $C_{18}H_{19}NO_4$.

In agreement with the molecular formula 18 carbon signals were observed in ^{13}CNMR spectrum that by using the information from DEPT experiment were determined as 7 aromatic methines, 2 aliphatic methines, 2 methylenes, 1 methoxyl and 6 quaternary sp2 carbon signals (Table 1). Four of the quaternary carbons had downfield chemical shifts in ^{13}CNMR spectra (δ_C 170.3, 148.6, 149.4, 156.9) which were concluded to be oxygenated.

Analysis of ^{1}HNMR spectra and correlating its information with that of ^{13}CNMR spectra, resulted to the identification of two aromatic rings, involving one *p*- substituted (2H doublets; δ_H 6.71 and 7.01, j=8.5 Hz) and one *m*- and *p*-substituted (1H: δ_H 6.76, j=8.5; δ_H 6.94, j=8.5, 2 and δ_H 7.38) ring (Table 1). Two sp^2 methines with the downfield chemical shifts of proton and carbon signals (1H doublets; δ_H 5.83 and 6.64, j=12.8) indicated the existence of a *cis* di-substituted double bond conjugated with one of the aromatic rings in the structure. The presence of the methoxy group and its position of attachment to the C6 in the structure was concluded respectively from the existence of the methoxy signal at ^{1}H and ^{13}CNMR spectra (3H singlet; δ_H 3.90, δ_C 56.4) and long range correlations were observed in HMBC experiment (Table 1). Finally, determining the heteroneuclear^{1}H–^{13}C connectivities by HSQC and HMBC experiment, the assignments were confirmed and the chemical structure of (**1**) defined as N-*cis*-feruloyltyramine (Fig.1).

Due to the great similarity of compound (**2**) spectra to that of compound (**1**), its chemical structure was elucidated based on the structure of compound (**1**). Compound (**2**) had a pseudomolecular ion peak of m/z 298 [M-H] in the ESIMS spectrum, which together with the ^{13}CNMR data (Table 1) suggested the molecular formula as $C_{17}H_{17}NO_4$. ^{1}H and ^{13}CNMR spectra were almost completely coincident to those of (**1**), except for the absence of a singlet (δ_H 3.90) at ^{1}HNMR spectrum, indicated that the structure of

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(**2**) has no methoxy groups in comparison to (**1**). More spectroscopic analyses indicated that C6 in the structure of (**2**) was substituted by a hydroxy group instead of methoxy compared to that of (**1**) (Table 1). Regarding to these data, the chemical structure of compound (**2**) was defined as N-*cis*-caffeoyltyramine (Fig.1)

Characterization of compound (3)

ESIMS spectra of compound (**3**) in the negative-ion mode showed a pseudomolecular ion peak at m/z 135 [M-H]. HNMR spectrum of (**3**) revealed the characteristic signals of aromatic protons including two doublets at δ_H 6.86 (2H, d, J= 8.8) and δ_H 7.90 (2H, d, J= 8.8) as well as a singlet at δ_H 2.54 (3H, s) indicative of protons of an acetyl group.

CNMR spectrum showed 6 signals, 2 double height sp2 aromatic methine carbon signals (δ_C 116.27 and 132.15), 3 quaternary sp2 carbon signals (δ_C 128.30, 163.95 and 199.80) and an sp3 carbon signal (δ_C 26.42) which in agreement with MS and HNMR spectral data confirmed the molecular formula as $C_8H_8O_2$. Analyzing the downfield chemical shift of two of the quaternary carbons exhibited that one of them had to be connected to an oxygen atom and the other must be a carbonyl group. All these data confirmed that the compound (**3**) should be a benzaldehyde derivative and its chemical structure ~~of it~~ was defined as *p*-hydroxyacetophenone (Piceol) (Fig. 1).

Table 1.^{1}H and ^{13}CNMR data of compound (1) and (2) (400 MHz, 100 MHz, CD_3OD).

Position	Compound (1)		Compound (2)		HMBC (C)
	δ_C (mult)	δ_H (int ,mult, J)	δ_C (mult)	δ_H (int ,mult, J)	
1	170.3	-	168.9	-	-
2	121.7	5.83 (1H,d,J=12.8)	120.9	5.83 (1H,d,J=12.8)	1,3,4
3	138.4	6.64 (1H,d,J=12.8)	138.3	6.64 (1H,d,J=12.8)	1,2,9
4	128.5	-	128.0	-	-
5	113.9	7.38 (1H, d, J=2)	114.0	7.33 (1H, d, J=2)	3,7,9
6	148.6	-	147.8	-	-
7	149.4	-	147.9	-	-
8	115.9	6.76 (1H, d, J=8.5)	116.0	6.77 (1H, d, J=8.5)	4,6
9	124.8	6.94 (1H, dd, J=8.5, 2)	124.7	6.83 (1H, dd, J=8.5, 2)	3,5,7
10	42.4	3.40 (2H, t ,J= 7.5)	42.4	3.42 (2H, t ,J= 7.5)	1,11,12
11	35.6	2.71 (2H, t ,J= 7.5)	35.8	2.72 (2H, t ,J= 7.5)	10,13,17
12	130.8	-	130.7	-	-
13-17	130.7	7.01 (2H, d ,J= 8.5)	131.0	7.01 (2H, d ,J= 8.5)	11,15
14-16	116.3	6.71 (2H, d ,J= 8.5)	116.2	6.71 (2H, d ,J= 8.5)	12
15	156.9	-	156.7	-	-
18	56.4	3.90 (3H, S)	-	-	6

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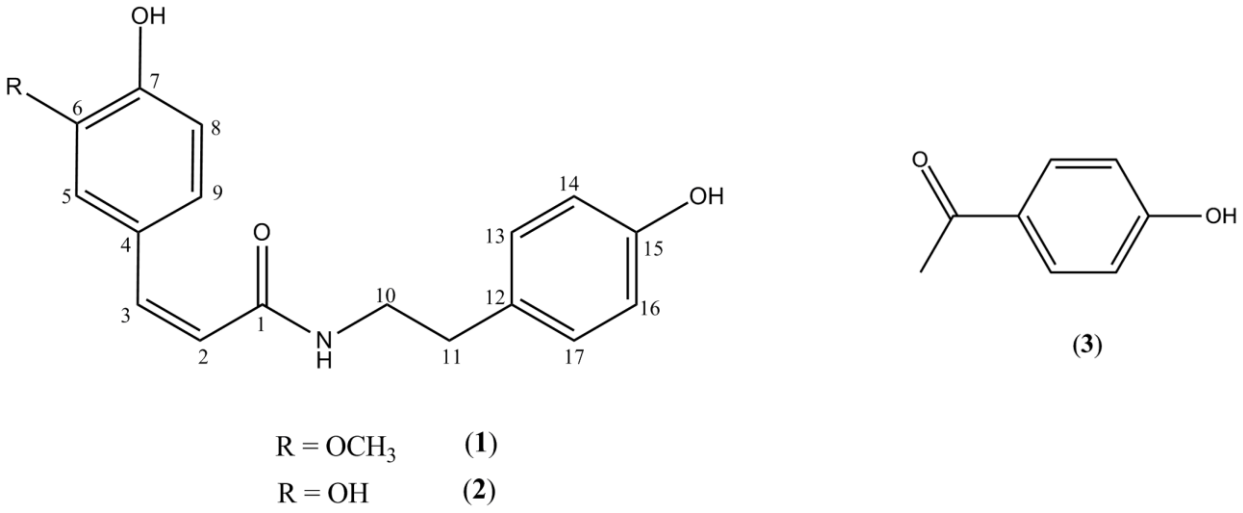

Fig. 1. Chemical structure of cinnamic acid and benzaldeyde derivatives isolated from the aerial parts of *S. rosamrinus*

Discussion

S. rosmarinus is a well-known woody plant that belongs to Chenopodiaceae family. It has many traditional and biological applications [1,2]. Phytochemical investigation of the aerial parts of the plant with a more focus on the isolation and identification of its pohenolic constituents was conducted in this study which led to the identification of cinnamic acid and benzaldehyde derivatives.

Cinnamic acid derivatives have been isolated from different plant species and shown to have important biological and pharmacological activities. This study reports the isolation of N-*cis*-feruloyltyramine (**1**), N-*cis*-caffeoyltyramine (**2**), two cinnamic acid derivatives as well as *p*-hydroxyacetophenone (Piceol) (**3**), from the aerial parts of *S. rosmarinus* for the first time.

Cinnamic acid derivatives are natural substances found especially in fruits, vegetables and flowers and form a part of our dietary phenolic intake. They have been also observed to be engaged in the formation of some commercially important intermediates which are used in the production of some important ingredients especially in pharmaceutical, cosmetic and perfumery industries [7] and have been reported to exhibit many interesting biological and pharmacological activities. These compounds especially those with the phenolic hydroxyl groups, have significant antioxidant [8]. Antimicrobial [9, 10], antibacterial [9], antiviral [11] and antifungal [12,13] activities and supposed to have several health benefits due to their strong free radical scavenging properties [8,14].

Cinnamic acid derivatives have been isolated from numerous plant species and shown to be of wide distribution in the plant families and species. According to the different biological and pharmacological activities have been demonstrated for cinnamic acid derivatives, it seems that these compounds are responsible for at least some of the medicinal benefits attributed to S. *rosmarinus* specially those related to antimicrobial, antioxidant and wound healing properties.

Cytotoxic and anticancer effects are other important pharmacological activities which have been reported for different natural or synthetic cinnamic acid derivatives. After the first clinical use in 1905, many attentions were attracted towards the Cinnamic acid derivatives and their potential anticancer effects [15,16]. Molecular analysis showed that the anti-tumor activity of these compounds may be some parts due to their capability to inhibit protein isoprenylation which is related to inhibition of mitogenic signal

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transduction [17]. Isolation of these compounds from *S. rosmarinus* supposed to be a good reason to candidate the plant and its compounds for evaluation of their cytotoxic effects.

Piceol (*p*-hydroxyacetophenone) is another penolic compound isolated and identified in this study from *S. rosmarinus*. Acetophenones are benzaldehyde derivatives and these compounds have also been reported to possess some important biological activities such as anti-microbial, cytotoxic, anticancer, analgesic, anti-inflammatory, diuretic and anticonvulsant effects [18-22]. It has shown also that mannich base derivatives of these compounds could exhibit anti-fungal activities against a panel of phytopathogenic fungi [19].

Piceol (4-hydroxy acetophenone) and its glycosilated form, Picein, are phenolic compounds were isolated firstly from the needles of Norway spruce (*Piceaabies*) and considered to be the indicators of plant stress and new growth substances bearing inhibitory activity [23,24]. However, pharmacological activities of piceol and its derivatives have been studied in many researches among them the hepatoprotective and choleretic activity and anti-hepatitis B virus (HBV) effects [25,26] seems to be the most important medicinal properties of Piceol that can be the subject of more studies. Piceol isolated from *Artemisia scoparia* (Compositae) is the main hepatoprotective and choleretic constituent of the plant which has been shown to be capable of increasing the amount of bile secretions with lesser toxicity on liver-bile system than common choleretic chemical medicines [25]. This compound was also revealed to have antiviral activity against the hepatitis B virus (HBV) [26]. Identification of piceol from the aerial parts of *S. rosmarinus* could be used for explanation of some of the biological and medicinal activities reported for the plant and candidate it for the evaluation of different pharmacological effects.

Conclusion

S. rosmarinus ("Oshnan" in Persian) was investigated phytochemically and two cinnamic acid derivatives; N-*cis*-feruloyltyramine, N-*cis*-caffeoyltyramine, together with *p*-hydroxyacetophenone (Piceol) were isolated from aerial parts of the plant. Identification of these compounds may provide some chemical basis for the explanation of pharmacological and biological activities attributed to the plant.

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Conflict of interest

Authors certify that there is no conflict of interest in relation to this article.

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