



## Isolation and identification of Astragalin and 2-methoxy tyrosol from the bulbs of *Allium paradoxum*

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### ABSTRACT

**Introduction:** *Allium paradoxum* is a perennial herb in northern Iran, especially in Mazandaran province. It is locally called "Alezi" and in addition to using as raw vegetable, is also used in the preparation of regional foods. This study was aimed to investigate the main phenolic constituents of the plant.

**Methods:** Bulbs of the plant were extracted respectively by hexane, chloroform, chloroform-methanol (9-1) and methanol in a stepwise method with increasing solvent polarity. Methanol extract was then partitioned between water and butanol. Chloroform-methanol and butanol extract constituents were isolated and purified by column chromatography and HPLC. Chemical structure of the compounds was elucidated unambiguously by spectroscopic methods, including 1D and 2D NMR and MS spectroscopy.

**Results:** Phytochemical investigation of *A. paradoxum* led to the isolation of two main phenolic compounds, a flavonoid glycoside and a tyrosol derivative. The isolated compounds were identified as kaempferol-3-O-glucoside (1) (Astragalin) and 2-methoxy-2-(4'-hydroxyphenyl) ethanol (2-Methoxy tyrosol) (2).

**Conclusion:** Isolation and identification of astragalin and 2-methoxy tyrosol from *A. paradoxum* is reported for the first time in this study and provide a chemical basis for the explanation of pharmacological and biological activities attributed to the plant.

#### Implication for health policy/practice/research/medical education:

Identification of the compounds from *A. paradoxum* provides a chemical basis for the explanation of pharmacological and biological activities of the plant.

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### Introduction

Plants are the oldest source of active pharmaceutical ingredients and many years before the discovery of chemical drugs, people have extensively used plant-based preparations and ingredients for treatment of different diseases. During the centuries numerous useful medicinal preparations have been prepared from different medicinal plants and over the years their therapeutic effects or ineffectiveness have been experienced and proved (1). Due to the specific climate conditions, Iran has historically a great amount of plant diversity including medicinal plants, which could be a main source of phytochemical studies, especially for native plants (2). *Allium* genus (Amarylidaceae) with more than 700 species of edible and medicinal species all around the world, is one of the most important plant families in Iran, its plant species have been used both as vegetable and medicinal plants for

thousands of years (3). This genus is composed of different groups of perennial plants which have recently attracted great attentions because of their both pharmaceutical and alimentary benefits (4).

*Allium* species are rich sources of phytonutrients and have been exhibited to possess many pharmacological activities including cholesterol lowering, anti-hypertensive, anti-spasmodic, anti-bacterial, anti-viral, antimicrobial and anti-cancer effects (5,6). They are also one of the richest sources of naturally occurring flavonoids that help us to intake a high level of phenolic compounds through the daily diet (7).

Phenolic compounds are a diverse group of naturally occurring secondary metabolites possessing one or more hydroxylated aromatic rings, involving over 8000 structurally different compounds. Beside of their roles in plant biosynthesis, a large spectrum of pharmacological

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and biological activities have been demonstrated for these group of natural compounds, the most important examples are antioxidant, anti-inflammatory, antibacterial, antiviral, antihypertensive, cholesterol lowering, anti proliferative, anticarcinogenic, antimutagenic and antiatherosclerotic effects (8).

As a member of Amaryllidaceae family, *Allium paradoxum* is an edible *Allium* species in northern Iran, especially in Mazandaran province. It is locally called "Alezi" and in addition to use as a raw vegetable, it is an important plant for making several local foods. *A. paradoxum* has also been traditionally used as a medicinal plant and some recent studies have been subjected and demonstrated its pharmacological activities including antioxidant activity (9,10), protective effects against gentamicin-induced nephrotoxicity (9), anti-hemolytic activity (10) and protective effects against liver toxicity induced by CCl<sub>4</sub> (11).

According to its wide use and the lack of comprehensive phytochemical study to identify its main constituents, as a part of our research program on phytochemical investigation of *Allium* species, main phenolic constituents of the bulbs of *A. paradoxum* were isolated and structurally identified.

## 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<sup>®</sup> RP-18 (25-40 $\mu$ m) and C-660 Buchi fraction collector. TLC performed on SiO<sub>2</sub> plates with BuOH:H<sub>2</sub>O:CH<sub>3</sub>COOH (60:25:15 v/v/v) (BAW) as a mobile phase and cerium sulfate in 2N H<sub>2</sub>SO<sub>4</sub> 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<sup>®</sup> 7.8  $\times$  300 mm) and analytical C18 column (Novapak<sup>®</sup> 3.9  $\times$  300 mm) in isocratic mode. H and C NMR spectra recorded by Bruker 400 MHz (H at 400 MHz and C at 100 MHz) spectrometer, using solvent signal for calibration (CD<sub>3</sub>OD:  $\delta$ H = 3.31,  $\delta$ C = 49.0). Distortionless enhancement by polarization transfer (DEPT) experiment was used to determine the multiplicities of C NMR resonances. 2D heteronuclear multiple bond correlation (HMBC), optimized for <sup>2</sup>J<sub>CH</sub> of 8 Hz, was used for determination of two and three bond heteronuclear <sup>1</sup>H-<sup>13</sup>C connectivities, while 2D heteronuclear single-quantum coherence (HSQC), interpulse delay set for <sup>1</sup>J<sub>CH</sub> of 130 Hz, used for the determination of one-bond heteronuclear <sup>1</sup>H-<sup>13</sup>C connectivities. ESIMS spectra were prepared by Shimadzu LCMS 2010 EV, using methanol as the solvent.

### Plant material

The whole plant of *A. paradoxum* L. was collected from Babol mountainous areas (Mazandaran, Iran), during

April 2014 and was identified by botanist, Mr. Joharchi. A voucher specimen (No. 2163) was deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences, Iran.

### Extraction and isolation

The air-dried aerial parts of *A. paradoxum* were finely powdered by means of a mill and the powder (1600 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 3 times with 6 L of solvent under occasional stirring. The chloroform-methanol (9:1) and methanol extracts of the sample were concentrated under vacuum. The methanol extract was dissolved in water and then by adding *n*-butanol, was distributed between two solvents. The resulting butanol phase was separated and concentrated under vacuum.

The crude dried chloroform-methanol (16 g) and butanol (11.5 g) extracts were fractionated by MPLC on RP-18 column (36  $\times$  460 mm) using a linear gradient solvent system of H<sub>2</sub>O to CH<sub>3</sub>OH. Fractions were analyzed by TLC (SiO<sub>2</sub>, BAW, reagents: cerium sulfate in 2N H<sub>2</sub>SO<sub>4</sub> and NP) and similar fractions were mixed together. The resulted final fractions were subjected to HNMR spectroscopy and based on TLC and preliminary NMR analysis, two fractions (A.P.B-7 and A.P.CM-3) were considered to be rich in phenolic compounds and selected for purification of the constituents by HPLC.

The first fraction (A.P.B-7) which was from butanolic extract of the plant, was subjected to purification by HPLC using a semi preparative C18 column (Novapak<sup>®</sup> 7.8  $\times$  300 mm) and H<sub>2</sub>O-CH<sub>3</sub>OH (50:50) mobile phase in isocratic mode, resulted in the compound (1) (144 mg).

The second fraction (A.P.CM-3), from chloroform-methanol extract, was purified by HPLC using a semi preparative C18 column (Novapak<sup>®</sup> 7.8  $\times$  300 mm) and H<sub>2</sub>O-CH<sub>3</sub>OH (35:65) isocratic mobile phase, resulted in the compound (2) (129 mg).

## Results

Based on TLC and preliminary NMR screening, two fractions derived from butanol and chloroform-methanol extract of the plant showed signals typical of phenolic compounds which were selected for further purification, resulted in the isolation and identification of a glycosilated flavonol (1) and a *p*-hydroxyphenyl ethanol derivative (2). Chemical structure of the isolated compounds was determined by comprehensive spectroscopic methods and also by comparison of the NMR spectral data with those reported in the literature.

### Characterization of compound (1)

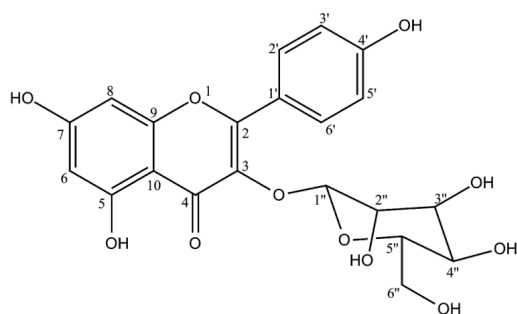
Compound (1) showed a pseudomolecular ion peak at *m/z* 447 [M-H] in the negative-ion ESIMS, which in agreement with the CNMR spectral data of 21 carbon signals suggested the molecular formula as C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>.

The HNMR spectrum of the (1) revealed the characteristic signals of a flavonol glycoside including two singlet proton signals at  $\delta_H$  6.22 (s, 1H, H-6) and  $\delta_H$  6.41 (s, 1H, H-8) and two doublet proton signals at  $\delta_H$  6.91 (d,  $j=8.5$ , 2H, H-2',6') and  $\delta_H$  8.07 (d,  $j=8.5$ , 2H, H-3,5') that revealed the kaempferol-like nature of the aglycon part of the compound (Table 1).

HNMR spectrum of (1) exhibited also the existence of an anomeric proton signal at  $\delta_H$  5.26 (d,  $j=7.2$ , 1H, Glc-1) together with overlapped proton signals at  $\delta_H$  2.5-4 that demonstrated the glycosylated nature of (1) and was further supported by the presence of carbon signals of sugar part at  $\delta_C$  103.87 (Glc-1), 75.68 (Glc-2), 77.94 (Glc-3), 71.31 (Glc-4), 78.41 (Glc-5), and 62.64 (Glc-6) (Table 1). The assignments were finally approved using heteronuclear  $^1H$ - $^{13}C$  correlations through HSQC and HMBC experiments. Specially, the attachment of glucose to C-3 of the aglycone was confirmed by HMBC experiment according to the  $H^{13}C$ -C3 cross-peak. According to these data and by comparing them with the

**Table 1.**  $^1H$  and  $^{13}C$ NMR data of Astragalins (1) (400 MHz, 100 MHz,  $CD_3OD$ )

Position	$\delta_C$ (mult)	$\delta_H$ (int., mult., J)
2	162.82 (C)	-
3	135.65 (C)	-
4	179.50 (C)	-
5	161.54 (C)	-
6	99.73 (CH)	6.22 (1H, s)
7	165.88 (C)	-
8	94.85 (CH)	6.41 (1H, s)
9	159.22 (C)	-
10	105.67 (C)	-
1'	122.58 (C)	-
2',6'	132.31 (CH)	6.91 (2H, d, $J=8.5$ )
3',5'	116.12 (CH)	8.07 (2H, d, $J=8.5$ )
4'	158.47 (C)	-
1''	103.87 (CH)	5.26 (1H, d, 7.2)
2''	75.68 (CH)	3.46 (1H, dd, 10.4, 3.6)
3''	77.94 (CH)	3.37 (1H, m)
4''	71.31 (CH)	3.24 (1H, m)
5''	78.41 (CH)	3.56 (1H, m)
6''	62.64 (CH <sub>2</sub> )	3.70 (2H, d, 11.5)



**Figure 1.** Chemical structure of Astragalins (1) isolated from the bulbs of *Allium paradoxum*.

NMR data of different flavonol glycosides reported in the literature (12), the chemical structure of (1) was defined as kaempferol-3-O-glucoside (Astragalins) (Figure 1).

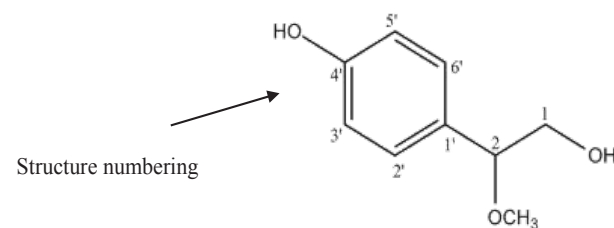
### Characterization of compound (2)

ESIMS spectra of compound (2) in the negative-ion mode showed a pseudomolecular ion peak at  $m/z$  167 [M-H], while the HNMR spectrum exhibited 2 characteristic aromatic proton signals at  $\delta_H$  6.79 (2H, d,  $J=8.5$  Hz) and  $\delta_H$  7.14 (2H, d,  $J=8.5$  Hz). CNMR spectrum of (2) showed 7 carbon signals including 2 double height  $sp^2$  aromatic methine carbons ( $\delta_C$  116.26 and 129.35), 2 quaternary  $sp^2$  carbon signals ( $\delta_C$  130.89, 158.39) and 3  $sp^3$  carbon signals ( $\delta_C$  56.85, 67.77, 85.98) which in agreement with MS and HNMR spectral data confirmed the molecular formula as  $C_9H_{12}O_3$  (Table 2).

Further analyses of the CNMR spectrum, specially observing the downfield chemical shift of one of the quaternary carbons ( $\delta_C$  158.39), exhibited that one of the quaternary carbons had to be connected to an oxygen atom. The same condition was observed for  $sp^3$  carbons, so that all  $sp^3$  carbons had significant downfield chemical shifts, indicated that they had to be connected to the electronegative oxygen atom, one of them was characteristic of a methoxy carbon signal ( $\delta_C$  56.85). Finally, determining the low and high range  $^1H$ - $^{13}C$  connectivities, respectively by HSQC and HMBC experiment, exact assignments specially the attachment of  $OCH_3$  to C2 were confirmed and the chemical structure of (2) was concluded to be of tyrosol derivatives and defined as 2-methoxy-2-(4'-hydroxyphenyl)ethanol (2-methoxy tyrosol) (Figure 2).

**Table 2.**  $^1H$  and  $^{13}C$ NMR data of 2-methoxy tyrosol (1) (400 MHz, 100 MHz,  $CD_3OD$ )

Position	$\delta_C$ (mult)	$\delta_H$ (int., mult., J)
1a	67.77(CH <sub>2</sub> )	3.64(1H, dd, $J=11.6, 8.2$ )
1b		3.51(1H, dd, $J=11.6, 3.9$ )
2	85.98(CH)	4.18(1H, dd, $J=8.2, 3.9$ )
1'	130.89(C)	-
2'	129.35(CH)	6.79(2H, d, $J=8.5$ )
3'	116.26(CH)	7.14(2H, d, $J=8.5$ )
4'	158.39(C)	-
5'	116.26(CH)	7.14(2H, d, $J=8.5$ )
6'	129.35(CH)	6.79(2H, d, $J=8.5$ )
OCH <sub>3</sub>	56.85	3.50



**Figure 2.** Chemical structure of 2-methoxy tyrosol (2) isolated from the bulbs of *Allium paradoxum*

## Discussion

*Allium paradoxum* is an edible vegetable in northern Iran, especially in Mazandaran province, which is locally called "Alezi" and is widely used as a raw vegetable, for making foods and as a medicinal plant (9). Pharmacological and biological activities of *A. paradoxum* have been studied in recent studies and it has been shown to have antioxidant activity (9,10), protective effects against gentamicin-induced nephrotoxicity (9), anti-hemolytic activity (10) and protective effects against liver toxicity induced by CCl<sub>4</sub> (11). Wide uses both as a vegetable and also as a medicinal herb and the absence of any comprehensive phytochemical investigation of the plant encouraged us for phytochemical study of the plant with a more focus on the isolation and identification of its main phenolic constituents. Bulbs of *A. paradoxum* were phytochemically studied which finally resulted in isolation and identification of kaempferol-3-O-glucoside (Astragalin) and 2-methoxy tyrosol as 2 main phenolic constituents of the plant.

*Allium* species are rich sources of phytonutrients and considered to be one of the richest sources of naturally occurring flavonoids, playing an important role in daily intake of phenolic compounds. As a known and widespread flavonoids, astragalin (1) is a flavonol glycoside that has been isolated from many famous *Allium* species like Garlic (*A. sativum*), Onion (*A. cepa*), Persian leek (*A. ampeloprasum* subsp. *persicum*) and victory onion (*A. victorialis*) (6,13,14). Astragalin has been subjected to pharmacological studies and shown to possess many pharmacological and biological activities including antioxidant effects, anti-atopic dermatitis effects, inhibitory effects on TNF- $\alpha$ , IL-1 $\beta$  and IL-6 production, inhibitory effects on histamine release in human blood cells (15-17), inhibition of LPS-induced production of nitrite oxide and prostaglandin E<sub>2</sub> and promising anti-inflammatory activity (15). It seems to be to some extent responsible for a part of medicinal effects observed from *A. paradoxum* specially antioxidant, nephroprotective and hepatoprotective effects of the plant.

Tyrosol (2-(4-hydroxyphenyl) ethanol) is also a well-known phenolic compound in the nature which is especially famous as one of the main phenolic constituents of the extra virgin olive oil. It is also found in different dietary sources and exerts mild antioxidant properties probably through the scavenging effects on free radicals of Nitrogen and oxygen (18). Tyrosol has also been reported to possess protective effect against oxidative stress in kidney cells (19), inhibitory effects on LPS-induced cytokine and leukotriene B<sub>4</sub> release, dose-dependent neuroprotective (18,20) and hypoglycemic effects (21). 2-methoxy tyrosol (2) has been previously isolated from various plant species such as *Dystaenia takeshimana* (22), *Angelica tenuissima* (23) *Ostericum koreanum* (24) and *Coriandrum sativum* (25) and *Allium victorialis* var. *platyphyllum* (26) and has been shown to be pharmacologically active as an anti-inflammatory natural compound (20). Other tyrosol derivatives have also been shown to have useful medicinal activities as *p*-tyrosyl

gallate exhibits potent anticancer (27) and 2-hydroxy tyrosol possess significant anti-inflammatory activity (20). Some of the medicinal effects of *A. paradoxum* especially those which are related to inflammation may be considered to be intermediated by 2-methoxy tyrosol.

## Conclusion

Two phenolic compounds including a flavonol glycoside, astragalin, and a tyrosol derivative, 2-methoxy tyrosol, were isolated from the bulbs of *A. paradoxum* for the first time in this study. According to wide spread uses of the plant specially in the north of Iran, identification of these compounds is of great importance and could be used as a basis for explanation of some of the biological and pharmacological activities observed from this plant, especially those which are related to antioxidant and anti-inflammatory effects.

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## Authors' contributions

The authors contributed equally to the study.

## Conflict of interests

The authors declared no competing interests.

## Ethical considerations

Common ethical issues were observed by the authors.

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