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Articles

Analysis of leaf flavonoid composition of some Iranian Cotoneaster Medik. (Rosaceae) species

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The genus *Cotoneaster* consists of unarmed shrubs that naturally distributed in north parts of Iran. It is a problematic genus, and there are many discussions about its species number in Iran as well the World. Recently, M Khatamsaz has been listed 19 species of it in Iran. In the current research, we studied leaf flavonoid composition of seven *Cotoneaster* species from Iran. These species were harvested from the northern provinces of the country and their leaf ethanolic extracts were subjected to HPLC, for detection the types and amounts of their flavonoid compounds. We identified four flavonoid compounds: rutin, myricetin, quercetin, and kaempferol. The concentration of these flavonoids differed between the species, moreover amount of each flavonoid also varied among the studied species. All of them were registered in the studied species, except for myricetin, which was not observed in *C. nummularius*. The highest amount of flavonoids were detected in *C. nummularius,* while *C.* discolor had the lowest one. The evaluated species divided into four distinct group in UPGMA tree. According to CA-Joined plot, each group was characterized by species amount of flavonoid(s). All of evaluated species belonged to the same section of the genus, therefore our findings revealed that the flavonoid data were useful at sectional level for identification of the species.

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Introduction

The genus *Cotoneaster* Medik.belongs to the tribe Pyreae of the subfamily Spiraeoideae-Rosaceae (Potter et al.2007). Its species number varies and ranges from about 50 to over 400 (Niaki et al. 2019). According to previous evaluations (Marshall and Brown 1981; Nogler 1984), the most prominent reason for the wide range of species number was the asexual seed production (apomixes) that is often associated with hybridization and polyploidy.

Dickoré and Kasperek (2010) have suggested that *Cotoneaster* distribution is often scattered and mainly concentrated in the mountains of the meridional and nemoral zones, while its center of

diversity is in China and the Himalayas.

Cotoneaster is a problematic genus, mainly because it comprises nearly 500 published binomials. Riedl (1969) listed 14 species in Iran, but Khatamsaz (1992) introduced 19 species. In Iran, these species are mainly distributed in the Alborz Mountains, high elevations in the northwest and northeast of the country (Raei Niaki et al.2009; Raei Niakiet al. 2019).

The genus includes numerous ornamentals, which are widely cultivated in landscaping for their attractive flowers and fruit (Bailey and Bailey 1976). Moreover, several species of the genus, for example *C. melanocarpus* Lodd., *C. nummularia* Fisch. et Mey, and *C. tricolor* Pajork, are used in folk medicine, in different parts of the world, to treat various disorders such as nasal hemorrhage, excessive menstruation, hematemesis, neonatal jaundice, fever and cough (Holzer et al. 2013; Esmaeili et al. 2015). It is important to know that most of these properties can be attributed to the presence of low-molecular weight polyphenols including simple phenolic acids and flavonoids (Khan et al. 2009; Sati et al. 2010).

Flavonoids are considered as polyphenolic secondary metabolites that play different roles in plants. These compounds are involved in the production of flower pigments and also in plant protection from various pathogens. For example, some kinds of flavonoids are antifungal and antibacterial agents, while other types have significant roles in the plants protection from insects and herbivorous mammals (Harborne and Williams 2000).

The chemical stability and widespread occurrence make the flavonoids as chemical markers in the classification of plants and they are considered as a useful tool for identification and taxonomy of higher plants (Noori et al. 2015a, b; Noori and Talebi 2017).

For these reasons, in the current study we investigated leaf flavonoid compounds of seven Iranian species of *Cotoneaster* inorder to identify their flavonoid compounds and species relationship based on these data. As far as we could search, no similar previous study was found on these species in Iran.

Material and method

Plant materials

Plant samples of seven *Cotoneaster* species were harvested from natural populations in two provinces of northern Iran (Table 1) and were identified according to descriptions provided in Flora of Iran (Khatamsaz 1992) and Flora of USSR (Pojarkova 1941). One of the collected samples did not match with descriptions in Flora of Iran and Flora of USSR, and it seems that the sample belongs to a new species of *Cotoneaster*from Iran. Therefore, we decide to definite it as C. new, until its legal name is validate. Voucher specimens were deposited at Herbarium of Islamic Azad University Tehran (IAUNT).

Voucher No.	Localities, elevation a.s.l.	Species
AUNT-17413	Mazandaran province, 72 km Varsk-Firouzkoh, 3346 m	C. discolor Pojark.
AUNT-17406	Golestan province, Kolaleh, Aziz Abad, 1140 m	C. insignis Pojark.
AUNT- 17408	Golestan province, Til Abad, Khosh Yeylagh, 1570 m	C. kotschyiKlotz, Wissensch. Zeitschr.
AUNT-17418	Golestan province, Til Abad, Khosh Yeylagh, 1570 m	C. morrisonensis Hayata.
AUNT-17411	Mazandaran province, Avard, Galogah, 1217 m	C. morulus Pojark.
AUNT-17421	Mazandaran province, Pole Akhondi,	C. new

	Galogah, 1034 m	
AUNT-17414	Mazandaran, Sefidchah, Galogah, 1054 m	C. nummulariusFisch. & C.A Mey.

Table 1. *Localities and herbarium numbers of the studied species of Cotoneaster.*

Preparation of Plant Extracts

About 0.5 g of fine powdered leaf sample of each species were extracted with 5 ml HPLC methanol (80%) through open reflux process at 40 °C for 24 h. Then the extracts were filtered through filter paper to remove free unextractable substances. The filtrates solution of plant extracts were preserved 4 °C for further process.

HPLC Analysis

The HPLC analysis of the extract was performed with Chromatographic system (Agilent – infinity II). The separation was performed on a SGE Protecol PC18GP120 (250mm×4.6 mm, 5µm) column at ambient temperature. The mobile phase consists of methanol to water (70:30 v v^{-1}) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 ml min^{-1}. The samples were run for 15 min and detection was done at 352 nm by UV detector. Rutin was used as standard and flavonoid content was determined as rutin equivalent. For this purpose, a calibration curve for rutin was drawn. From the standard rutin solution the dilutions of 0.080, 0.040, 0.064, and 0.126 mg ml^{-1} concentrations were prepared in methanol.

Statistical analysis

For clustering of the investigated species, the flavonoid data were standardized (mean = 0, variance = 1) and used for multivariate analyses, including UPGMA (Unweighted Pair-Group Method with Arithmetic mean) and Correspondence analysis (CA-joined plot). Plots clustering were performed in Multivariate Statistical Package (MVSP) software (Podani 2000).

Results

The extracted flavonoids extracted from species leaves were presented in Table 2. Among the studied species, *C. nummularius* had the highest total amount of flavonoids, while *C. discolor*. had the lowest total amount. Kaempferol and myricetin were the most and less abundant flavonoids respectively.

Species	Rutin	Myricetin	Quercetin	Kaempferol
C. discolor	11.99	0.13	7.24	0.14
C. insignis	16.75	0.06	34.10	0.06
C. kotschyi	7.94	4.06	7.14	0.89
C. morrisonensis	9.61	4.72	7.13	0.47
C. morulus	6.30	2.32	255.51	0.10
C. new	13.98	5.13	417.92	0.32
C. nummularius	10.58	n/a	4.00	7516.7

Table 2. *Flavonoid compounds were found from methanolic extract of leaves (all values are mg g^{-1} dried tissue).*

The highest (16.75 mg g^{-1}) and lowest (6.30 mg g^{-1}) amounts of rutin were registered in *C. insignis* and *C. morulus,* respectively. Myricetin was not observed in *C. nummularius*, while its highest amount was reported from *C. new* (5.13 mg g^{-1}). Moreover, *C. new* had the highest amount (417.92 mg g^{-1}) of quercetin, whereas the lowest amount (4.00 mg g^{-1}) of it was registered from *C. nummularius*. We recorded the largest amount (7516.7 mg g^{-1}) of kaempferol in *C. nummularius*,

whereas *C. insignis* had the lowest amount (0.06 mg g^{-1}) (Fig.1).

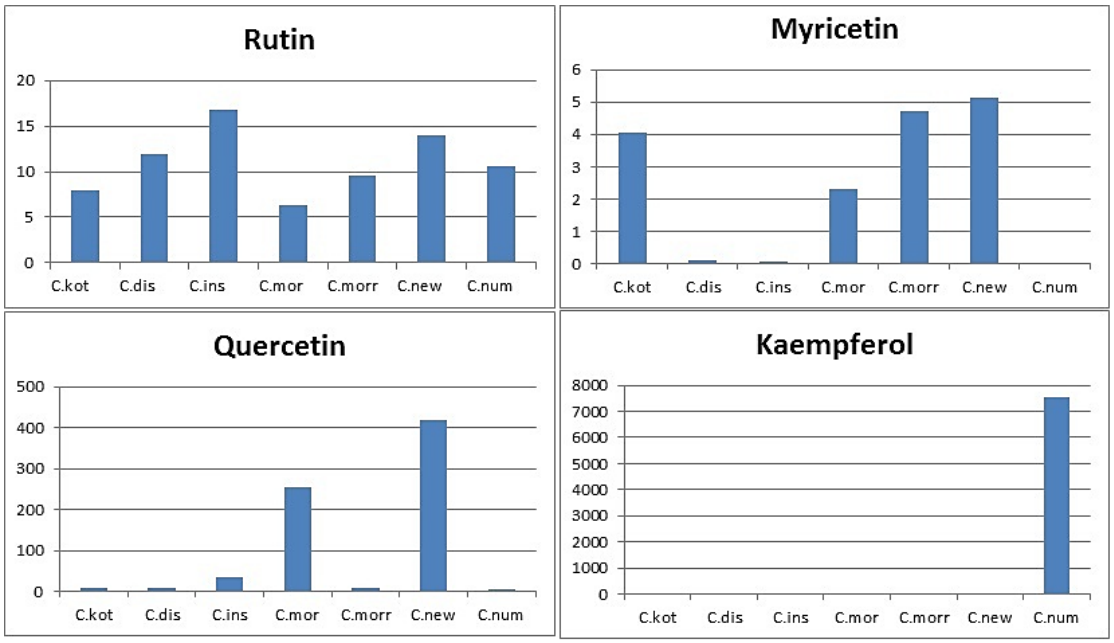

Figure 1. *Pattern of flavonoids existence among the studied Cotoneaster species.*

The studied species were clustered in UPGMA tree of phytochemical data (Fig. 2). The tree had two clades: *C. nummularius* placed separately in the small clade, while the rest populations were in the large clade, which were divided into two branches. One branch was consisted of *C. new* and *C. morulus*, while another one had two groups. *C. insignis* was registered in a group, however the rest species place closely in another group. Therefore, the studied species were observed in four groups.

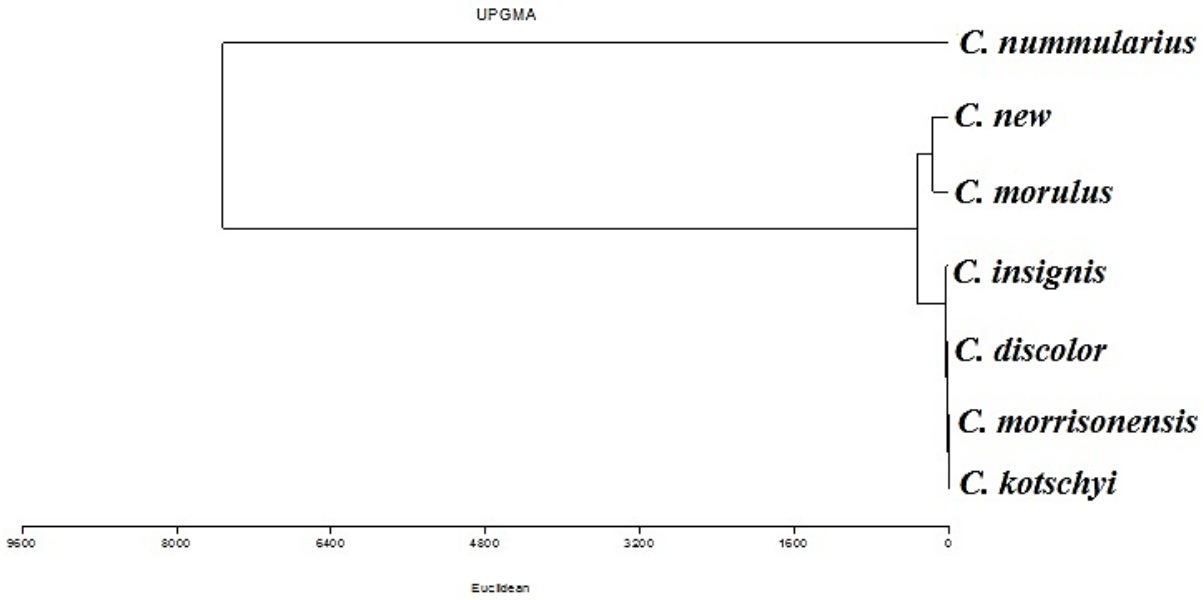

Figure 2. *UPGMA tree of the studied species according to phytochemical data*

Ca-joined plot revealed these group had species flavonoid type that was useful in identification of them. For example, *C.nummularius* was characterized by kaempferol, or *C. morulus*and*C. new* had the highest amount of quercetin (Fig. 3).

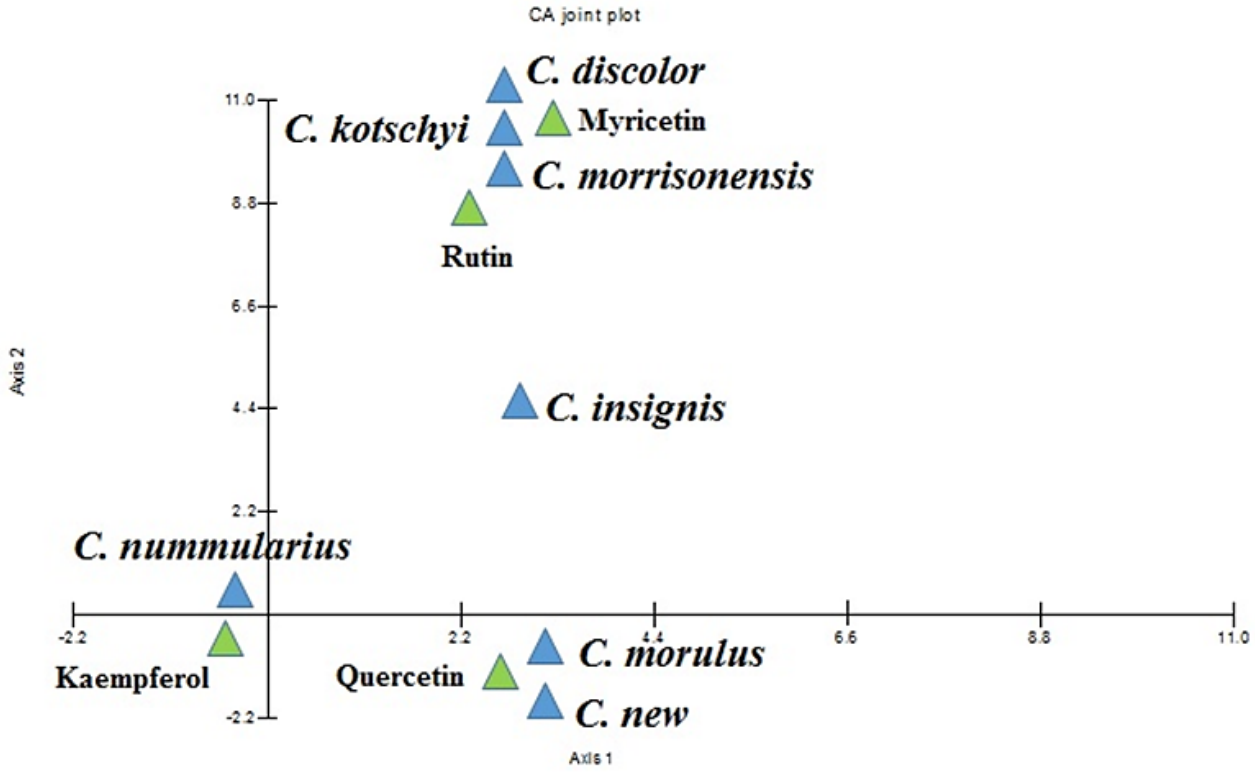

Figure 3. *CA- joined plot of the phytochemical data and species (the blue and greed symbols are species and chemical compounds, respectively).*

Discussion

In the current study, we detected flavonoids from the leaf ethanolic extract of seven Iranian *Cotoneaster* species. In total, four flavonoids were identified: myricetin, kaempferol, rutin, andquercetin. All of these flavonoids belong to flavonols subgroup. Flavonoids can be divided into several subgroups according to the substitution patterns of the ring C, and flavonoids within the same class can be differentiated by the substitution of A and B. These subgroups are: flavonoids, flavanones, isoflavonoids, flavones, flavans-3-ol, and anthocyanins (Prasain et al. 2010; Havsteen 2002).

According to previous studies, these flavonoids have several medicinal properties. For example, Godse et al. (2010) have suggested that myricetin has antihypertensive effect. This flavonoid hindered the progression of high blood pressure and also turned the metabolic alternations in rats on fructose-induced diet. In addition, according to Chang et al. (2012) findings myricetin suppresses body weight increment and accumulation of fat by maximizing the fatty acids oxidation that was due to enhance the regulation of hepatic peroxisome proliferator activated receptor and decrease the regulation of hepatic sterol regulatory element- binding protein expressions in rats on high fat diet.

Kaempferol was the most abundant flavonoid among the studied species. Previous investigations (Vinson et al. 1995; Asif and Khodadadi 2013) have revealed that kaempferol has various properties such as inhibitory effect on HIV protease, anti-inflammatory, antioxidant, antiulcer activity and antitumor.

Quercetin was the second most abundant flavonoid in our studied species. It is the most active flavonoid and many medicinal plants owe their functions to their high content of this flavonoid. Lim et al. (2007) have believed that the significant anti-inflammatory property of this compound is related to direct inhibition of the inflammation initial processes. Moreover, investigations have revealed that quercetin has anticancer property, including in inhibiting the proliferation and also migration of cancer cell.

Rutin is a more effective antioxidant than vitamins and has antioxidant and free radical scavenging effect in foods (Vinson et al. 1995; Hollman et al. 1997). Rutin and quercetin were extracted from leaf extracts of several *Cotoneaster* species such as *C. zabelii*, *C. bullatus*, *C. splendens*, *C. dielsianus*, *C. hjelmqvistii*, *C. horizontalis*, *C. divaricatus*, *C. lucidus*, *C. melanocarpus*, *C. tomentosus*, and *C. integerrimus* (Kicel et al. 2016).

According to UPGMA tree of flavonoids data, the studied species were divided into four groups. Group I including *C. nummularius*, group II had *C. new* and *C. morulus,* group III consisted of*C. insignis* and group IV has been consisted of *C. discolor*, *C. kotschyi* and *C. morrisonensis.* CA-Joined plot revealed that each of these groups had specific compound(s) that was useful in identification of them. This results revealed that the flavonoid data are useful in identification of species of the genus. In addition, all of our examined species belonged to the same section of *Cotoneaster*, therefore flavonoids can be used as the phytochemical marker in sectional level of the genus. Our findings were in agreement with previous similar works. Webb and Harborne (1991) have suggested that flavonoid data were meaningful in infra-generic classification of the plants, especially at sectional level. In addition, it is well revealed that the flavonoid data on flowering plants are often of value for resolving the evolutionary relationships among the taxa (Markham et al. 1970). For instance, flavonoid data can be used as a taxonomic features of the genus *Chromolaena*, and the chemotaxonomic significance of these compounds was useful for resolving the infra-generic complexity of the genus (de Oliveira et al. 2017). Furthermore, Braunberger et al. (2015) have reported that flavonoids were used as chemotaxonomic characteristics in *Drosera*for

the species classification in sections and for the improvement of infra-generic taxonomy in this genus.

As mentioned in material and methods section, one of plant samples that was harvested from Mazandaran province did not morphologically match with the descriptions of identified *Cotoneaster* species in Iran and neighboring countries. Therefore, we considered it as a new species for Iran and contemporary definite it as *C. new*. In present study, this species closely grouped with *C. morulus*, according to flavonoid data. The harvesting localities of these species are close to each other, so there can be two issues in this regard. (1) *C. new* can be a population of *C. morulus*, which is very distinct in the morphological characteristics, or (2) it is a new species of the genus for Iran created by the sympatric speciation. However, further molecular, cytological, anatomical, and palynological investigations is needed to verify this.

Christensen (1992) has stated that several new species have been described from North America by taxonomists, and *Cotoneaster* has more than 1000 species. Nevertheless, the current number of accepted species is about 200 and many of the described taxa have been recently reduced to synonyms. According to Dönmez (2004) suggestion, polymorphism, hybridization, and apomictic breeding strategies caused great differences among the populations of *Cotoneaster* species. Therefore, most of the taxa described by taxonomists should be regarded as synonyms because of genus diverse nature.

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