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# **Chemotype variations among lichen ecotypes of *Umbilicaria aprina* revealed by LC-ESI-MS/MS: a survey of antioxidant phenolics**

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## Abstract

In the present study, we characterized the phytochemical profile of six distinct ecotypes of *Umbilicaria aprina* Nyl. from Iran (include: Kivarestan, Mishan, Takht-e Nader, Tochal, Sabalan, and Sahand ecotypes) in order to ascertain whether the environmental differences can affect the chemical profile of this species. Based on the results of LC-ESI-MS/MS, 9 major chemical substances were identified: Orsellinic acid (1), Lecanoric acid (2), Evernic acid (3), Gyrophoric acid (4), Umbilicatic acid (5), Hiassic acid (6), Stictic acid (7), a member of Hiassic acid complex (8), and an unknown substance (9). The MS/MS fragmentation patterns of each substance were determined and proposed. Broad variability was observed in the chemical profiles of lichen samples according to the presence/absence of these substances. The results corroborate that the influence of ecological locality conditions for example altitude, slope aspect, light intensity, and temperature, on phytochemical variations among ecotypes of a particular lichen species like *U. aprina* are of great importance.

**Keywords:** *Umbilicaria aprina*, *Umbilicariaceae*, phytochemical profile, LC-ESI-MS/MS, Fragmentation pattern, Depsides, ecological factors, slope aspect

## 1. Introduction

Lichen, as a form of mutualistic symbiosis life between algae/cyanobacteria (as photobiont/cyanobiont) and fungi (as mycobiont), is one of the biotechnological interesting issues that attracted much consideration due to its potential for producing multifarious pharmaceutical secondary metabolites especially phenolics such as depsides, depsidones and depsones which are specifically originating from the mycobiont (Oetl et al., 2014).

Depsides, such as Gyrophoric acid, umbilicinic acid, and lecanoric acid, which are the eventual products of esterification of orcinol rings through acetyl polymalonyl pathway are mainly produced by most species of family *Umbilicariaceae* (Nguyen et al., 2013; Oetl et al., 2014; Ranković, 2015). Many studies have demonstrated the presence of high concentrations of phenolic compounds in different *Umbilicaria* species (Fernández-Moriano et al., 2016).

The family *Umbilicariaceae* contains two main genera, *Lasallia* and *Umbilicaria*. In accordance with climatic conditions in Iran, the genus *Lasallia* is not a member of the lichen flora of this country, but the *Umbilicaria* as a worldwide boreal and sub-boreal genus occurs chiefly in alpine zones of Iran. According to the latest checklist of lichen flora in Iran published by Seaward et al. (2008) the genus *Umbilicaria* has 11 distinguished species in Iran including *U. aprina*, *U. cinerascens*, *U. cinereorufescens*, *U. cylindrica*, *U. decussata*, *U. freyi*, *U. hirsuta*, *U. microphylla*, *U. nylanderiana*, *U. polyphylla* and *U. vellea*. Among the mentioned species, the *U. aprina* is a highly distributed species in alpine regions of Iran.

Phytochemical analysis of *Umbilicaria* species by several authors, has revealed the presence of ca. 16 compounds including gyrophoric acid, lecanoric acid, umbilicinic acid, hiassic acid, ovoic acid, crustinic acid, lasallic acid, usnic acid, norstictic acid, salazinic acid, atraric acid, atranorin, unknown terpenoids, methyl orsellinate, methyl  $\beta$ -orcinolcarboxylate and ethyl hematamate (Hestmark, 1991; Krog and Swinscow, 1986; Krzewicka, 2004; Krzewicka and Osyczka, 2002; Manojlović et al., 2012; Narui et al., 1998, 1996; Poelt and Nash, 2006; Posner et al., 1992; Serina et al., 1996; Zlatanović et al., 2017).

Despite the wide distribution of *U. aprina* throughout the world, there isn't any clear report about its phytochemical profile. Based on thin-layer chromatography (TLC) results in few studies (Hestmark, 1991; Krog and Swinscow, 1986; Krzewicka, 2004; Krzewicka and Smykla, 2004), the major secondary product in *U. aprina* is gyrophoric acid which is always accompanied by lecanoric acid. Therefore, gyrophoric acid and lecanoric acid are the only reported secondary substances from *U. aprina* up to now.

On the other hand, qualitative and quantitative polymorphism of secondary metabolites is expected in various ecotypes of a particular species due to geographical distribution according to results of several studies

(Bjerke et al., 2005, 2003; Culberson, 1967; Fahselt and BeGora, 2001; Piercey-Normore, 2007, 2003). Whereas *U. aprina* is a widespread species in Iran, we can anticipate certain phytochemical variations between different ecotypes of this species.

The purpose of this study was to investigate the discrepancies between qualitative phytochemical profiles of various *U. aprina* ecotypes distributed throughout the boreal regions of Iran, in order to confirm the role of ecology in imposing alterations on phytochemical traits of lichens. To the best of our knowledge, liquid chromatography (LC) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS), is a reliable analytical method, applicable for accurate identification and quantification of lichen secondary metabolites. Therefore, in this study, an attempt was made to determine the individual phenolic compounds of six *U. aprina* ecotypes from Iran, for the first time based on LC-ESI-MS/MS.

## **2. Experimental**

### **2.1. Chemicals and reagents**

All the commercial chemicals and reagents including analytical acetone for extraction, LC-MS grade water, acetonitrile, and formic acid were purchased from Merck (Germany).

### **2.2. Lichen material**

Thalli of *U. aprina* ecotypes were collected from siliceous alpine rock surfaces on six different alpine zones of Iran during May and June 2018. According to table 1, three ecotypes were collected from Alvand Mt. in Hamadan province (Kivarestan, Takht-e Nader, Mishan), one ecotype from Tochal Mt. in Tehran province (Tochal), one ecotype from Sabalan Mt. in Ardabil province (Sabalan) and one ecotype was collected from Sahand Mt. in East Azerbaijan province (Sahand). After cleaning, the sample identification was substantiated by data from examinations of macroscopic, microscopic and chemical (thalline reaction with para-phenylenediamine (P), potassium hydroxide (K) and sodium hypochlorite (C) or/and KC reagents) characters and ultimately by comparison with the literature. Voucher specimens for each ecotype were deposited at ICH lichen herbarium of Iranian research organization for science and technology (IROST), and their identity was confirmed by Dr. Mohammad Sohrabi. The thalli were air-dried for one week in room temperature and then grounded to a fine powder using pestle and mortar under liquid nitrogen and stored at room temperature in closed tubes.

### 2.3. Sample preparation

An extraction of 5g dry *U. aprina* powder for each ecotype was performed in 50 mL of acetone in an ultrasonic bath during 30 minutes in 20°C. The extracts were filtered (Whatman filter paper grade 2) and then concentrated under reduced pressure in a rotary evaporator. The dry extracts of ecotypes were stored at -20°C until they were used for phytochemical screening.

### 2.6. LC-ESI-MS/MS analysis

The chromatographic analyses of lichen compounds were conducted on a Waters Alliance e2695 separation module (Waters Corporation, Milford, MA, USA) through an Atlantis T3 C18 column (2.1mm × 100 mm, 3 µm; Waters Corp., Milford, MA, USA) which was kept at 30°C. The MS/MS spectra were acquired from a Quattro micro API mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an ESI source. Immediately before analysis, 5mg of the dry residue of the extract was dissolved in 5 mL methanol. Then, the samples were filtered through PTFE membrane filters (pore size 0.45 µm, Simplepure, China). Chromatographic separations were performed at a flow rate of 0.25 ml/min using a gradient elution program starting from 95% of eluent A (water with 0.1% formic acid, v/v) and gradually changing to 5% A over 20 min. Elution was continued for 5 min by 95% of eluent B (acetonitrile). This allowed elution of all analytes within 25 minutes. The injection volume was 10 µL.

Mass detection conditions were as follows: source temperature 120°C, desolvation temperature 300°C, capillary voltage 3.5 kV, cone voltage 30 V. Nitrogen was used for both nebulizing and drying the gas. The samples were measured in negative ionization mode and scanned in the range of 50-600 *m/z*. The MS/MS analyses were performed with the collision energy of 30 eV. The MassLynx V 4.1 software (Waters) was used for the data evaluation.

### 2.7. Aspect map

For generating the aspect map of Iran and study areas we used topographic data of Iran provided by ArcGIS Online. Using ArcGIS (ArcMap) 10.6.1 software we could convert the topographic data to digital elevation map (DEM). Spatial analyst tools were used for engendering the aspect map of Iran from DEM.

## 3. Results and discussion

### 3.1. LC-ESI-MS/MS peak identification and fragmentation pathways

In the present study detection of 9 major compounds eventuated from qualitative analysis of the phytochemical profile of six *U. aprina* ecotypes using LC-ESI-MS/MS in negative ion mode (Table 2). The identification of major compounds was facilitated by their MS/MS spectra and comparison to literature. Major compounds identified in this species were mainly depsides, depsidones, and monocyclic aromatic compounds.

One monocyclic aromatic compound was identified based on its MS<sup>2</sup> spectra. Peak 1 was detected as orsellinic acid which indicated an [M-H]<sup>-</sup> ion at *m/z* 167.210 whose cleavage produced 2 perceptible MS<sup>2</sup> ions at *m/z* 81.385 and ..... Our results are in great accordance with those reported by other authors (Brakni et al., 2018; Castro et al., 2017; Kumar et al., 2018; Musharraf et al., 2015; Parrot et al., 2015; Torres-Benítez et al., 2017).

Five depsides were identified using MS<sup>2</sup> analysis. Peak 3 was identified as umbilicatic acid whose molecular ion was detected at *m/z* 481.646. Major observable daughter MS<sup>2</sup> ions of umbilicatic acid were detected at *m/z* 181.050, 167.075, 162.810, 148.882 and 136.950. Although umbilicatic acid is a well-known tridepside, its negative ion mode mass fragmentation pathway has not been reported up to now. Here we have proposed a cleavage pathway for umbilicatic acid in Fig 1. The cleavage of ester bonds between aromatic units leads to the generation of representative MS<sup>2</sup> ions at *m/z* 181.050, 167.075 and 148.882. Separation of CO<sub>2</sub> and H<sub>2</sub>O from *m/z* 181.050 and 167.075 results in other MS<sup>2</sup> fragments for umbilicatic acid at *m/z* 136.950 and 148.882. Peak 4 indicated a molecular ion at *m/z* 331.398 which was identified as evernic acid on the basis of its diagnostic MS<sup>2</sup> daughter ions at *m/z* 180.952, 162.723, 148.915, 136.915 and 104.848.. Our results for Evernic acid are in suitable concordance with other researches (Brakni et al., 2018; Castro et al., 2017; Jin et al., 2018; Torres-Benítez et al., 2017). A molecular ion at *m/z* 483.702 was detected for peak 4 which made the identification of hiascic acid feasible for us by the production of four cleaved diagnostic MS<sup>2</sup> ions at *m/z* 183.005, 166.974, 148.910 and 122.840. This is the first report on hiascic acid identification by means of MS fragmentation pattern in negative ion mode, therefore we deduced a reasonable fragmentation pathway for this tridepside in Fig 2. The most prevalent fragmentation pattern for tridepsides is the separation of aromatic units due to ester bond cleavage and there is no exception in the case of hiascic acid. Thus, 3 representative MS<sup>2</sup> ions at *m/z* 183.005, 166.974 and 148.910 are results of ester bonds cleavage. Further losses such as CO<sub>2</sub> and H<sub>2</sub>O from *m/z* 166.974 ion leads to MS<sup>2</sup> fragments at *m/z* 148.910 and 122.840. Peak 5, indicated a deprotonated molecular ion at *m/z* 497.736 which was identified as a member of the hiascic acid complex on the basis of its fragmentation pattern with 3 representative MS<sup>2</sup> ions at *m/z* 196.962, 152.860 and 108.898. There are 3 compounds with the same molecular ion mass in hiascic acid complex including 2-O-Methylhiascic acid, 4-O-Methylhiascic acid, and 5-O-Methylhiascic acid and the main difference between these 3 compounds is the situation of the methyl group on

ring A, therefore all we could determine was that the ion at  $m/z$  497.736 is a member of the hiassic acid complex. As we have proposed in Fig 3 separation of ring A leads to an ion at  $m/z$  196.962 and loss of a  $\text{CO}_2$  from this fragment results in an ion at  $m/z$  152.860. Further separation of rings B and C provides other ions. The aromatic ring C produces 2 other diagnostic  $\text{MS}^2$  fragments at  $m/z$  152.860 and 108.898 through the loss of methyl and a  $\text{CO}_2$  respectively. Peak 6 demonstrated a molecular ion at  $m/z$  317.400 which is characteristic of lecanoric acid. We observed 3 representative ions at  $m/z$  166.970, 148.920 and 122.883 which facilitated the identification of lecanoric acid for us. These results for lecanoric acid are in great conformity with those from other studies (Brakni et al., 2018; Castro et al., 2017; Kumar et al., 2018; Musharraf et al., 2017, 2015; Parrot et al., 2015; Salgado et al., 2018; Torres-Benítez et al., 2017). Peak 7 as gyrophoric acid was detected at  $m/z$  467.612. Precise investigation of its cleavage pattern resulted in recognition of 3 major  $\text{MS}^2$  ions at  $m/z$  166.949, 148.912 and 122.920. A proportionate correspondence was observed between our findings and other studies (Castro et al., 2017; Cornejo et al., 2016; Kumar et al., 2018; Musharraf et al., 2015; Salgado et al., 2018; Torres-Benítez et al., 2017).

Peak 2 represented a molecular ion at  $m/z$  385.419 whose  $\text{MS}^2$  analysis revealed 3 diagnostic fragments at  $m/z$  167.020, 148.932 and 122.950. These  $\text{MS}^2$  ions are correspondent to those of stictic acid, therefore peak 2 was identified as the stictic acid, which is a depsidone. Our proposed fragmentation pattern for stictic acid in Fig 4 does not conform well to findings from other studies (Kumar et al., 2018; Matteucci et al., 2017; Parrot et al., 2013; Salgado et al., 2018; Torres-Benítez et al., 2017; Xu et al., 2017). Generally, after  $\text{CO}_2$  separation from the molecular ion ( $[\text{M}-\text{H}] = 385.419$ ), a fragment will appear at  $m/z$  341.07 whose cleavage results in the production of 2 fragments at  $m/z$  148.932 and 196.04. The detachment of  $\text{CH}_2\text{O}$  from the latter generates an ion at  $m/z$  167.020 and further loss of  $\text{CO}_2$  from this ion brings forth another fragment at  $m/z$  122.950.

In this study, we have detected gyrophoric acid and lecanoric acid in all the investigated ecotypes of *U. aprina* (Table 2). Orsellinic acid, umbilicinic acid, hiassic acid and the member of the hiassic acid complex were also found in all the ecotypes. Gyrophoric acid, lecanoric acid, umbilicinic acid, and norstictic acid are the most prevalent lichen substances in genus *Umbilicaria* (Serina et al., 1996). Gyrophoric acid is the most prominent secondary metabolite in *Umbilicaria* genus, commonly accompanied by lecanoric acid as a concomitant compound in lower concentrations (Posner et al., 1992). An informative result reported by Serina et al (1996) is the coincidence of gyrophoric acid and hiassic acid in their samples, so the co-occurrence of these two tridepsides in our investigated ecotypes was plausible. Based on the literature (Hestmark, 1991; Krog and Swinscow, 1986; Krzewicka, 2004; Krzewicka and Osyczka, 2002; Manojlović et al., 2012; Narui et al., 1998, 1996; Poelt and



Nash, 2006; Posner et al., 1992; Serina et al., 1996; Upreti and Pant, 2008; Zlatanović et al., 2017) presence of umbilicic acid in all of the *U. aprina* ecotypes is also admissible.

Orsellinic acid as a monocyclic aromatic compound, evernic acid as a depside and the member of hiassic acid complex as a tridepside are secondary metabolites detected in this study and they have not been reported from *Umbilicaria* to date. Posner et al (1992), emphasized the systematic significance of stictic acid or norstictic acid in *Umbilicaria* species and they ascertained the presence of stictic acid in *U. torrefacta* and here we detected the presence of stictic acid in *U. aprina* for the first time.

### 3.2. phytochemical discrepancies between ecotypes

In this study, Kivarestan, Tochal, and Sahand ecotypes indicated a similar secondary metabolite profile and we could elucidate their compounds as orsellinic acid, stictic acid, umbilicic acid, hiassic acid, putative hiassic acid complex member, lecanoric acid and gyrophoric acid on the basis of their MS/MS spectra and fragmentation patterns (Table 2). Sabalan ecotype showed the same secondary metabolites with foresaid ecotypes but it lacked stictic acid. For Takht-e Nader ecotype an unknown compound was observed at  $m/z$  347.400 we could not identify, however ignoring this extra substance, the secondary metabolite pattern for this ecotype showed similarities with Kivarestan, Tochal and Sahand ecotypes (Table 2). Mishan ecotype indicated the most multifarious metabolic profile by the presence of two extra substances including evernic acid and an unknown compound at  $m/z$  347.400 while it lacked stictic acid (Table 2).

Qualitative differences (e.g. presence or absence of a particular compound) in the phytochemical profile of some ecotypes, for example, absence of stictic acid in Mishan and Sabalan ecotypes or existence of evernic acid and an unknown compound in Mishan ecotype (Table 2) can be justified as a type of quantitative variation. Presence or absence of a specific compound, or at least our inability in detecting that particular compound, may be related to a status of drastic quantitative fluctuation i.e. the concentration of that compound have endured intense fluctuations in a way that we can't detect it readily with the aid of common approaches. The prominent example of such condition in our study is stictic acid. Fahselt (2008), on the basis of results from previous studies by Clayden (1992) and Culberson et al. (1993) (Clayden, 1992; Culberson et al., 1993), emphasizes on down-regulation of synthetic enzymes or significant attenuating changes in coding regions which can result in severe quantitative fluctuations. Such chemical plasticity is a considerable strategic reaction of lichens to different ecological zones. It is substantiated by data from number of studies that secondary metabolite fluctuations in thalli of lichens, especially in ecotypes of a particular species, can be affected by various environmental factors such as

allelopathy (Fahselt, 2008), geographical distribution (Bjerke et al., 2005; Culberson, 1967; Piercey-Normore, 2007, 2003), light and UV intensity (Rubio et al., 2009), habitat elevation (Shukla et al., 2016; Swanson et al., 1996), seasonality (Ranković and Kosanić, 2015) and temperature oscillations (Bjerke et al., 2003; Culberson et al., 1983; Hamada, 1991, 1984, 1982). Therefore, we expect phytochemical alterations in a specific impressionable species under various climatic conditions.

Due to its preference, *U. aprina* grows mostly in elevated alpine habitats, where the distinguishing characteristic of the environment is the labile climatic condition which predispose *U. aprina* ecotypes to various sequential abiotic stresses. Among all factors Altitude, light intensity and moisture might be the most effective driving forces in changing the chemical profile of lichens. As the radiation intensity can be affected by the topographic factors like slope aspect on which the lichen is growing, we can consider an important role for the efficacy of this factor (Shukla et al., 2016). Moreover, the intensity of radiation can modify the amount of moisture in a particular locality. Despite the limited distance between Takht-e Nader, Mishan and Kivarestan ecotypes, certain qualitative chemical differences were observed between them. The two main differences between the localities of these three ecotypes were the altitude and the slope aspect on which they were growing. The aspect map of the Takht-e Nader, Mishan and Kivarestan ecotypes are presented in Fig 5. The localities of Takht-e Nader and Kivarestan ecotypes are both southeast-facing while locality of Mishan ecotype is east-facing which can modify the intensity of radiation for each ecotype. Moreover, there is tangible difference between the locality altitude of these three ecotypes (Table 1) as if the locality of Kivarestan ecotype lies in a lower altitude (2449 m) and the elevation rises for localities of Mishan (2691 m) and Takht-e Nader (3028 m) ecotypes. The rise in elevation can result in different temperatures and UV intensity for each locality. As we can see in Fig. 11, locality of the Tochal ecotype lies on a south-facing aspect which means that this locality receives much more direct radiation compared with a locality which is lied on a north-facing aspect like the one for Sabalan ecotype. The locality of Sahand ecotype shows a southwest-facing aspect. According to Fig 5, it seems that the only south-facing localities belong to Tochal and Sahand ecotypes and therefore they might have received much more direct radiation compared to other ecotypes. Therefore, it might be possible that ecotypes with more similar slope aspect indicate much more resembling chemical profiles. Since the localities of Sabalan and Mishan ecotypes are likely to receive lower intensities of direct radiation maybe we could consider this factor as a reason for lack of the Stictic acid in both of these ecotypes. Moreover, Sabalan ecotype belongs to a geographical region that long periods of cold and fridity pervades the area for most of the year, therefore this persistent freezing state may influence the quality of chemical profile in Sabalan ecotype. Accordingly, the variation of chemical profile of our

investigated ecotypes may be attributable to all these environmental and topographic differences between their localities.

Based on our results in this study, which indicated differences between phytochemical profiles of investigated *U. aprina* ecotypes (Table 2), and also results from previously mentioned studies, perhaps we could ascribe such phytochemical variations to the effect of a set of environmental and endo-lichenic interactions that lead to suppression or increase in expression of some genes that subsequently affects the activity of certain enzymes in the biosynthetic pathway of various compounds.

#### 4. Conclusions

A phytochemical analysis including metabolic profiling of six *U. aprina* ecotypes was performed in this study. To the best of our knowledge, this study represents the first metabolic profiling of *U. aprina* making use of LC-ESI-MS/MS approach which resulted in identification of 9 major secondary metabolites (orsellinic acid, umbilicic acid, evernic acid, stictic acid, hiassic acid, hiassic acid complex member, lecanoric acid, gyrophoric acid and an unknown compound), mainly on the basis of their fragmentation patterns. Cleavage pathways are proposed for umbilicic acid, hiassic acid and hiassic acid complex member for the first time. Certain ecotypes were indicated to have similarities in chemical profile but there were also considerable variations between them. Orsellinic acid, umbilicic acid, hiassic acid, hiassic acid complex member, lecanoric acid and gyrophoric acid were ubiquitous compounds as if they were detected in all the investigated ecotypes, but the majority of differences between ecotypes can best be elucidated by presence or absence of stictic acid, evernic acid, and an unknown compound. Perhaps these 3 compounds are the key secondary metabolites for *U. aprina*'s chemotaxonomy. The most diverse metabolic profile was detected for the Mishan ecotype which indicated the presence of 8 out of 9 identified compounds.

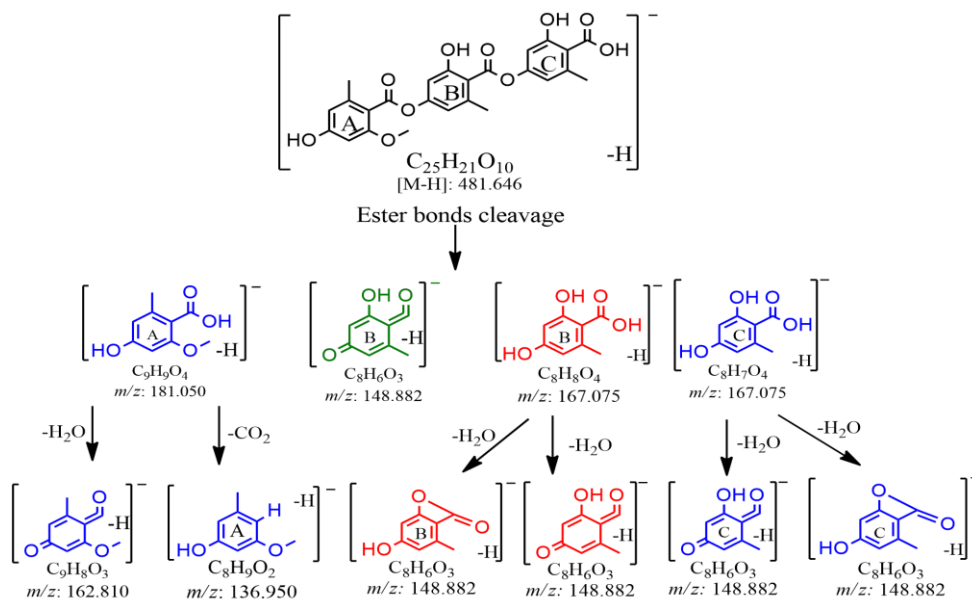
Phytochemical differences revealed in this study, suggest that a particular lichen species, like *U. aprina* in our study, possess potent sentience which enables them to perceive the changes in environmental factors and geographical distribution. Based on the variations described previously, we can assert that ecological differences impose alterations on the metabolic profile of *U. aprina* but we need to study the effect of different environmental factors and also genetic variabilities between ecotypes so that the main reason for chemical differences will be clearly established. Moreover, the structure and nature of the unknown compound at  $m/z$  347.020 remain to be studied by accurate approaches.

**Table 1**

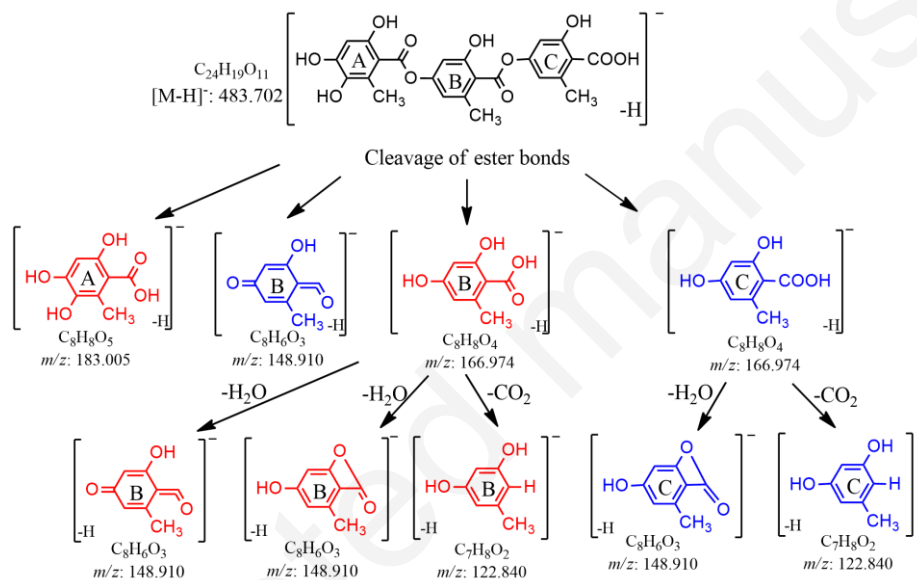
Lichen material investigated in this study: details of collection sites

	U. aprina ecotype	Voucher No.	GPS coordinates of localities	Altitude <sup>a</sup>
1	Kivarestan	18723	34.759 N 48.432 E	2449
2	Takht-e Nader	18755	34.769 N 48.409 E	3028
3	Mishan	18759	34.768 N 48.426 E	2691
4	Tochal	18731	35.858 N 51.396 E	3070
5	Sabalan	18740	38.321 N 47.845 E	3032
6	Sahand	18750	37.610 N 46.417 E	3032

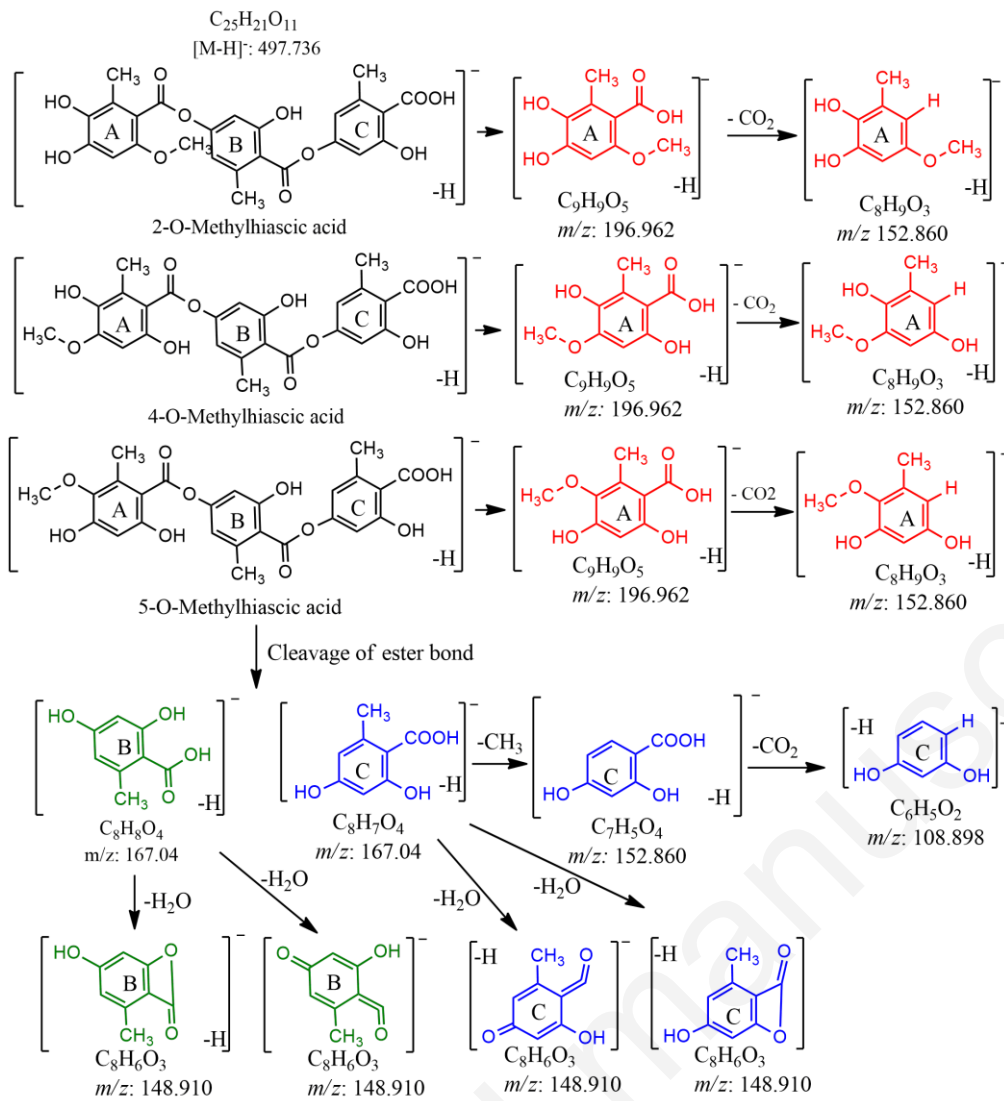
<sup>a</sup> Altitude expressed in meters above sea level



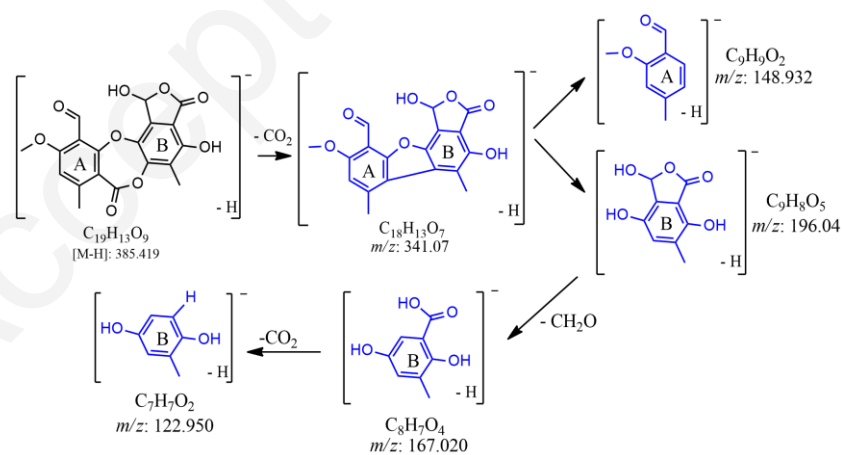
**Fig 1** Proposed fragmentation scheme of umbilicic acid



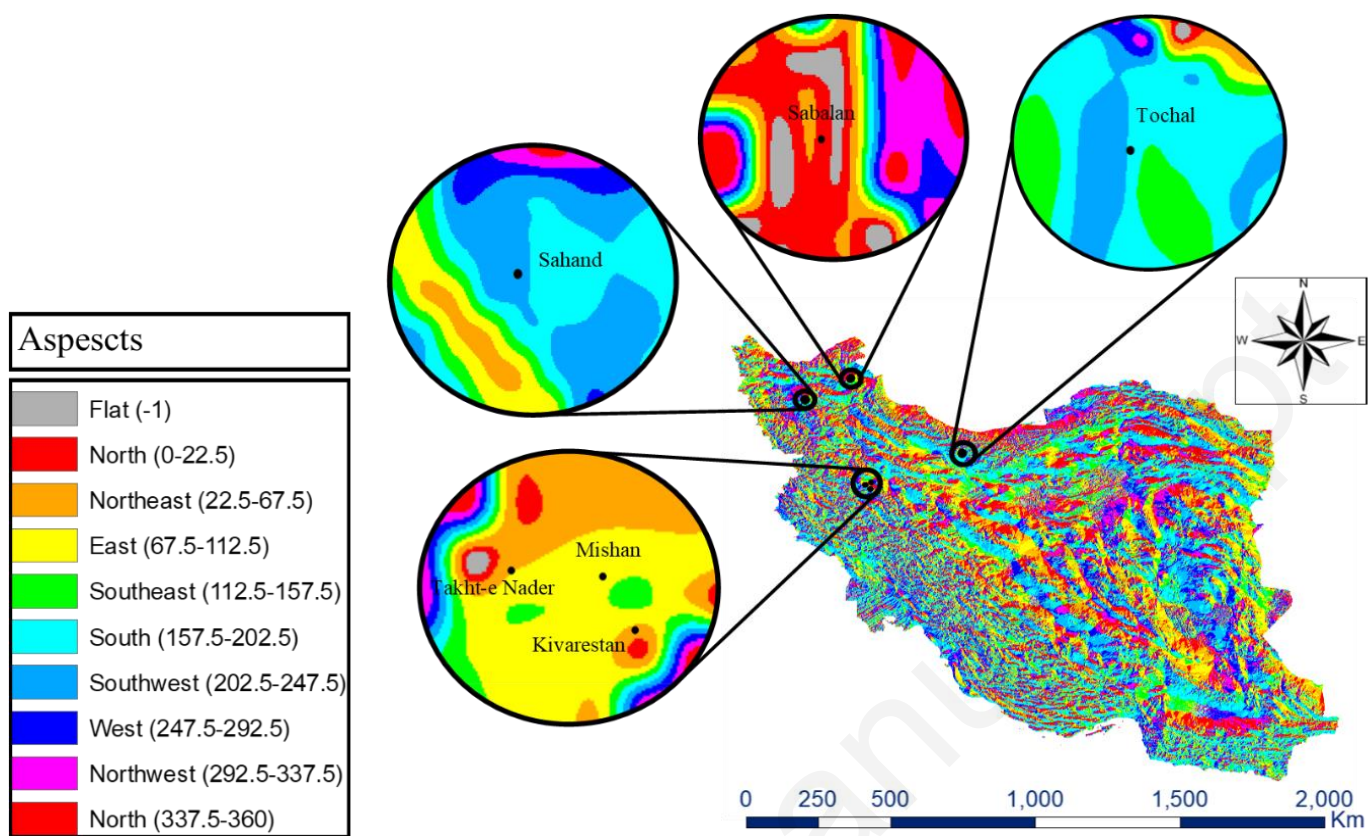
**Fig 2** Proposed fragmentation scheme of hiassic acid



**Fig 3** Proposed Fragmentation scheme of hiassic acid complex member



**Fig 4** Proposed fragmentation scheme of stictic acid



**Fig 5** Aspect map of Iran and localities of investigated ecotypes

**Table 2**

Characterization of major compounds from 6 *U. aprina* ecotypes identified using LC-ESI-MS/MS. [M-H]<sup>-</sup>: *m/z* of the molecular ion; Rt (min): retention time for each compound; MS/MS ions (at 30 eV): product ions for each compound; (R.I. %): relative intensities %; Presence or absence of identified secondary metabolites in each *U. aprina* ecotype is indicated respectively by + and -

Peak no.	Molecular formula	[M-H] <sup>-</sup> <i>m/z</i>	Rt (min)	MS/MS ions ( <i>m/z</i> ) at 30 eV (R.I. %)	Identification	Ecotypes					
						Kivarestan	Takhte-e Nader	Mishan	Tochal	Sabalan	Sahand
1	C <sub>8</sub> H <sub>7</sub> O <sub>4</sub>	167.210	14.72	81.385 (100)	Orsellinic acid	+	+	+	+	+	+
2	C <sub>19</sub> H <sub>13</sub> O <sub>9</sub>	385.419	14.78	167.020 (100)/ 148.932 (30)/ 122.950 (25)	Stictic acid	+	+	-	+	-	+
3	C <sub>25</sub> H <sub>21</sub> O <sub>10</sub>	481.646	15.02	181.050 (100)/ 167.075 (20)/ 162.810 (40)/ 148.882 (25)/ 136.950 (30)	Umbilicatic acid	+	+	+	+	+	+
4	C <sub>17</sub> H <sub>15</sub> O <sub>7</sub>	331.398	15.03	180.952 (15)/ 162.723 (35)/ 148.915 (18)/ 136.915 (100)/ 104.848 (25)	Evernic acid	-	-	+	-	-	-
5	C <sub>24</sub> H <sub>19</sub> O <sub>11</sub>	483.702	16.37	183.005 (10)/ 166.974 (100)/ 148.910 (10)/ 122.840 (5)	Hiascic acid	+	+	+	+	+	+
6	C <sub>25</sub> H <sub>21</sub> O <sub>11</sub>	497.736	16.56	196.962 (95) / 152.860 (100)/ 148.910 (30)/ 108.898 (10)	Hiascic acid complex member	+	+	+	+	+	+
7	-	347.313	16.60	197.251 (12)/ 152.867 (100)/ 148.992 (30)/ 108.883 (60)	Unknown	-	+	+	-	-	-
8	C <sub>16</sub> H <sub>13</sub> O <sub>7</sub>	317.400	17.70	166.970 (90)/ 148.920 (50)/ 122.883 (100)	Lecanoric acid	+	+	+	+	+	+
9	C <sub>24</sub> H <sub>20</sub> O <sub>10</sub>	467.612	17.70	166.949 (100)/ 148.912 (20)/ 122.920 (5)	Gyrophoric acid	+	+	+	+	+	+



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