Research in Pharmaceutical Sciences, December 2022; 17(6): 723-727
Received: 21-02-2021
Peer Reviewed: 10-07-2021
Revised: 05-04-2022
Accepted: 17-10-2022
Published: 29-10-2022

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Short Communication

Isolation and identification of 6-methoxy parillin and coniferin from the bulbs of *Allium affine* L.

Narges Vakili[1], Mahsa Esmaeilifallah[2], and Masoud Sadeghi Dinani[1,*]

[1]Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[2]Department of Medical Parasitology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: Phytochemically, *Allium* species are a rich source of important secondary metabolites especially steroidal saponin and sapogenins, flavonoids, and sulfur compounds. As a member of this genus, *Allium affine*, which is locally known as "tareh kouhi", is an endemic plant of middle Asian countries.
Experimental approach: Bulbs of *A. affine* were collected and air-dried in the shade. The chloroform-methanol (9:1) extract of the sample was subjected to purification by MPLC and HPLC. Structure elucidation of isolated compounds was done using comprehensive spectroscopic methods including 1D-NMR, 2D-NMR, and MS.
Findings/Results: A steroidal saponin structurally related to parillin and a phenylpropanoid glycoside (coniferin) were isolated and identified from the plant chloroform-methanol extract.
Conclusion and implication: To the best of our knowledge isolation of these potentially medicinal compounds from *A. affine* was reported for the first time in this study.

Keywords: *Allium affine* Ledeb, Structure Elucidation, Steroidal Saponin, Phenylpropanoid.

INTRODUCTION

Allium is a member of the Amaryllidaceae family, which is one of the largest and most diverse families of monocots with roughly 750 species worldwide (1,2). Medicinal and nutritional usage of *Allium*s goes back thousands of years ago. Their edible and ornamental usage made them well-known all over the world (1,3,4).

Phytochemically, *Allium*s are the main source of phytonutrients and some important secondary metabolites like steroidal saponins, sapogenins, flavonoids, and sulfur compounds, which saponins are of high importance due to their pharmacologic effects (5-7).

Allium affine (*A. affine*), which is locally known as "tareh kouhi", is endemic to middle Asian countries, and grows widely in the western mountains of Iran. So far, saponins and sapogenins such as diosgenin, tigogenin, and ruscogenin have been isolated from this plant and recent studies suggest its antioxidant, fibrinolytic, and cytotoxic activities (4,8,9).

MATERIALS AND METHODS

Plant material

The whole plant of *A. affine* was collected from Borujen, Chaharmahal and Bakhtiari Province, Iran, in May 2019 and identified by the botanist, Mohammad Reza Joharchi (Ferdowsi University of Mashhad). A voucher specimen (No. 3403) was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.359438

*Corresponding author: M. Sadeghi Dinani
Tel: +98-931026719, Fax: +98-3136680011
Email: m_sadeghi@pharm.mui.ac.ir

Extraction, isolation, and structure elucidation

Bulbs of *A. affine* were separated, air-dried in the shade, and powdered using a mill. The powder (930 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 the maceration method, performing each step four times with 3 L of solvent under occasional stirring.

The chloroform-methanol (9:1) extract of the sample was concentrated under vacuum, yielding a crude dried extract (17 g) which was fractionated by medium-pressure liquid chromatography (MPLC) on an RP-18 column (36 × 460 mm, LiChroprep® silica gel, Merck, Germany) using a linear gradient solvent system of H_2O to CH_3OH. Fractions were analyzed by thin-layer chromatography (TLC; SiO_2, BAW 60:15:25 v/v/v) and similar fractions were mixed. Based on TLC and preliminary nuclear magnetic resonance (NMR) analysis, the fractions F19 and F20 were considered richer in steroidal saponins and subjected to further purification by high-performance liquid chromatography (HPLC; (C18 column, Novapak® 7.8 × 300 mm ,WATERS, USA, as the stationary phase and H_2O:CH_3OH (20:80) in isocratic mode as the mobile phase) resulted in compound **(1)**. The fraction F2 was also determined rich in phenolic compounds which was subjected to HPLC (C18 column; Novapak® 7.8 × 300 mm as the stationary phase and H_2O:CH_3OH [85:15] in isocratic mode as the mobile phase) for the final purification, yielded compound **(2)**.

Proton and carbon-13 (^{1}H- and ^{13}C-NMR) spectra were recorded by Bruker (Avance 400, Germany) 400 MHz (H at 400 MHz and C at 100 MHz) spectrometer, using the 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. 2D heteronuclear single-quantum coherence (HSQC), interpulse delay set for 1 JCH of 130 Hz, used for determination of one-bond heteronuclear ^{1}H-^{13}C connectivities. Electrospray ionization mass spectroscopy (ESIMS) spectra were prepared by Shimadzu LCMS 2010 EV (Japan), using methanol as the solvent.

RESULTS

Saponin-rich fractions of the plant extract were selected for further purification, resulting in the isolation and identification of a steroidal saponin (compound **1**) which was structurally related to parillin, already isolated from *Smilax aristolochiifolia* (10). Final purification of the phenolic fraction (F2) resulted in the isolation of a pure phenylpropanoid glycoside (compound **2**) which was identified as coniferyl β-d-glucopyranoside (coniferin, abietin).

Characterization of compound (1)

The steroidal saponin nature of compound **(1)** was confirmed by ^{1}H- and ^{13}C-NMR spectra of the compound, including those related to the steroidal part, a bundle of overlapped signals at δ_H 3 to 5 ppm, and the existence of diagnostic and characteristic signals of saponins especially two tertiary methyls (3H singlets: δ_H 0.85 and 1.00; C-NMR: δ_C 14.92 and 16.92), two secondary methyls (3H doublets: δ_H 0.75 (J = 6 Hz), 0.91 (J = 6.8 Hz)); in the ESIMS spectra, compound **(1)** showed a pseudomolecular ion peak at m/z 1071.6 [M+Na] in the positive-ion mode that together with the ^{13}C-NMR data, suggested its molecular formula as $C_{51}H_{84}O_{22}$. Using the MS and NMR spectral data and comparing them with those reported in the literature, compound **(1)** was recognized as substantially similar to parillin (11,12).

To deduce the glycon part of the compound **(1)**, starting from the first anomeric proton (H_1^I; δH 4.33) and using the HSQC spectral data, the first sugar was determined as β-D-glucopyranoside. Doing the same type of analysis for remained three sugars resulted in the identification of another β-D-glucopyranoside, an α-L-rhamnopyranoside and a β-D-xylopyranoside, and the completion of sugar chain structure elucidation.

Based on these data, the chemical structure of compound **(1)** was determined as 6-methoxy parillin, a steroidal saponin analogous to parillin with an additional methoxy group in the aglycon part (C_6) (Fig. 1).

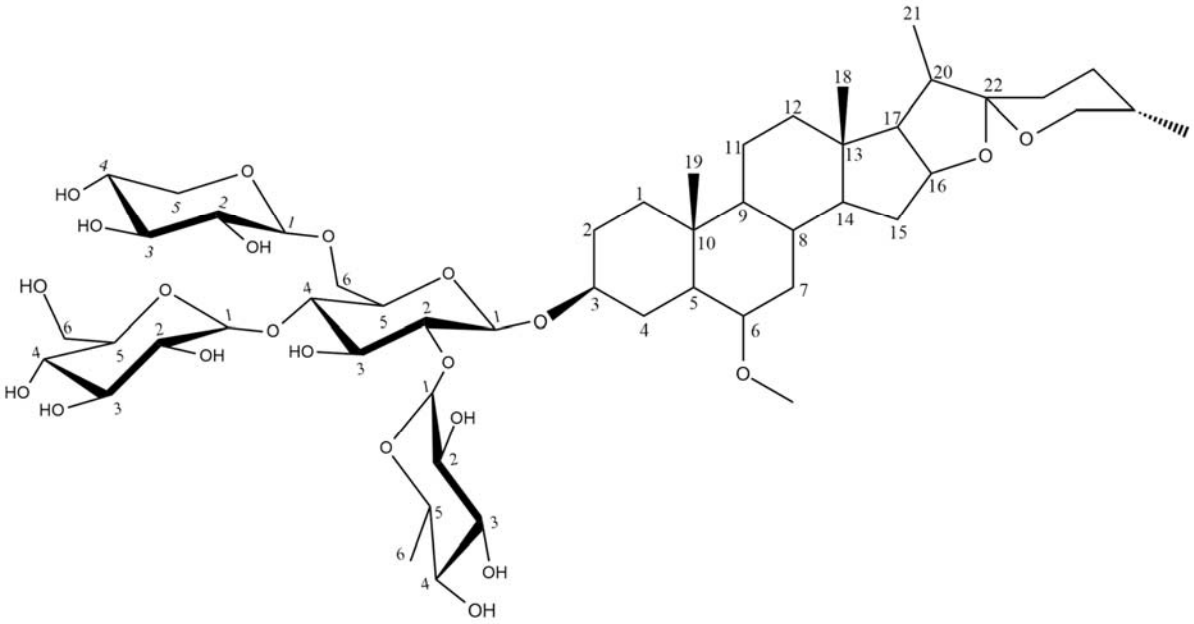

Fig. 1. Chemical structure of compound **(1)** isolated from the bulbs of *Allium affine*.

^{1}H-NMR data of compound **(1)** (400 MHz, CD_3OD): δ_H 0.75(3H, d, 21), 0.84 (H,9), 0.85 (3H, s, 18), 0.91 (3H, d, 27), 1.00 (3H, s, 19), 1.18 (H, 14), 1.32 (H, 5), 1.77 (H, 25), 1.85 (H, 17), 1.92 (H, 20), 2.90 (H, 8), 3.38 (6-OCH_3), 3.45 (H, 6), 3.51 (H, 3), 4.41 (H, 16), 1.24 (6-Rah), 4.33 (1-Glc^I), 4.40 (1-Xyl), 4.63 (1-Glc^{II}), 4.90 (1-Rah).

^{13}C-NMR data of compound **(1)** (100 MHz, CD_3OD): δ_C 14.92 (18), 16.23 (21), 16.92 (19), 17.54 (27), 22.01 (11), 29.06 (24), 29.88 (2), 31.38 (8), 31.48 (25), 32.41 (15), 32.72 (23), 35.82 (10), 36.52 (1), 36.86 (4), 41.13 (12), 41.74 (13), 42.90 (20), 45.76 (5), 50.92 (6-OCH_3), 55.70 (9), 57.28 (14), 63.84 (17), 67.86 (26), 82.17 (16), 82.23 (3), 86.30 (6), 110.58 (22), 1.24 (6-Rah), 104.27 (1-Glc^I), 104.94 (1-Rha), 105.21 (1-Xyl), 105.25 (1-Glc^{II}).

Characterization of compound (2)

^{1}H-NMR spectrum of compound **(2)** exhibited the characteristic signals of aromatic protons (δ_H 6.28-7.14) as well as an isolated doublet at δ_H 4.15 (J = 5.6), an anomeric proton signal at δ_H 4.82 (J = 7.37) and overlapped proton signals of sugar residue (δ_H 3.25-3.65), altogether suggested the chemical structure of compound **(2)** as a glycosylated phenolic compound.

^{13}C-NMR spectrum of compound **(2)** in agreement with ^{1}H-NMR spectral data confirmed the glycosylated phenolic nature of compound **(2)** and suggested the molecular formula as $C_{16}H_{22}O_8$.

^{1}H-NMR data of compound **(2)** (400 MHz; CD_3OD): δ_H 3.81 (OCH_3), 4.15 (3'), 6.24 (2'), 6.56 (1'), 6.89 (6), 7.01 (2), 7.05 (5)

^{13}C-NMR data of compound **(2)** (100 MHz; CD_3OD): δ_C 56.64 (OCH_3), 63.73 (3'), 111.17 (2), 117.68 (5), 120.76 (6), 128.82 (2'), 131.30 (1'), 133.58 (1), 147.57 (4), 150.75 (3), 102.61 (1''), 62.43 (6''), 71.27 (4''), 74.84 (2''), 77.78 (3''), 78.18 (5'').

Finally by comparing the spectral data with those reported for similar compounds in the literature (11), the assignments especially the attachment of OCH_3 to C_3 and glucose residue to C_4 were confirmed and the chemical structure of compound **(2)** was defined as coniferyl β-D-glucopyranoside (coniferin, abietin) (Fig. 2).

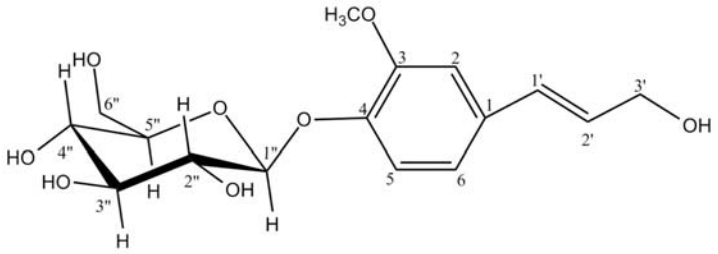

Fig. 2. Chemical structure of compound (2) isolated from the bulbs of *Allium affine*.

DISCUSSION

Phytochemical investigation of *A. affine* resulted in the isolation and identification of one steroidal saponin, 6-methoxy sarsapogenin 3-O-{β-D-glucopyranosyl-(1→2) -[α-L-rhamnopyranosyl-(1→4)]- [β- D-glucopyranosyl-(1→6)]-β-D-xyloyranoside}, and one phenylpropanoid glycoside, coniferyl β-D-glucopyranoside (coniferin), from the chloroform-methanol extract of bulbs of the plant. The result was in agreement with previous studies, which have reported the isolation of some steroidal saponins including tigogenin, diosgenin, ruscogenin (12), and 226-O-β-D-glucopyranosyl-22-O-methyl- (25R)-5α-furustane-2α, 3β, 26-tetrol 3-O-{O-α-L- rhamnopyranosyl- (1→2)-O-[β-D-glucopyranosyl-(1→4)] -β-D-glucopyranoside}, and cinnamic acid derivatives such as (4-hydroxyphenyl)-3-(4-hydroxyphenyl) prop-2-enoate-4-α-L-rhamnopyranoside-(1→2)-β-D-glucopyranoside (13) from *A. affine*.

Coniferin is an astringent phenylpropanoid glycoside that has been isolated from some conifers, citrus, asparagus, and *Alliums*. It has been used in cough syrups and has been claimed to have antiasthmatic effects. Coniferin is a metabolic precursor of podophyllotoxin, which has anticancer activity (13-16).

CONCLUSION

Phytochemical investigation of chloroform-methanol extract of *A. affine* resulted in isolation and identification of 6-methoxy parillin and coniferin from this plant for the first time. It could be used as a basis for new studies on the pharmacological effects of these compounds.

Acknowledgments

The content of this paper is extracted from the Pharm. D thesis which was conducted by Narges Vakili and financially supported by the Vice-Chancellery of Research of Isfahan University of Medical Sciences, Isfahan, Iran through Grant No. 398452.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

All the authors contributed equally to this work. The final version of the manuscript was approved by all authors.

REFERENCES

1. Sadeghi M, Zolfaghari B, Senatore M, Lanzotti V. Antifungal cinnamic acid derivatives from Persian leek (*Allium ampeloprasum* Subsp. *Persicum*). Phytochem Lett. 2013;6(3):360-363. DOI: 10.1016/j.phytol.2013.04.007
2. Miryeganeh M, Movafeghi A. Scape anatomy of *Allium sect*. *Allium* (Alliaceae) in Iran. J Sci. 2009;35(1):1-5.
3. Ozturk M, Gucel S, Altay V, Altundag E. *Alliums*, an underutilized genetic resource in the east Mediterranean. ISHS Acta Horticulturae 969: VI International Symposium on Edible Alliaceae; 2012. DOI: 10.17660/ActaHortic.2012.969.39.
4. Sadeghi M, Safaeian L, Ghazvini MA, Ramezani M. Evaluation of fibrinolytic and antioxidant effects of *Allium affine* hydroalcoholic extract. Res Pharm Sci. 2017;12(4):299-306. DOI: 10.4103/1735-5362.212047.
5. Chehri Z, Zolfaghari B, Dinani MS. Isolation of cinnamic acid derivatives from the bulbs of *Allium tripedale*. Adv Biomed Res. 2018;7:60-1-5. DOI: 10.4103/abr.abr_34_17.
6. Rezaee F, Zolfaghari B, Dinani MS. Isolation of dioscin-related steroidal saponin from the bulbs of

Allium paradoxum L. with leishmanicidal activity. Res Pharm Sci. 2018;13(5):469-475. DOI: 10.4103/1735-5362.236875.
7. Cui M, Song F, Zhou Y, Liu Z, Liu S. Rapid identification of saponins in plant extracts by electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2000;14(14):1280-1286. DOI: 10.1002/1097-0231(20000730)14:14<1280::AID-RCM26>3.0.CO;2-C.
8. Kazemi M, Zolfaghari B, Keivanloo Shahrestanaki M, Sadeghi Dinani M. Cytotoxic effects of *Allium affine* Ledeb butanolic fraction on breast and ovary cancer cell lines. J Med Plants. 2017;16(64):83-90.
9. Azizi H, Keshavarzi M. Ethnobotanical study of medicinal plants of Sardasht, Western Azerbaijan, Northwestern Iran. J Herb Drugs. 2015;6(2):113-119.
10. Challinor VL, Parsons PG, Chap S, White EF, Blanchfield JT, Lehmann RP, *et al*. Steroidal saponins from the roots of *Smilax* sp.: structure and bioactivity. Steroids. 2012;77(5):504-511. DOI: 10.1016/j.steroids.2012.01.009.
11. Sobolewska D, Michalska K, Podolak I, Grabowska K. Steroidal saponins from the genus *Allium*. Phytochem Rev. 2016;15(1):1-35. DOI: 10.1007/s11101-014-9381-1.
12. Challinor VL, Parsons PG, Chap S, White EF, Blanchfield JT, Lehmann RP, *et al*. Steroidal saponins from the roots of *Smilax* sp.: structure and bioactivity. Steroids. 2012;77:504-511. DOI: 10.1016/j.steroids.2012.01.009.
13. Ghaffari H, Sadeghi DM. Isolation of phenyl propanoid glycosides from *Allium tripedale* Trautv. J Rep Pharm Sci. 2018;7(2):116-122.
14. Tatum JH, Berry RE. Coniferin-an astringent glycoside in citrus. Phytochemistry. 1986;26(1): 322-323. DOI: 10.1016/S0031-9422(00)81540-X.
15. Savidge RA. Coniferin, a biochemical indicator of commitment to tracheid differentiation in conifers. Can J Bot. 1989;67(9):2663-2668. DOI: 10.1139/b89-343.
16. Lin HW, Kwok KH, Doran PM. Development of *Linum flavum* hairy root cultures for production of coniferin. Biotechnol Lett. 2003;25(7):521-525. DOI: 10.1023/a:1022821600283.