

Isolation of Cinnamic Acid Derivatives from the Bulbs of *Allium tripedale*

Abstract

Background: *Allium* genus with 750 species is the most diverse genus in the Amaryllidaceae family. Historically, *Allium* species have been used as medicinal plants, especially for prevention and treatment of cardiovascular diseases and considered as valuable sources of phytonutrients. Phytochemical investigation of *Allium tripedale*, locally called “Anashq,” which is an edible plant of the “Zagros” region (west of Iran) was conducted in the present study. **Materials and Methods:** Air-dried bulbs of the plant were extracted in a four-step extraction method with increasing polarity using hexane, chloroform, chloroform–methanol (9:1), and methanol. Chloroform-methanol (9:1) extract was fractionated by medium-pressure liquid chromatography on a RP-18 column using a linear gradient solvent system of H₂O to MeOH. Phenolic-rich fractions were subjected to the final isolation and purification of the constituents by reversed-phase high-performance liquid chromatography method. Structure elucidation of the compounds was performed through comprehensive methods including 1D- and 2D-NMR and mass spectroscopy. **Results:** Two cinnamic acid derivatives were isolated from the bulbs of *A. tripedale*; using spectroscopic methods, their chemical structures were determined as 6,7-dimethoxy N-trans-caffeoyltyramine (1) and N-trans-feruloyltyramine (2). **Conclusion:** Cinnamic acid derivatives are pharmacologically active phenolic compounds, which have been isolated from different *Allium* species. Isolation of these compounds from *A. tripedale* is reported for the first time in this study and could be used as a chemical basis for explanation of the plant biological and pharmacological activities.

Keywords: *Allium tripedale*, *Anashq*, cinnamic acid derivatives, phytochemistry, structure elucidation

Introduction

Allium species are very important edible plants, and they have always been considered as edible vegetables and medicinal plants all over the world.^[1,2] Historically, these plants have been mainly used for the prevention and treatment of cardiovascular disease, hypertension, and diabetes.^[3-5]

Allium genus with more than 750 species is the most diverse plant genus in the Amaryllidaceae family and spreads around the globe although the place of origin is supposed to be the central Asia where they were used from about 3000 BC. Iran accounts as one of the main resources of *Allium* plants with the most distribution of this genus in the west, Zagros Mountains, and also northeast of the country.^[3,6,7]

Alliums are also main sources of phytonutrients. These plants are valuable sources of important secondary plant metabolites including steroidal saponins and

sapogenins, flavonoids, and organosulfur compounds,^[1,2] and numerous studies have been conducted on the isolation and identification of these compounds from different *Allium* species in recent years. Cinnamic acid derivatives are valuable phenolic compounds isolated from different *Alliums* and have been demonstrated to bear several biological activities including antifungal,^[8] antioxidant,^[9] anti-inflammatory,^[10] and anticancer^[11] effects.

Allium tripedale is an edible plant grows wildy in the mountainous areas of Iran, especially west and northwest regions.^[12] This plant is locally called “Anashq” in the west of Iran and grows wildy in Zagros mountainous regions, and its leaves which have a very strong odor and taste are used widely as an edible vegetable and to make dishes. *A. tripedale* is also considered as a medicinal plant and it has been traditionally used for the treatment of various infectious conditions; some of its

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Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_34_17

Quick Response Code:



How to cite this article: Chehri Z, Zolfaghari B, Sadeghi Dinani M. Isolation of Cinnamic Acid Derivatives from the Bulbs of *Allium tripedale*. *Adv Biomed Res* 2018;7:60.

Received: February, 2017. **Accepted:** September, 2017.

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pharmacological effects including antifungal, antidiabetic, and hepatoprotective activity have been recently demonstrated.^[13,14]

As a part of our research program on phytochemical investigation of different *Allium* species, identification of main phenolic constituents of the plant was conducted and this paper reports the isolation and identification of two main cinnamic acid derivatives from the bulbs of *A. tripedale* for the first time.

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 (25–40 µm) and C-660 Buchi fraction collector. Thin-layer chromatography (TLC) performed on SiO₂ plates with n-BuOH: CH₃COOH: H₂O (60:15:25 v/v/v) (BAW) as a mobile phase and cerium sulfate in 2N H₂SO₄ and natural product (NP) as reagents for visualizing the spots.

¹H and ¹³C NMR spectra recorded by Bruker 400 MHz (H at 400 MHz and C at 100 MHz) spectrometer, using solvent signal for calibration (CD₃OD: δ_H = 3.31, δ_C = 49.0). Distortionless enhancement by polarization transfer (DEPT) experiments was used to determine the multiplicities of ¹³C NMR resonances.

2D heteronuclear multiple bond correlation (HMBC), optimized for^[2-3] J_{CH} of 8 Hz, was used for determination of two- and three-bond heteronuclear ¹H–¹³C connectivities while 2D heteronuclear single-quantum coherence (HSQC), interpulse delay set for ¹J_{CH} of 130 Hz, and COSY were used for determination of one-bond heteronuclear ¹H–¹³C connectivities and homonuclear ¹H–¹H connectivities, respectively. ESIMS spectra were prepared by Shimadzu LCMS 2010 EV, using methanol as the solvent.

Plant material

The whole plant of *A. tripedale* was collected from Khorramabad, Lorestan province, on April 2014. The plant was identified by botanist and a voucher specimen (No. 3579) deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences, Iran.

Extraction and isolation

Air-dried bulbs of *A. tripedale* were finely powdered by means of a mill, and the powder (1650 g) 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 maceration method, performing each step four times with 5 L of solvent.

The chloroform-methanol (9:1) extract was concentrated by rotary evaporator, yielded a dried extract (12 g)

which was then fractionated by MPLC on a RP-18 column (36*460 mm) using a linear gradient solvent system of H₂O: MeOH (90:10) to MeOH (100%). Fractions were analyzed by TLC (SiO₂, BAW 60:15:25 v/v/v) and visualized by 1% cerium sulfate in 2N H₂SO₄ or NP reagents, and similar fractions were mixed together.

Based on TLC and preliminary NMR analysis, fraction 8 was rich in phenolic compounds, which subjected again to MPLC with same conditions and resulted in two impure white compounds. Final purification by recrystallization yielded the pure compounds 1 (ATCM-8-4; 7 mg) and 2 (ATCM-8-5; 20 mg), respectively.

Results

Final purification of two main phenolic-rich fractions resulted in the isolation of two pure phenolic compounds, finally identified as cinnamic acid derivatives.

Identification of compounds

Compound (1) showed a pseudo molecular ion peak at m/z 327.15 [M-H] in the negative-ion ESIMS, indicated the presence of a nitrogen atom in the chemical structure.

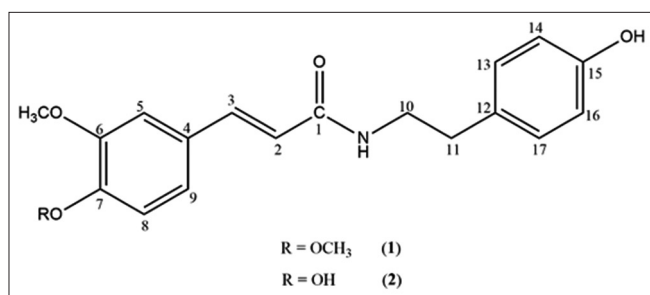
Nineteen carbon signals were observed in ¹³CNMR spectrum that using the information from DEPT experiment, were determined as 7 aromatic methines, 2 aliphatic methines, 2 methylenes, 2 methoxys, and 6 quaternary sp² carbon signals [Table 1], suggested that the molecular formula is C₁₉H₂₁NO₄. Four of the quaternary carbons had downfield chemical shifts in ¹³CNMR spectra (δ_C 169.22, 149.80, 149.30, 156.90) which were concluded to be oxygenated.

Analysis of ¹HNMR spectra and correlating its information with that of ¹³CNMR spectra resulted to the identification of two aromatic rings, involving one *p*-substituted (2H doublets; δ_H 6.76 and 6.99, *j* = 8.5 Hz) and one *m*- and *p*-substituted (2H doublets; δ_H 6.82 and 7.05, *j* = 8.5 and a broad singlet; δ_H 7.03) ring [Table 1]. Two sp² methines with the downfield chemical shifts of proton and carbon signals (1H doublets; δ_H 6.46 and 7.48, *j* = 15.7) indicated the existence of a di-substituted double bond conjugated with one of the aromatic rings in the structure. The attachment of two methoxy groups to the structure was concluded from the existence of the methoxy signals at ¹H and ¹³CNMR spectra (3H singlets; δ_H 3.79 and 3.82; δ_C 56.53) [Table 1]. Finally, determining the heteronuclear ¹H–¹³C connectivities by HSQC and HMBC experiment, the assignments were confirmed and the chemical structure of (1) defined as 6,7-dimethoxy N-trans-caffeoyltyramine [Figure 1].

Besides to the great similarity of spectra to that of compound (1), compound (2) had a pseudo molecular ion peak of m/z 313.13 [M-H] in the ESIMS spectrum, which together with the ¹³CNMR data [Table 1] suggested the molecular formula as C₁₈H₁₉NO₄. ¹H, and ¹³CNMR spectra were almost completely coincident to those of (1),

Table 1: ¹H and ¹³CNMR data of compound (1) and (2) (400 MHz, 100 MHz, CD₃OD)

Position	Compound (1)		Compound (2)	
	δ_c (mult)	δ_H (int, mult, J)	δ_c (mult)	δ_H (int, mult, J)
1	169.25	-	169.22	-
2	118.97	6.46 (1H, d, J=15.6)	118.90	6.38 (1H, d, J=15.6)
3	142.22	7.48 (1H, d, J=15.6)	142.10	7.40 (1H, d, J=15.6)
4	128.37	-	128.35	-
5	111.67	7.02 (1H, s)	111.62	7.04 (1H, s)
6	149.74	-	149.80	-
7	149.27	-	149.30	-
8	116.65	6.81 (1H, d, J=8.5)	116.56	6.75 (1H, d, J=8.5)
9	123.40	7.05 (1H, d, J=8.5)	123.33	6.96 (1H, d, J=8.5)
10	42.66	3.48 (2H, t, J=7.2)	42.39	3.42 (2H, t, J=7.2)
11	35.87	2.74 (2H, t, J=7.2)	35.84	2.70 (2H, t, J=7.2)
12	131.43	-	131.37	-
13-17	130.92	6.99 (2H, d, J=8.5)	130.82	6.99 (2H, d, J=8.5)
14-16	116.49	6.76 (2H, d, J=8.5)	116.38	6.68 (2H, d, J=8.5)
15	156.84	-	156.90	-
6-OCH ₃	56.53	3.79 (3H, s)	56.48	3.77 (3H, s)
7-OCH ₃	56.53	3.82 (3H, s)	-	-

**Figure 1: Chemical structure of cinnamic acid derivatives isolated from the bulbs of *Allium tripedale***

except for the absence of a singlet (δ_H 3.82) at ¹H NMR spectrum, indicated the chemical structure of (2) to possess a methoxy group less than that of (1). Complementary 1D and 2D NMR analysis showed that C7 in (2) was substituted by a hydroxyl group instead of methoxy compared to that of (1) [Table 1]. Regarding these data, the chemical structure of compound (2) was defined as N-feruloyltyramine [Figure 1].

Discussion

Many natural compounds with well-defined biological and pharmacological activities have been isolated and structurally characterized from different medicinal plants. Phenolic compounds are a very interesting group of these naturally occurring compounds with a broad spectrum of biomedical, food, and industrial applications. *Allium* species have recently attracted great attention due to high content and diversity of their phenolic compounds which due to their abundant use as edible vegetables and medicinal plants could be of great importance for explanation of their protective and medicinal effects on different diseases.

Allium tripedale, locally called “Anashq”, is an important edible plant of “Zagros” region in the west of Iran. The leaves of the plant are used widely as a vegetable, to make dishes and also as a medicinal plant, especially for the treatment of infectious diseases. Pharmacological activities of *A. tripedale* have been evaluated through different studies and the antifungal effects of the aqueous extract, hepatoprotective effects of hydroalcoholic extract accompanied by reducing aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and blood urea nitrogen, antidiabetic and cholesterol- and triglyceride-lowering effects of hydroalcoholic extract of the plant have been recently demonstrated.^[13,14]

A. tripedale has been phytochemically investigated for its volatile organosulfur constituents that resulted to the isolation and identification of three new cysteine-based 1-butenyl derivatives including homoisoalliin and desoxyhomoisoalliin (12). However, nonvolatile secondary metabolites of the plant including phenolic compounds and steroidal saponins have not been studied yet. Hence, as one of the important edible *Alliums* of Iran, *A. tripedale* was subjected to phytochemical investigation in this study, especially for its phenolic constituents, resulted to the isolation and identification of two cinnamic acid derivatives from bulbs of the plant.

Cinnamic acid derivatives are natural substances mainly found in fruits, vegetables, and flowers and are a part of our daily dietary intake of phenolic compounds.^[15,16] Besides, these compounds are important intermediate molecules in the production of different pharmaceutical ingredients. Cinnamic acid derivatives have been isolated from different plant species; among them, *Alliums* have been recently shown to be a source of these compounds,^[1,2] for example,

Allium sativum (N-feruloyltyramine, N-caffeoyltyramine), *A. ampeloprasum* Subsp. *Persicum*, Persian leek (Persicoimide, N-feruloyltyramine, 6,7-dimethoxy N-caffeoyltyramine), and *A. fistulosum* (N-trans-feruloyl-3'-methoxytyramine, N-cis-feruloyl-3'-methoxytyramine, N-trans-p-coumaroyltyramine).^[8,17,18] Biological activities of cinnamic acid and its derivatives have been also the subject of many studies and shown to possess antioxidant, hepatoprotective, anxiolytic, insect repellent, antiviral, antibacterial, antidiabetic, anticholesterolemic, anti-inflammatory, and anticancer effects.^[9,10,11]

N-trans-feruloyltyramine and 6,7-dimethoxy N-trans-caffeoyltyramine are known cinnamic acid derivatives, have been previously isolated from an important edible *Allium* species of Iran, Persian Leek (*Allium ampeloprasum* Subsp. *Persicum*), and shown to bear significant antifungal activity, especially against the antagonistic fungi, *Trichoderma harzianum*.^[8] Although the mechanism of action is unclear, the synthesis of these compounds by *Alliums* may be the reason of their resistance against the plant fungal diseases and could candidate the cinnamic acid derivatives as potential antifungal compounds.^[19,20] Recently, antifungal effects of the aqueous extract of *A. tripedale* against *Candida albicans* were demonstrated which these compounds could at least be responsible for some part of this activity.^[14]

N-feruloyltyramine has also been isolated from garlic (*Allium sativum*) and exhibited to be capable of inhibiting COX enzyme and suppressing P-selectin expression on platelets.^[21,22] Similarly, N-caffeoyltyramine was exhibited to inhibit COX enzyme and bear anti-inflammatory effects. It has been isolated from *Tribulus terrestris* and demonstrated to prevent inflammation process by inhibiting COX-2 enzyme, suppressing COX-2 expression, and inhibiting the synthesis of PGE-2. Since these compounds are potentially safe natural anti-inflammatory compounds which could be candidate for the treatment of inflammatory diseases.^[23]

Cinnamic acid derivatives are also potentially potent natural cytotoxic compounds, and their cytotoxic effects have been investigated in numerous studies. N-caffeoyltyramine has been shown to bear potent antiproliferative effects through the induction of cell death, activating caspase-3 and inhibiting protein tyrosine kinases. Recent studies have indicated that the hydroxyl groups of the phenylpropenyl group of the structure are responsible for the potent antiproliferative effects of N-caffeoyltyramine.^[24] Substitution of the hydroxyls with methoxyl in 6,7-dimethoxy N-trans-caffeoyltyramine can significantly change the antiproliferative activity which needs more studies for the evaluation of cytotoxic activity.

Conclusion

Two cinnamic acid derivatives were isolated from the bulbs of *A. tripedale* for the first time in this study. Identification

of these compounds is in agreement with previous studies have been done on other *Allium* species and could be used as a chemical basis for explanation of some of the biological activities observed from this plant, especially its antifungal effects, and to candidate it for investigation of cytotoxic activity.

Acknowledgment

The authors are grateful to Isfahan University of Medical Sciences for financial supporting of the project.

Financial support and sponsorship

This study was supported by Isfahan University of Medical Sciences for financial and technical supporting of the project.

Conflicts of interest

There are no conflicts of interest.

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