International Journal of Modern Botany 2014, 4(1): 1-7
DOI: 10.5923/j.ijmb.20140401.01

Fruit Flavonoids of Some Species of Subgenera Esula and Chamaesyce (Euphorbia) in Iran

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Abstract In this research, fruit flavonoids characters of some collected *Euphorbia* species (subgenera *Esula* and *Chamaesyce*) from Markazi province, Iran area were studied using 2-Dimentional Paper Chromatography (2-DPC), Thin Layer Chromatography (TLC) and available standard flavonoids. Voucher specimens were prepared for reference as herbarium voucher. Our studies showed that all of studied *Euphorbia* species had flavonoids considerably. Flavonoid sulphates and flavone *C*- and *C-/O*-glucosides existed in fruit of studied taxa, while dihyro flavonol 3-*O*-monoglycosides just were found in *E. falcata*. Kaempferol were detected in all of studied species in contrast to rhamnetin which was found only in *E. ozyridiforma*. Concenteration and variety of flavonoid componds in studied taxa of subgenera *Esula* were more than subgenera *Chamaesyce*. Some flavonoid compounds including myricetin, naringenin and rhamnetin were not detected in *Euphorbia* studied species of subgenera *Chamaesyce*.

Keywords Subgenera *Esula*, Subgenera *Chamaesyce*, *Euphorbia*, Flavonoids compounds, Chromatography

1. Introduction

The Euphorbiaceae is one of the larger families of flowering plants with ca. 300 genera and 8000 species [1, 2]. The genus *Euphorbia* belongs to the subfamily Euphorbioideae and comprises ca. 2000 species, mainly distributed in subtropical and warm temperate regions [3, 4]. *Euphorbia* subgenus *Esula* is the largest subgenus within *Euphorbia,* which largely corresponds to Boissier's *Euphorbia* sect. *Tithymalus* and has about 500 species. Subgenera *Chamasyce* is the large segregate genus from *Euphorbia* with about 300 species. *Euphorbieae* and *Euphorbia* are generally considered taxonomically difficult, and a considerable degree of uncertainty has always existed about the relationships of the groups within them [5]. Plant chemosystematics is the application of chemical data to systematic problems. It is a rapidly expanding interdisciplinary field concerned with using chemical constituents for explaining relationships between plants and inferring phylogeny [6]. Flavonoids are popular compounds for chemotaxonomic surveys of plant genera and families because of their almost ubiquitous presence in vascular plants, structural variety, ease of detection and relative ease of identification [7]. They are the most numerous of the phenolics and are found throughout the plant kingdom [8]. To date, more than 6400 different flavonoid compounds have been identified [9] and the pathways responsible for their synthesis have been characterized in detail in numerous plant species [10, 11, 12, 13, 14]. Flavonoids are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. They are widely distributed in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa, and wine [15, 16]. Within the subgroups of the flavonols and the flavones, the flavonol quercetin is the most frequently occurring compound in foods and also kaempferol, myricetin, and the flavones apigenin and luteolin are common [17]. Different classes of flavonoids and their conjugates have numerous functions during the interactions of plant with the environment, both in biotic and abiotic stress conditions [18, 19]. Many flavonoids are active principles of medicinal plants and exhibit pharmacological effects [20]. Flavonoids play a variety of significant functions in plants as signal molecules [21], agents for pollen germination [22, 23, 24, 25, 26, 27], and seed germination [28, 29], pollinator attractants [30], regulators of auxin transport [18, 31, 32, 33], UV filters [34, 35], antimicrobial [36, 37] and antiherbivory agents [38]. Some flavonoids such as quercetin, kaempferol, myricetin, apigenin, and leuteolin also have antioxidative activity in many *in vitro* studies [39]. Moreover, plants use the huge variety of secondary metabolites as tools to overcome stress [40]. Flavonoids are a diverse group of natural product found in all plants [41, 42]. Bryophytes (mosses), liverworts, hornworts are the oldest plant group to produce chalcone,

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Published online at http://journal.sapub.org/ijmb

flavonols, and flavones among the types of flavonoids [43]. Leguminosae produce about 28% of all known flavonoids, 95% of all isoflavonoid aglycones and about 850 compounds, including 362 isoflavones which were known in this family [44, 45] .Thirty-three kinds of flavonoids were reported from Polygonaceae species leaves [46]. Flavonoid compounds were found widely in Euphorbiaceae and there are some studies in this connection. Noori et al (2012) showed that all studied populations of *Chrozophora tinctoria* and *C. hierosolymitana* in Markazi Province contain flavonoid sulphates, flavone *C* and *C-/O*-glycosides and aglycon. Also all studied populations have apigenin and quercetin, while rutin was just found in 4 populations of *C. tinctoria* species [47]. Five flavonoid glycosides were reported in the methanol extract of the aerial parts of *Chrozophora tinctoria* [48]. Four flavonoids were isolated from the butanolic extract of the aerial parts of *Croton campestris* St. Hill. (Euphorbiaceae) [49]. Eleven flavonoid compounds -one *C*-glycosyl flavone and ten flavonol glycosides- were isolated and identified from leaf material of *Cnidoscolus aconitifolius, C. souzae*, and *C. spinosus* [50]. Several studies by different researchers indicated that flavonoids occurred widely in various species of *Euphorbia* [41, 42, 51-72]. The aim of this study is to describe fruit flavonoids of 18 *Euphorbia* species from Iran in two subgenera *Chamaesyce* and *Esula*.

2. Materials and Methods

2.1. Sampling and Identification

Mature fresh fruits of 18 *Euphorbia* species were collected from some area, described in Table 1. Plants identified using available references [73, 74, 75]. Samples were air dried for detection and identification of flavonoids.

2.2 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 fruit 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).

2.3. Flavonoid analysis by 2-Dimensional Paper Chromatography (2-DPC)

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.

2.4. Methods of Identification of the Flavonoids

After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from 18 *Euphorbia* species, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids [76, 77] 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, naringenin, apigenin, luteolin, and rhamnetin (all obtained commercially, rutin from Merck and the rest from Fluka).

2.5. 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 with a pipette, evaporated to dryness, dissolved in 0.5 ml of MeOH and applied as spots on thin layer chromatograms=TLC (cellulose). The TLC plates were run in three solvents alongside standards to identify the aglycone moiety [78].

3. Results

All studied *Euphorbia* species contained flavonoid compounds in their fruits. Data in Table 1 and 2 show the collection information and also two-dimentional paper and thin layer chromatographical data of 18 studied *Euphorbia* species in two subgenera *Esula* and *Chamaesyce* from central of Iran. Table 1 shows 2-DPC and Table 2 show TLC data of studied *Euphorbia* species in two subgenera *Esula* and *Chamaesyce* from Markazi Province, Iran. The results showed existing flavonoid sulphates and flavone *C*-and *C-/O*-glucosides in all of studied taxa fruits but dihyro flavonol 3-*O*-monoglycosides were not found in them with the exception of *E. Falcata* (Table1 1). The most flavonoids number was observed in *E. macroclada* species fruits and *E. osyridiformis* showed the lowest (Table1 1). Kaempferol was found in all of studied species fruits. While rhamnetin was detected only in *E. osyridiformis* and myercetin existed in *E. falcata* and *E. szovitsii* (Table 2). All of studied samples had not quercetin with the exception of 5 species (*E. chamaesyce*, *E. macroclada*, *E. seguieriana*, *E. splendida* and *E. aleppica*). For other flavonoids is refered to Table 2. Our investigation into studied *Euphorbia* species illustrated that the variety and concentration of flavonoid compounds in studied *Euphorbia* species in subgenera *Esula* is more than subgenera *Chamaesyce* (Table 1 and Table 2).

Table 1. Collection information and 2-DPC data of studied *Euphorbia* species in two subgenera *Esula* and *Chamaesyce* from Markazi Province, Iran

Voucher data	Taxon	Sampling locality		Flavonoid type			
		Latitutde	Longitude	Number of total flavonoids	Number of flavonoid sulphates	Number of flavone *C*-and *C*-/*O*-glucosides	Number of dihyroflavonol 3-*O*-monoglycosides
Subgenera *Chamaesyce*							
*CMK63	*E. chamaesyce*	49° 47′ N	34° 05′ E	3	2	1	-
CMK65	*E. maculata*	50° 02′ N	34° 08′ E	3	2	1	-
Subgenera *Esula*							
CMK 75	*E. aleppica*	49° 02′ N	34° 14′ E	4	2	2	-
CMK 23	*E. bungei*	50° 07′ N	34° 36′ E	4	3	1	-
CMK 57	*E. cheiradenia*	49° 22′ N	33° 56′ E	3	2	1	-
CMK 60	*E. esula*	49° 47′ N	34° 01′ E	4	1	3	-
CMK 59	*E. falcata*	49° 45′ N	34° 03′ E	4	2	1	1
CMK 32	*E. helioscopia*	49° 47′ N	34° 09′ E	6	4	2	-
CMK 26	*E. heteradena*	49° 41′ N	34° 29′ E	3	1	2	-
CMK 54	*E. macroclada*	49° 16′ N	34° 02′ E	7	4	3	-
CMK 70	*E. microsciadea*	49° 48′ N	34 ° 13′ E	5	4	1	-
CMK 66	*E. osyridiformis*	50° 03′ N	34° 12′ E	2	1	1	-
CMK 62	*E. peplus*	49° 37′ N	34° 01′ E	3	1	2	-
CMK 74	*E. petiolata*	50° 20′ N	34° 22′ E	5	4	1	-
CMK 16	*E. seguieriana*	49° 55′ N	34° 01′ E	3	2	1	-
CMK 10	*E. splendida*	49° 42′ N	34° 02′ E	3	2	1	-
CMK 48	*E. szovitsii*	49° 11′ N	34° 23′ E	3	2	1	-
CMK 34	*E. teheranica*	49° 43′ N	35° 08′ E	6	3	3	-

*CMK: Mahdi Kaveh collection numbers

Table 2. Thin Layer Chromatography data of studied *Euphorbia* species in two subgenera *Esula* and *Chamaesyce* from Markazi Province, Iran

Voucher data	Identification							
	Rutin	Quercetin	Kaempferol	Myricetin	Naringenin	Apigenin	Luteolin	Rhamnetin
Subgenera *Chamaesyce*								
CMK63	*-	+	+	-	-	-	-	-
CMK 65	+	-	+	-	-	+	+	-
Subgenera *Esula*								
CMK 75	-	+	+++	-	-	+	+++	-
CMK 23	+	-	+	-	-	+	-	-
CMK 57	-	-	++	-	++	-	-	-
CMK 60	-	-	+++	-	+	-	-	-
CMK 59	+	-	+	+++	-	+++	+++	-
CMK 32	-	-	+	-	-	+++	+	-
CMK 26	-	-	+	-	+	-	-	-
CMK 54	-	+	+++	-	+	-	-	-
CMK 70	-	-	+++	-	-	-	-	-
CMK 66	-	-	+++	-	-	-	+	+
CMK 62	+	-	+	-	-	+	++	-
CMK 74	-	-	+++	-	-	+	-	-
CMK 16	-	++	+++	-	+	-	-	-
CMK 10	-	+	++	-	-	-	+	-
CMK 48	+	-	+++	+++	-	+	-	-
CMK 34	-	-	+	-	-	+	++	-

*-: (non flavonoid), +: (few flavonoid), ++: (high concentration of flavonoid), +++: (very high concentration of flavonoid).

4. Discussion and Conclusions

As table 1 shows, flavonoid sulphates and flavone *C*-and *C*-/*O*-glucosides were detected in fruit of studied taxa, but dihyro flavonol-3-*O*-monoglycosides were not found in studied taxa except *E. falcata* (Table 1). Flavonoid solphates and flavone *C*-and *C*-/*O*-glucosides were reported in leaf of studied *Euphorbia* species [41]. Kaempferol was predominant flavonoid in extracts of studied taxa (Table 2). Quercetin, and kaempferol were reported as the most representative compounds for the genus *Euphorbia* [41]. Kaempferol was reported in *E. helioscopia* [55], *E. hirta* [69], *E. chamaesyce*, *E. cordifolia*, *E. osyridiformis*, *E. heteradena*, *E. bungei*, *E. peplus*, *E. esula*, *E. falcata*, *E. szovitsii*, and *E. seguieriana* [41]. Kaempferol 3-*O*-glucoside and quercetin 3-*O*-glucoside were obtained from *E. larica*, *E. virgata*, *E. chamaesyce,* and *E. magalanta* [61]. Papp et al (2005) studies showed that arial parts of *Euphorbia cyparissias* had 2 main flavonoids: kamfpherol-3-glucuronide and quercetin-3-glucuronide [68]. The hypotensive principales of *E. maddeni* were found to be kaempferol-4'-*O*-glucose and hyperin [57]. Result showed that quercetin was identified in *E. macroclada*, *E. seguieriana*, *E. splendida*, *E. Chamaesyc* and *E. aleppica* among studied species (Table 2). Quercetin was found in *Euphorbia pilulifera* [52], *E. helioscopia* [55], *E. hirta* [69, 71], *E. chamaesyce*, *E. ozyridiforma*, *E. heteradena*, *E. bungei*, *E. helioscopia*, *E. petiolata*, *E. peplus*, *E. esula*, *E. falcata*, *E. szovitsii*, *E. microsciadea*, *E. cheiradenia*, *E. macroclada*, *E. seguieriana*, *E. splendida* [41] and *Euphorbia wallichii* [42]. Some known derivatives of quercetin were isolated from different *Euphorbia* species by researchers [58, 60, 62, 67, 68, 72]. In this study, rutin was detected in *Euphorbia bungei*, *E. peplus*, *E. falcata*, *E. szovitsii* and *E. maculata* (Table 2). Rutin was identified in *E. larica*, *E. virgata*, *E. magalanta* [61], and leaves extracts of *E. chamaesyce*, *E. cordifolia*, *E. ozyridiforma*, *E. heteradena*, *E. bungei*, *E. helioscopia*, E. *peplus*, *E. esula*, *E. falcata*, *E. szovitsii*, *E. teheranica*, *E. microsciadea*, *E. cheiradenia*, *E. seguieriana*, *E. splendida* [41] and *E. hirta* [71]. As table 2 shows, *Euphorbia falcata* and *E. szovitsii* among studied species just have myricetin. Myricetin was reported in leaves extract of *E. macroclada* and *E. petiolata* [41] and in *E. hirta* [71]. Murillo and Jakupovic (1998) identified myricetin-3-rhamnoside and one flavonoid glycosides in *E. aucherii* which was collected in Iran [65]. Ghanadian et al (2012) identified myricetin 3-*O*-β-D-galactopyranoside in aerial parts of *Euphorbia microsciadia* [72]. Result shows that there are luteolin in some studied species, including *E. ozyridiforma*, *E. helioscopia*, *E. peplus*, *E. teheranica*, *E. falcata*, *E. splendida*, *E. aleppica* and *E. maculata* (Table 2). Omurkhamzinova and Erzhanova (1985) identified luteolin-3-rhamnoside and luteoline-3-galactoside from the aerial parts of *E. soongarica* and *E. alatavica* [59]. Our study indicated that rhamnetin was absent in all studied taxa except *E. ozyridiforma* (Table 2). Muller and Pohl (1970) isolated six new flavonoids all being glycosides of rhamnetin from *E. amygdaloides* [54]. Apigenin was detected in this study in *E. maculata*, *E. bungei*, *E. helioscopia*, *E. petiolata*, *E. peplus*, *E. falcata*, *E. szovitsii*, *E. teheranica*, and *E. aleppica* (Table 2). Apigenin was characterized in nineteen *Euphorbia* species [79]. The major flavonoids of *Euphorbia prostrata* are apigenin, luteolin, apigenin-7-glucoside and luteolin-7-glucoside [63]. Five apigenin glycosides were identified in *E. humifusa* [80]. As table 2 shows, naringenin were detected in *Euphorbia heteradena*, *E. esula*, *E. cheiradenia*, *E. macroclada* and *E. sequieriana*. Roshchin et al. (1970) found naringenin 7-*O*-β-D glucofuranoside for the first time in genus *Euphorbia* [81]. Myricetin, naringenin, and rhamnetin were not detected in fruits extracts of *Euphorbia maculata* and *E. chamaesyce* of subgenera *Chamaesyce* (Table 2). Finally, this study showed that the diversity of flavonoids in studied taxa is considerable and the concentration and variety of flavonoid compounds in studied species in subgenera *Esula* is more than studied taxa in subgenera *Chamaesyce*. It is believed that the high variety of flavonoid compounds in studied *Euphorbia* species is to their response to environmental stresses because all of studied taxa were weed and grow in destroyed pasture. Flavonoids protect plants against various biotic and abiotic stresses and play an important role in the interaction between the plant and their environment [13, 39, 82, 83]. However, further work is needed for attaining an obvious mechanism of flavonoid roles against stress protection.

ACKNOWLEDGEMENTS

The authors would like to thank of Organic Chemistry Laboratory Experts, Chemistry Department in University of Arak.

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