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Original Article

Investigation of Leaf Flavonoids of *Reseda* (Tourn.) et L. (Resedaceae) Members in Markazi Province, Iran

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Abstract

Reseda (Tourn.) et L. is a genus in Resedaceae family with 153 species and subspecies in the world and 14 species in Iran. Some flavonoid compounds have been reported from the *Reseda* genus. Flavonoids are as one set of the polyphenolic compounds among secondary metabolites in different organs of plants that are used in plant chemotaxonomy. They have basic role in pollination and life cycle of anthmophyllus plants and also their spreading abroad and survival. Phytochemical studies on 10 collected *Reseda* populations of 3 species (*R. lutea* L., *R. bungei* Boiss. and *R. buhseana* Mull-Arg.) from different parts of Markazi Province, Iran area were done using 2-dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC). Voucher specimens of each population were prepared for reference as herbarium vouchers. Results showed all of populations contain flavonoid sulphates and flavone *C* and *C-/O*-glycosides. All of studied *R. lutea* populations had kaempferol and quercetin. While both myricetin and luteolin were found in *R. buhseana* and *R. bungei* species, where as *R. lutea* lack.

Key words: *Reseda*, Resedaceae, Flavonoids, Chromatography.

Introduction

The Resedaceae are represented by six genus, five of them namely, *Reseda*, *Caylusea*, *Oligomeris*, *Ochradenus*, *randonia* are distributed in the Saharian regions. The *Reseda* genus is found in the Mediterranean and the South Western Asian areas [1,2]. There are about 14 *Reseda* species in Iran and 3 species *R. lutea* L., *R. bungei* Boiss. and *R. buhseana* (Mull-Arg.) in Markazi Province [3-5]. Secondary metabolites specially flavonoids are valuable and widely and effectively used in chemosystematics [6]. Flavonoids are a diverse group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, leaves, flowers, tea and wine [7,8]. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified [9]. In contrast to earlier studies, all these compounds are no longer judged as waste products, nor as evolutionary remnants without current function, nor as mere metabolic end products that are toxic to the plant and are therefore to be stored away in vacuoles. Moreover, they possess a wide range of biological activities. One of them is their contribution to human health which has made them prominent in the past 10 years [10]. Many flavonoids are active principles of medicinal plants and exhibit pharmacological effects [11]. Woelfle et al (2010) showed *Reseda luteola* L. extract displays antiproliferative and pro-apoptotic activities that are related to its major flavonoids [12]. Flavonoids are also beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents, and, in general, by their significant role in plant resistance [13].

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Flavonoids protect plants against various biotic and abiotic stresses and play an important role in the interaction between the plant and their environment [14]. Noori et al (2012) showed phytochemical changes consisting of flavonoids kind and number varieties are defensive reactions of plant against physiological stresses such as UV-C [15]. Also these compounds serve essential functions in plant reproduction by recruiting pollinators and seed disperses. They are also responsible for the beautiful display of fall color in many plant species, which has recently been suggested to protect leaf cells from photo-oxidative damage, thereby enhancing the efficiency of nutrient retrieval during senescence [16]. Flavonoids are popular compounds for chemotaxonomic surveys of plant genera and families. Several studies indicated that flavonoids occurred in various species of *Reseda*. Eight flavone, 15 flavonols and one isoflavone have been reported from the *Reseda. Reseda luteola* contains 40% flavonoids, primarily luteolin, but also luteolin-7-*O* glucoside and apigenin [12]. Moiteiro et al (2008) found luteolin 4́-*O*-glucoside in *Reseda luteola* for first time [17]. Berrahal et al (2006) reported five flavonoid glycosides, quercetin-7-*O*-α-L-rhamnosyl-3-*O*-β-D-glucoside, isorhamnetin-3 *O*-β-D-glycosyl-7-*O*-α-Lrhamnoside, kaempferol-7-*O*-α-L-rhamnoside, kaempferol-7-*O*-α-L-rhamnosyl-3-*O*-β-D-glucoside and kaempferol-3, 7-*O*-α-L-dirhamnoside from aerial parts of *R. villosa* for first time [18]. El-Sayad et al. (2001) isolated aglycone flavonols, kaempferol and quercetin from the Mediterranean *Reseda* species [19]. Also Yuldashev et al. (1996) reported flavonol diglycosides of kaempferol, quercetin and isorhamnetin from four other *Reseda* species [20]. Rzadkowska (1969) isolated four 3-*O*-glycosides from *R. lutea* [21].

The aim of this study was to compare the leaf flavonoid profiles of 10 populations from three *Reseda* species (*R. lutea* L., *R. bungei* Boiss. and *R. buhseana* Mull-Arg.) in Markazi Province, Iran. Leaf flavonoid compounds of *R. buhseana* and *R. bungei* are reported for the first time.

Materials and Methods

Collection of plant material and praperation

Mature fresh leaves of 10 *Reseda* populations from 3 species (*R. lutea* L., *R. bungei* Boiss. and *R. buhseana* (Mull-Arg.) were collected from Markazi Province, Iran area during 2009 as described in Table 1. Plants identified using available references [4, 5, 22]. Specimens of each sample were prepared for reference as herbarium vouchers that were deposited at the Arak University herbarium. Samples were air dried for detection and identification of flavonoids.

Extraction of the plant material

For a comparative analysis of the flavonoids, small extracts of all the accessions were prepared by boiling 200 mg of powdered air dried leaf material for 2 min in 5 ml of 70% EtOH. The mixture was cooled and left to extract for 24 h. The extract was then filtered, evaporated to dryness by rotary evaporation at 40°, and taken up in 2 ml of 80% MeOH for analysis by 2-Dimensional Paper Chromatography (2-D PC).

Flavonoid analysis by 2-Dimensional Paper Chromatography (2-D PC)

For the detection of flavonoids, ca 20 µl of each of the small extracts was applied to the corner of a quarter sheet of Whatman No 1 chromatography paper as a concentrated spot (10 applications of 2µl). The chromatogram for each sample was developed in BAW (n-BuOH-HOAc-H_2O=4:1:5; V/V; upper layer), 1st direction, and HOAc (=15% aqueous acetic acid), 2nd direction, with rutin (= quercetin 3-*O*-rutinoside) as a standard. After development, the chromatograms were viewed in longwave UV light (366 nm) and any dark absorbing and fluorescent spots were marked. R_f -values in BAW and 15% HOAc were calculated.

Methods of identification of the flavonoids

When sufficient amounts of purified flavonoids had been obtained, as in the case of the flavonoids from 10 Reseda populations, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids [23,24] and by acid hydrolysis to identify the aglycone and sugar moieties. Cochromatography with standards was also performed where possible. Flavonoid standards available for comparison during the study were rutin, kaempferol, quercetin, myricetin, luteoline, apigenin and rhamnetin (all obtained commercially, rutin from Merck and the rest from Fluka).

Acid hydrolysis and identification of flavonoid aglycones

A small amount of each purified flavonoid (ca 0.5 mg) was dissolved in 0.5 ml of 80% MeOH in a test tube. To this sample 2 ml of 2M HCl were added and the mixture was heated in a water bath at 100°C for 0.5 h. The solution was cooled, 2 ml of EtOAc

were added and thoroughly mixed with the aqueous layer using a whirley mixer. The upper EtOAc layer was removed, evaporated to dryness, dissolved in 0.5 ml of MeOH and applied as spots on thin layer chromatograms (cellulose). The TLC plates were run in three solvents alongside standards to identify the aglycone moiety [25].

Results and Discussion

All studied *Reseda* populations contained flavonoid compounds in their leaves. Woelfle et al (2009) reported 8 flavone, 15 flavonols and 1 isoflavone from the genus [12]. Populations flavonoid profiles show a wide variety between the species. Data in Table 1 shows the sampling and also 2-dimentional paper and thin layer chromatographical data of 10 studied *Reseda* populations from Markazi Province, Iran and Figure 1 shows stacked column with a 3-D visual effect histogram for comprising leaf flavonoids data (number of total leaf flavonoids, number of flavonoid sulphate, number of flavone *C*- and *C-/O*-glucosides), number of aglycones and occurrence and concentrations of rutin, quercetin, kaempferol, myricetin, luteolin, apigenin and rhamnetin) in the populations. Secondary metabolites specially flavonoids are valuable and widely and effectively used in chemosystematics and phytochemistry [6]. In addition, Table 1 and Figure 1 show a wide variation in leaf flavonoid profiles in all studied samples. There are flavonoid sulphate and flavone *C* and *C-/O*-glycosides in all species with the exception CMG_{10}. Three populations (CMG_1, CMG_9 and CMG_{21}) have the highest number of total flavonoid compounds (10) and CMG_{13} has the lowest number of flavonoid compounds (5) in their leaves. Also all taxa studied, except 2 populations (CMG_1 and CMG_{21}) had not any aglycones (Table 1 and Figure 1). Identification of flavonoids by standards showed all of studied *Reseda* populations contain kaempferol and quercetin with the exception of CMG_{21} and CMG_{22} that do not have quercetin. Both quercetin and kaempferol are flavonols. The flavonols may be among the most important flavonoids, they are the most ancient and widespread of the flavonoids, synthesized even in mosses and ferns, and have a wide range of potent physiological activities [26]. Harborne and Baxter (1999) reported that quercetin is widely distributed in various plant families [27]. As El-Sayad et al (2001) isolated aglycone flavonols, kaempferol and quercetin from the Mediterranean *Reseda* species [19]. Also Yuldashev et al (1996) reported flavonol diglycosides of kaempferol, quercetin and isorhamnetin from four other *Reseda* species [20]. Rzadkowska (1969) isolated four 3-*O*-glycosides from *R. lutea* [21]. As Table 1 shows, all taxa studied, except 2 populations (CMG_9 and CMG_{21}) do not have myricetin. There are rutin in three CMG_9, CMG_{11} and CMG_{13} populations and others lacked. Apigenin was not found, but there was in CMG_{16} and CMG_{20} populations. Luteolin was obtained from CMG_9, CMG_{11}, CMG21 and CMG_{22}. There is doubt in existing rhamnetin in CMG_{11}, CMG_{13}, CMG_{14} and CMG_{16} populations and further work is needed.

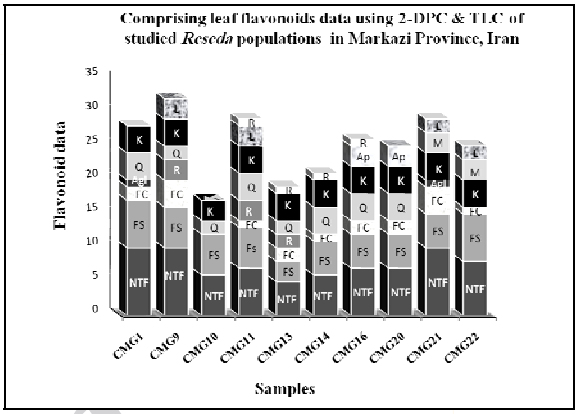

Fig 1 Stacked column with a 3-D visual effect histogram for comprising leaves flavonoids data using 2-Dimentional Paper and Thin Layer Chromatography of studied *Reseda* populations from Markazi Province, Iran. Scored characters for drowing 3-D column histogram in Excel based on Table 1 data: -0 (non flavonoid), ± 1 (UV absorbance < 0.1), + 2 (few flavonoid), ++ 3 (high concentration of flavonoid) and +++ 4 (very high concentration of flavonoid).
Abbreviation: NTF=number of total flavonoids, FS=number of flavonoid sulphate, FC=number of flavone C-and C-/O-glucosides, Agl=number of aglycones, R=rutin, Q=quercetin, K=kaempferol, M=myricetin, L=luteulin, A=apigenin, R=rhamnetin.

Conclusions

Finally, chemical study of 10 *Reseda* populations using two dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed all studied *Reseda* populations contained flavonoid compounds in their leaves and kempferol is the most representative compound for the genus. They may be useful taxonomic markers within the genus. Also *Reseda* flavonoids are very important for their toxicity and some different potential clinical applications such as their anti-atherosclerotic, anti-inflammatory, antitumor, anti-thrombogenic, anti-osteoporotic and antiviral effects [28].

Table 1 Collection information and 2-Dimentional Paper and Thin Layer Chromatographical data of 10 studied *Reseda* populations from Markazi Province, Iran area.

				Flavonoid type				**Identification**						
Voucher data	Taxon	Latitude	Longitude	Number of total flavonoids	Number of flavonoid sulphates	Number of flavone C- and C-/O-glucosides	Number of aglycones	Rutin	Quercetin	Kaempferol	Myricetin	Luteolin	Apigenin	Rhamnetin
* CMG_1	*R. lutea* L.	49° 46′ N	34° 06′ E	10	7	2	1	-	+++	+++	-	-	-	-
CMG_9	*R. lutea* L.	50° 05′ N	33° 39′ E	10	6	4	-	++	+	+++	-	++	-	-
CMG_{10}	*R. lutea* L.	49° 46′ N	34° 06′ E	6	6	-	-	-	+	++	-	-	-	-
CMG_{11}	*R. lutea* L.	50° 29′ N	33° 53′ E	7	6	1	-	++	+++	+++	-	++	-	±
CMG_{13}	*R. lutea* L.	50° 29′ N	33° 53′ E	5	3	2	-	+	+	+++	-	-	-	±
CMG_{14}	*R. lutea* L.	50° 05′ N	33° 39′ E	6	5	1	-	-	+++	+++	-	-	-	±
CMG_{16}	*R. lutea* L.	49° 25′ N	33° 56 E	7	5	2	-	-	+++	+++	-	-	++	±
CMG_{20}	*R. lutea* L.	49° 46′ N	34° 06′ E	7	5	2	-	-	+++	+++	-	-	++	-
CMG_{21}	*R. buhseana* Mull-Arg.	50° 20′ N	35° 03′ E	10	5	4	1	-	-	+++	++	+	-	-
CMG_{22}	*R. bungei* Boiss.	50° 20′ N	35° 03′ E	8	7	1	-	-	-	+++	++	+	-	-

*CMG: Masomeh Ghorbani collection numbers, - (non flavonoid), ± (probably flavonoid), + (few flavonoid), ++ (high concentration of flavonoid) and +++ (very high concentration of flavonoid).

The presence of quercetin and absence of myrestin in *R. lutea* are taxonomic characters for separation of the species from two other species (*R. buhseana* and *R. bungei*). Among the many functions of flavonoids at the interface between plant and environment, their activity as signals was intensively studied. Flavonoids are also beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents, and, in general, by their significant role in plant resistance [13]. Our studies showed all collected *Reseda* populations are weed and grow in poor soils and destroyed pasture. Progress continues to be made in understanding the roles of flavonoids in stress protection, as well as in defining the mechanisms that control the amount and varieties of flavonoids that are produced in plants in responses to diverse environmental cuse [29]. But, further work is needed using high performance liquid chromatography with diode array detection, atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy to evaluate all flavonoid profiles in studied and other *Reseda* species.

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References

1. Dogan Y, Mert H, Akcan K. Anatomical studies of *Reseda lutea* (Resedaceae), Phytologia. 2008;14:91-95.
2. Dougan A. A Study on the Autecology of *Reseda lutea* L. (Resedaceae) Distributed in Western Anatolia, Turk J Bot. 2001;25:137-148.
3. Ghahreman A. Iranian Chormophytes, Markaze Nashre Publications, Tehran, Iran. 1993;205-214.
4. Ghahreman A. Flore de l'Iran, A join project by the Research Institute of Forests and rangelands (Iran) and Tehran University, Published by RIFR, Ministry of Reconstruction Jahad. Volumes 1-24, 1979-2006.
5. Rechinger KH. Flora Iranica, Akodemische Druck–U. Verlagsanstalt Graz–Austria. 1979;23:3–34.
6. Noori M. Characterization of the Iranian species of *Sophora* and *Ammodendron* (Leguminosae; Sophoreae), PhD Thesis, University of London and Royal Botanic Gardens, Kew, UK. 2002.
7. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function, Adv Exp Med Biol. 1998;439:175-182.
8. Robles C, Greff S, Pasqualini V, Garzino S, Bousquet-Melou A, Fernandez C, Korboulewsky N, Bonin G. Phenols and flavonoids in Aleppo pine needles as bioindicators of air pollution, J Environ Qual. 2003; 32:2265-2271.
9. De Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol. 1998;12:249-255.
10. Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancment of the diet by modifying the phenols content or profile, Journal of the Science of Food and Agriculture, 2000;80:985–1012.
11. Yilmaz Y, Toledo RT. Health aspects of functional grape seed constituents, Trends Food Sci Technol. http://dx.doi.org/10.1016/j.tifs. 2004.04.006.
12. Woelfle U, Simon-Haarhaus B, Merfort I, Schempp CM. *Reseda luteola* L. extract displays antiproliferative and pro-apoptotic activities that are related to its major flavonoids, Phytother Res. 2010; 24:1033-1036.
13. Treutter D. Significance of flavonoids in plant resistance: a review, Environ Chem Lett. 2006;4:147-157.
14. Pourcel L, Routaboul JM, Cheynier V, Lepiniec L, Debeaujon L. Flavonoid oxidation in plants: from biochemical properties to physiological functions, Trends Plant Sci. 2007;12:29-36.
15. Noori M, Poorimani R, Khodaee M. Studies of UV-C effects on Coronilla varia L. chlorophyll and flavonoids, J Cell Tiss. 2012;3:43-53.
16. Field TS, Lee DW, Holbrook NM. Why leaves turn red in autumn, The role of anthocyanins in senescing leaves of Red-Osier Dogwood, Plant Physiol. 2001; 127:566–574.
17. Moiteiro C, Gaspar H, Rodrigues Al, Lopes JF, Carnide V. HPLC quantification of dye flavonoids in *Reseda luteola* L. from Portugal. J Sep Sci. 2008;31 :3683-3687.
18. Berrahal D, Kabouche A, Kabouche Z, Bruneau C. Flavonoid glycosides from *Reseda villosa* (Resedaceae), Biochem Syst Ecol. 2006;34:777-779.
19. El-Sayad NH, Omara NM, Yosef AK., Farag AM, Mabry TJ. Kaempferol triosides from *Reseda muricata*, Phytochemistry 2001;57:575-578.
20. Yuldashev MP, Batirov EKh, Malikov VM, Yuldashev NP. Flavonoids of *Psoralea drupaceae* and *Reseda luteola*. Khim Prir Soedin. 1996;6:949-951.
21. Rzadkowska-Bodalska H. Flavonoids in flowers of weld (Reseda lutea). P.3. Identification of compound C Dissertationes Pharmaceuticae et Pharmacologicae 1969; 21: 169-172

Journal of Medicinal Plants and By-products (2012) 2: 171-176

22. Mozafarian V. Plant Classification, Nashre Daneshe Emroz publication, 1994;2;192-193.
23. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids, Springer Verlag, Berlin. 1970.
24. Markham KR. Techniques of Flavonoid Identification, Academic Press, London. 1982.
25. Harborne JB. Phytochemistry Methods, 3rd ed. Chapman and Hall, London. 1998.
26. Stafford HA. Flavonoid evolution: an enzymic approach, Plant Physiol. 1991;96:680–685.
27. Harborne JB, Baxter H. The handbook of natural flavonoids, Vol. 1, Wiley, Chichester, 1999.
28. Nijveldt RJ, Van Nood E, Van Hoof EC, Boelens PG, Van Norren, K, Van Leeuwen PAM. Flavonoids: a review of probable mechanisms of action and potential applications, Am J Clin Nutr. 2001;74:418-425.
29. Chalker-Scott L. Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol. 1999;70:1–9.