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Flavonoids in some Iranian Angiosperms

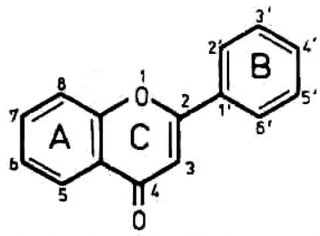
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1. Introduction

Flavonoids are as one set of the polyphenolic compounds among secondary metabolites in different organs of plants that possess a wide range of biological activities [Parr and Bolwell 2000, Noori 2002, Noori et al 2009]. Their distribution in plants, synthesis and mode of action have been extensively studied [Shirley 1996].

1.1 Structure, biosynthesis and variety

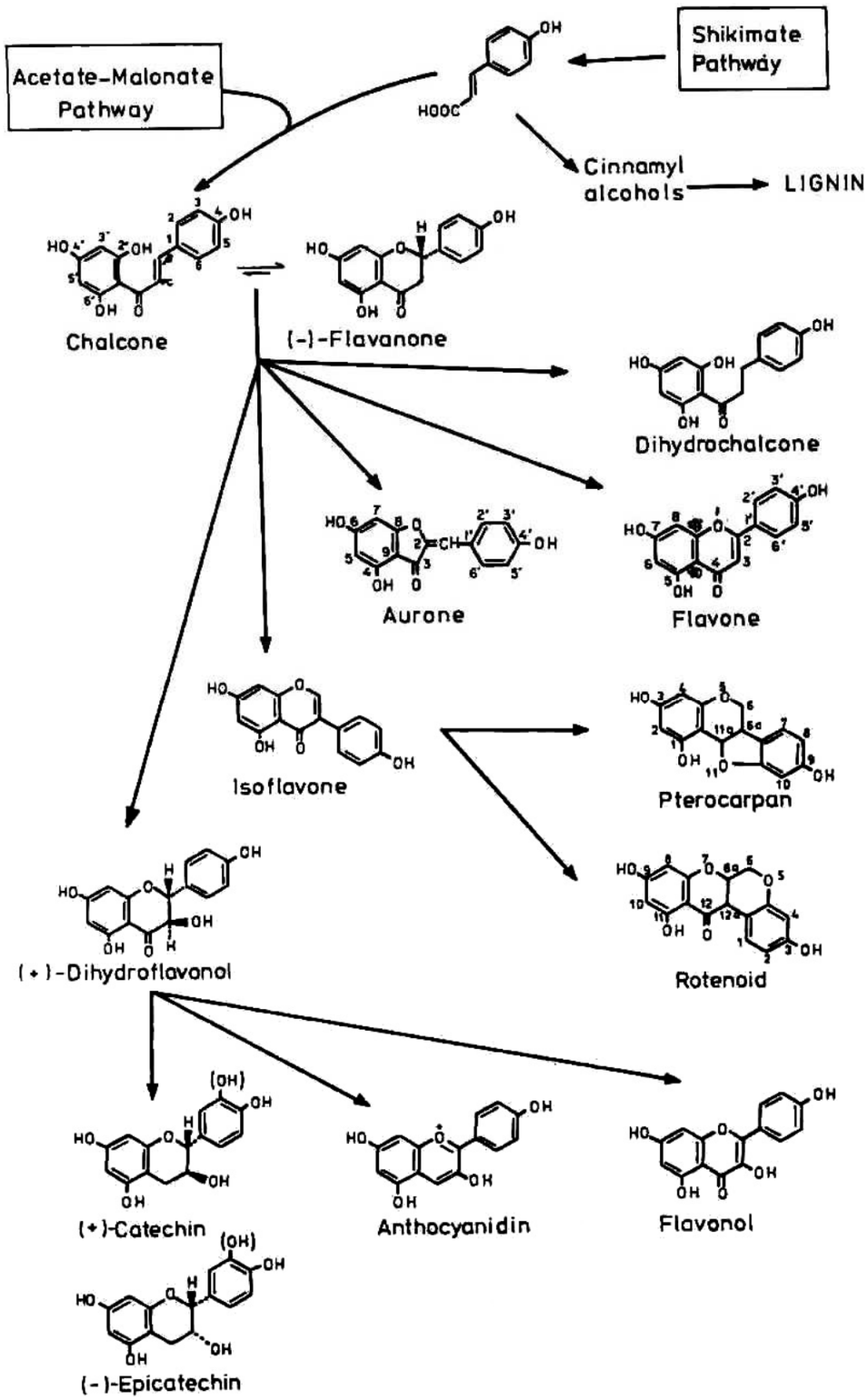
All flavonoids contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 configuration, that is, two aromatic rings linked by a three carbon unit which may or may not form a third ring. They are divided into different groups depending on the configuration of the rings and substitutions on these rings of a variety of side-groups which characterize the individual compounds [Stace 1980] (Scheme 1).



Scheme 1. The basic nucleus of flavonoids (Stace 1980)

The flavonoid variants are all related by a common biosynthetic pathway which incorporates precursors from both the "Shikimate" and "Acetate-Malonate" pathways [Hahlbrock and Grisebach 1975; Wong 1976], the first flavonoid produced immediately following confluence of the two pathways (Scheme 2).

The flavonoid initially formed in the biosynthesis is now thought to be the chalcone and all other forms are derived from this by a variety of routes [Hahlbrock 1981] (Scheme 2). More than 4000 varieties of flavonoids have been identified in different higher and lower plant species (De Groot and Rauen 1998). The main flavonoid groups are flavones (e.g. luteolin), flavanone (e.g. naringenin), flavonols (e.g. kaempferol), anthocyanidins (e.g. pelargonidin) and chalcones (e.g. butein) [Harborne et al 1975].



Scheme 2. Showing two biosynthetic pathways of flavonoids (Hahlbrock and Grisebach 1975; Wong 1976).

1.2 Occurance

Flavonoids are found in fruit, vegetables, grains, bark, roots, stems, leaves, flowers, tea and wine [Middleton 1998, Robles et al 2003]. The flavonoid nucleus is normally linked to a sugar moiety to form a water-soluble glycoside. Most flavonoids are stored in the plant cell vacuoles, although they also occur on the surfaces of leaves and stems (Farman 1990). 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 [Parr and Bolwell 2000].

1.3 Biological activities and their usages

Flavonoids possess a wide range of biological activities, medicinal and pharmacological effects [Parr and Bolwell 2000, Noori 2002, Noori et al 2009].

1.3.1 Biological activities

A large variety of colours such as orange, scarlet, crimson, mauve, violet, blue and purple that we encounter in different part of plants, especially flowers and fruits, are caused by anthocyanins (=anthocyanidin glycosides). Chalcones and some flavones and flavonols also absorb light in the visible region and are associated with bright yellow or cream coloured flowers. Other flavones account for the whiteness in most white flowers, without which they would perhaps appear translucent. Even some of the brown and black pigments found in plants are either due to oxidative products of flavonoids or related phenolic compounds. [Farman 1990]. They are 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 [Treutter 2006]. 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 [Field et al 2001].

1.3.2 Medicinal and pharmacological effects

Flavonoids medicinal and pharmacological effects are their contributions to human health which has made them prominent in the past 10 years (Parr and Bolwell 2000). Many flavonoids are active principles of medicinal plants and exhibit pharmacological effects [Yilmaz and Toledo 2004].

1.4 Chemotaxonomy

Flavonoid compounds are taxonomically important. They are popular characters for chemosystematic studies because: the almost universal presence of flavonoids in vascular plants; 2. Their structural diversity; 3. The fact that each species usually contains several flavonoids; 4. The chemical stability of many flavonoids in dried plant material enabling herbarium material to be used; 5. Flavonoid profiles using different chromatographic techniques are easily obtained. 6. Flavonoids are reasonably easy to identify using published

UV spectra data and available standards; 7. Flavonoids often show correlations with existing classifications at the family, genus and species level, and support revisions of existing classifications at the family, genus and species level. However, flavonoids rarely provide ″key″ characters (the flavonoid may be absent in one or more members of the taxon, and the same flavonoid may occur in an unrelated taxon, e.g. isoflavonoids occur in the Leguminosae and Iridaceae and biflavonyls in the Gymnospermae and some Angiospermae) [Harborne and Turner 1984].

1.5 Flavonoids in Leguminosae

The Leguminosae is economically the single most important family in the dicotyledonae, and also of major significance in nature The family is especially rich in flavonoids, producing about 28% of all known flavonoids and 95% of all isoflavonoid aglycones (Hegnauer and Grayer-Barkmeijer 1993). The importance of the phenolic constituents in the family has been stressed by Bate-Smith (Bath-Smith 1962). As Gomes et al (1981a) showed in "Advances in Legume Systematics" the Leguminosae are especially well endowed with flavonoid constituents, many of which are only known in these plants. Whithin the Leguminosae, some 850 compounds, including 362 isoflavones, are known [Dewick 1993]. There is a basic structures, Such as genistein (4´, 5, 7-trihydroxyisoflavone), 5-dexy derivatives (some 66% of structures), prenylated derivatives (some 51% of structures) and compounds with extra hydroxylation (e. g. at the 6-, 8- or 2´-positions). Isoflavonoids usually occur in the free state, and are obtained from root, wood, bark or seed rather than leaf or flower [Ingham 1981, 1983]. Flavonoids, as distinct from isoflavonoids and neoflavonoids, are widespread in the *Papilionoidaea* and there is little doubt that they occur not only in the species of the some tribes, but will eventually be found in all tribes [Gomes et al 1981b]. Harborn (1965) obtained quercetagetin from hydrolyzed petal of *Coronilla glauca* L.. He also found halogenin, 3-*O* rutinoside and limocitrin, 3- *O* rutinoside from C. glauca flower (Harborn 1981). Catechin, epigallocatechin, leucodelphinidin and 3, 3´, 4´, 5, 5´, 7-hexahydroxyflavan have been identified from *Alhaji maurorum* Medikus ground parts [Islambeko et al 1982]. Malvidin from hydrolyzed flower, myricitrin from flower and laef of *Cercis siliquastrum* L. have been isolated [Torck et al 1969, Sagareishvili and Ananiya 1990].

1.6 Polygonaceae flavonoids

Based on Isobe and Noda (1987) flavonoids and flavonol glycosides are of wide-spread occurrence in the genus *Polygonum*. Among them, glycosylation at C-3 of the quercetin nucleus has been found to be the most common trend, and present in all species of this genus [Park, 1987]. While rhamnose, gloucose, arabinose and rhamnosyl-rhamnose are the most common sugars found as aglycones of the flavonol glycosides [Mun and Park 1995], galactosylation is rather uncommon in the genus *Polygonum* or in the family polygonaceae [Collins et al 1975]. Kawasaki et al (1986) isolated thirty-three kinds of flavonoids from Polygonaceae species leaves. Quercetin glycosides were commonly found in the family. In the quercetin glycosides, 3-*O*-rhamnoside was most frequently found, 3-*O*-glucuronide is also distributed widely. Myricetin glycosides were rare. Methylated flavonols were found in some species of the section *Echinocaulon* and *Persicaria* [Kawasaki et al 1986]. The aerial exudate of *Polygonum senegalense* has been reported to contain 12 flavonoids of the chalcone

and flavanone types, and they are distinctly different from internal tissue aglycones [Midiwo et al 2007]. Also Hsu (2006) studies revealed that *Polygonum aviculare* L. extract has high phenolics and flavonoid contents.

Trichopoulou et al (2000) showed that some wild edible species of *Rumex* such as *R. acetosa* L. and *R. japonicas* Houttuyn have a very high flavonol content. Hasan et al (1995) studies showed besides rutin, quercetin 3-rhamnoside and kaempferol 3-rhamnosyl (1 -> 6) galactoside, a new flavonol glycoside, quercetin 3-glucosyl (1 -> 4) galactoside, and 1, 6, 8-trihydroxy-3-methyl anthraquinone (emodin) have been characterized from leaves of *R. chalepensis*.

1.7 Euphorbiaceae flavonoids

Several studies indicated that flavonoids occurred in various species of *Euphorbia*. Nagase reported the isolation of 5, 7, 4'-trihydroxy-flavone-7-glucoside from the leaves and stems of *E. thymifolia* [Nagase et al 1942]. Ten years later, quercetin was isolated from ethyl acetate extract of aqueous solution of hydroalcoholic extract of *E. pilulifera* [Hallett and Parks 1951]. Sotnikova and his coworkers identified 3′, 4′-pentahydroxyflavone 3β-D-galactopyranoside, steppogenin stepposide, isomyricitrin and nineteen other flavonoids in *E. stepposa* [Sotnikova and Litvinenko 1968, Sotnikova et al. 1968]. Muller and Pohl (1970) isolated six new flavonoids all being glycosides of rhamnetin from *E. amygdaloides*. The qualitative composition of flavonoids in alcoholic extract of *E. helioscopia* indicated fifteen substances with flavonoidal nature, by two-dimentional paper chromatography. Acid hydrolysis of *E. helioscopia* alcoholic extract by Volobueva (1970) yielded quercetin and kaempferol. Quercetin-3-xylosidoglucoside has been identified as one of the two flavonoids found in methanolic extract of *E. chamaesyce* and quercetin-3β-D-galactopyranoside gallate has been reported in *E. verrucosa* and *E. platiphyllos* [Singla and Pathak 1990]. Chromatography on cellulose of methanolic extract of *E. lucida* yielded quercetin and its derivatives, viz., isoquercetin, avicularoside, hyperoside and rutoside [Burzanska 1975]. The hypotensive principales of *E. maddeni* were found to be kaempferol-4'-*O*-glucose and hyperin (Sahai et al 1981). In an effort to identify the constituents responsible for the antiviral activity of *E. grantii*. Van Hoof et al. (1984) isolated derivatives of 3-methylquercetin. Polyphenolic components from the aerial parts of *E. soongarica* and *E. alatavica* have been identified as the esters of gallic acid, luteolin-3-rhamnoside and luteoline-3-galactoside [Omurkhamzinova and Erzhanova 1985]. Gautam and Mukhraya (1981) have isolated quercetin-3-O-β-D-glucopyranosyl (1-4)-O-α-L-rhamnopyranoside from the leaves of *E. dracuncoloides*. Kaempferol 3-O-glucoside and quercetin 3-O-glucoside were obtained from *E. larica*, *E. virgata*, *E. chamaesyce* and *E. magalanta*. Also all these taxa, except *E. chamaesyce* contained kaempferol 3-rutinoside and rutin. *E. larica* also yielded 6-methoxyapigenin while *E. virgata* and *E. magalanta* yielded kaempferol. There is an unknown acytylated kaempferol derivative in *E. chamaesyce* (Ulubelen et al 1983). Murillo and Jakupovic (1998) identified myricetin-3-rhamnoside and one flavonoid glycosides in *E. aucherii* which was collected in Iran. Aimova et al (1999) found quercetin 3-(2‴-Galloylglucosyl) (1→2)-α-L-arabinofuranoside in *E. pachyrrhiza*. Studies of Halaweish et al (2003) are the first report of quercetin-3-O-β glucuronic acid in *E. esula*. They separated and identified kaempferol-3-O-β glucuronic acid and quercetin-3-O-β glucuronic acid from the species. Papp et al (2005) studies showed arial

parts of *Euphorbia cyparissias* had 2 main flavonoids: kamfpherol-3-glucuronide and quercetin-3-glucuronide. Adedapo et al (2005) showed arial branch extract of *E. hirta* contains kaempferol, quercitol and quercitrin. The phytochemical studies of Falodun et al (2006) on *E. heterophylla* extract revealed the presence of flavonoids in the extract.

Abdel-Sattar (1985) reported existing flavonoids in *Chrozophora*. Then Hashim et al (1990) found kampferol, acacetin, luteolin and apigenin glycosides in *Chrozophora* species. Isorhamnetin and quercetin glycosides were separated from *C. oblique* [Mohamed 2001]. Talischi et al (2005) reported apigenin and quercetin glycosides from metanolic extract of *C. tinctoria* aerial parts. Also Delazar et al (2006) separated 5-flavonoid glycosid from aerial parts metanolic extract of *C. tinctoria*. Shi et al (2006) has reported 7-flavonoid glycoside from *C. sabulosa* species. Vassallo et al (2006) were separated three new flavon glycosids from *C. senegalensis* leaf metabolic extract. Apigenin and loteulin glycosids were reported from leaf aqueous-etanolic extract of *C. brocchiana* species [Hawas 2007].

1.8 Resedaceae flavonoids

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 [Woelfle et al 2009]. Moiteiro et al (2008) found luteolin 4́-O-glucoside in *Reseda luteola* for first time. 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-L-rhamnoside, 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. El-Sayad et al (2001) isolated aglycone flavonols, kaempferol and quercetin from the Mediterranean *Reseda* species. Also Yuldashev et al (1996) reported flavonol diglycosides of kaempferol, quercetin and isorhamnetin from four other *Reseda* species. Rzadkowska (1969) isolated four 3-*O*-glycosides from *R. lutea*.

1.9 Cyperaceae flavonoids

Clifford and Harborne (1969) studies showed identification of the flavonoid pigment aureusidin from *Scirpus nodosus*. Quercetin, kaempferol, apigenin and luteolin were reported from *S. wichurai* [Ahmed et al 1984]. Naser et al (2000) identified lupeol betulin, betulinalaldehyde and apigenin from *Scirpus tuberosus*. Also β-sitosterol, quercetin 3- β-glucoside, quercetin 3, 7- β-diglucoside and isorhamnetin 3, 7- β-glucoside were identified from *Scirpus litoralis* using spectroscopic analyses [Naser et al 2000]. Yang et al (2010) used a developed capillary electrophoresis with amperometric detection method for the determination of some phenolic compounds in the rhizome of *Scirpus yagara* Ohwi. Their work determined existing four phenolic compounds: transresveratrol, scirpusin A, scirpusin B, and p-hydroxycinnamic acid in the species rhizome.

1.10 Aim

The aim of this study was to compare the leaf flavonoids profiles of some Iranian Angiosperm species from Leguminosae, Polygonaceae, Euphorbiaceae, Resedaceae and Cyperaceae.

2. Materiales and methodes

2.1 Collection of plant material and praperation

Mature fresh leaves of eight Legumes, seven *Polygonum*, seven *Rumex*, seventeen *Euphorbia*, two *Chrozophora*, four *Reseda* and five *Scirpus* species from different parts of Iran were collected during 2006-2010 as described in Table 1. Plants identified using available references [Rechinger 1964, Mobayen 1979, 1980, Ghahreman 1979-2006]. 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.

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 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).

2.3 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.

2.4 Methods of identification of the flavonoids

When sufficient amounts of purified flavonoids had been obtained, as in the cases of the flavonoids from studied samples, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids [Mabry et al. 1970, Markham 1982] 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 obtained commercially from Merck, Sigma and 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 (cellulose). The TLC plates were run in three solvents alongside standards to identify the aglycone moiety [Harborne 1998].

Voucher data	Taxon	Number of total flavonoids	Number of flavonoid sulphates	Number of flavone C-and C-/O-glucosides	Number of aglycones
	Chrozophora				
*CAM2	*C. tinctoria*	3	3	2	8
CAM22	*C. hierosolymitana*	3	2	3	8
	Euphorbia				
*CMK 23	*E. bungei* Boiss.	5	2	3	0
CMK 65	*E. chamaesyce* L.	8	4	4	0
CMK 57	*E. cheiradenia* Boiss. et Hohen.	7	5	2	0
CMK 63	*E. cordifolia* Ell.	9	3	6	0
CMK 60	*E. esula* L.	7	3	4	0
CMK 59	*E. falcata* L.	7	3	4	0
CMK 32	*E. helioscopia* L.	5	3	2	0
CMK 26	*E. heteradena* Jaub,et Spach.	9	3	6	0
CMK 54	*E. macroclada* Boiss.	6	1	5	0
CMK 70	*E. microsciadeae* Boiss.	9	5	4	0
CMK 69	*E. ozyridiforma* Parsa.	7	4	3	0
CMK 62	*E. peplus* L.	8	4	4	0
CMK 74	*E. petiolata* Banks et Soland	9	6	3	0
CMK 16	*E. seguieriana* Necker.	8	5	3	0
CMK 10	*E. splendida Mobayen.*	7	3	4	0
CMK 48	*E. szovitsii* Fisch.& Mey.	8	5	3	0
CMK 34	*E. tehranica* Boiss.	8	6	2	0
	Papilionoideae				
*CMJ148	*Alhagi camelorum* Fisch.	2	1	1	0
CMJ149	*Cercis siliquastrum* L.	2	1	1	0
CMF1	*Coronilla varia* L.	5	4	1	0
CMJ150	*Glycyrrhiza glabra* L.	2	1	1	0
CMJ151	*Medicago sativa* L.	0	0	0	0
CMJ152	*Robina peseudo-acacia L.*	4	3	1	0
CMJ153	*Sophora alopecuroides ssp. alopecuroides*	0	0	0	0
CMN1	*Sophora alopecuroides ssp. tomentosa*	0	0	0	0
	Polygonum				
*CEM1	*P. aviculare*	4	0	4	0
CEM2	*P. convolvolus*	1	0	1	0
CEM3	*P. hyrcanicum*	4	0	4	0
CEM4	*P. patulum*	3	0	3	0
CEM5	*P. alpestre*	3	0	3	0
CAM6	*P. arenastrum*	4	0	4	0
CAM7	*P. persicaria*	3	1	2	0
	Reseda				
*CMG27	*Reseda aucheri*	8	5	3	0
CMG21	*R. buhseana* Mull-Arg.	10	5	4	1
CMG22	*R. bungei* Boiss.	8	7	1	0
CMG11	*R. lutea* L.	7	6	1	0
	Rumex				
*CMR2	*R. chalpensis*	10	4	6	0
CMR4	*R. crispus*	6	2	4	0
CMR6	*R. obtusifolius*	10	4	6	0
CMR7	*R. tuberosus*	10	5	5	0
CMR9	*R .pulcher*	8	2	6	0
CMR12	*R. acetosella*	8	2	6	0
CMR14	*R.conglomeratus*	9	2	6	1
	Scirpus				
*CNM4	*S. holoschenus* L.	8	4	1	3
CMN23	*S. lacustris* L.	6	5	0	1
CMN8	*S. littoralis* Kuntze	10	8	1	1
CMN6	*S. maritimus* L.	9	5	3	1
CMN18	*S. multicaule*	12	5	2	5

Table 1. The sampling and also two-dimensional paper and thin layer chromatographically data of 48 studied plant samples from Markazi Province, Iran.

Voucher data	Identification									
	Apigenin	Chrycin	Kaempferol	Luteolin	Myricetin	Naringenin	Quercetin	Rhamnetin	Rutin	Vitexin
Chrozophora										
*CAM2	**++++**	-	-	-	-	-	**+++**	-	**++**	-
CAM22	**++++**	-	-	-	-	-	**++++**	-	**-**	-
Euphorbia										-
*CMK 23	-	-	++	-	-	-	+++	-	++	-
CMK 65	-	-	-	-	-	-	+++	-	+++	-
CMK 57	-	-	-	-	-	-	+++	-	+++	-
CMK 63	-	-	-	-	-	-	-	-	++	-
CMK 60	-	-	+++	-	-	-	+++	-	+	-
CMK 59	-	-	+++	-	-	-	++	-	+++	-
CMK 32	-	-	-	-	-	-	+++	-	+	-
CMK 26	-	-	+++	-	-	-	++	-	++	-
CMK 54	-	-	-	-	+++	-	++	-	-	-
CMK 70	-	-	-	-	-	-	+++	-	+++	-
CMK 69	-	-	+++	-	-	-	++	-	+	-
CMK 62	-	-	++	-	-	-	+	-	++	-
CMK 74	-	-	-	-	+++	-	+++	-	-	-
CMK 16	-	-	+++	-	-	-	+++	-	+++	-
CMK 10	-	-	-	-	-	-	+	-	+	-
CMK 48	-	-	++	-	-	-	+++	-	++	-
CMK 34	-	-	-	-	-	-	-		+	-
Papilionoideae										
*CMJ148	-	-	++	-	-	-	--	-	++	-
CMJ149	-	-	++		+++		+++		++	-
CMF1	-	-	-	-	±	-	+	+	±	-
CMJ150	-	-	--	-	-	-	++	-	++	-
CMJ151	-	-	-	-	-	-	-	-	-	-
CMJ152	++		++		-	-	-	-	++-	-
CMJ153	±	-	-	-	-	-	-	-	-	-
CMN1	±	-	-	-	-	-	-	-	-	-
Polygonum										
*CEM1	-	-	**+**	-	+	-	+	-	**+**	-
CEM2	-	-	**-**	-	**-**	-	+	-	**-**	-
CEM3	-	-	**+**	-	+	-	+	-	**-**	-
CEM4	-	-	**+**	-	-	-	+	-	**-**	-
CEM5	-	-	**+**	-	**-**	-	+	-	**+**	-
CAM6	-	-	**+**	-	+	-	+	-	**+**	-
CAM7	-	-	**-**	-	+	-	+	-	**-**	-
Reseda										
*CMG27	-	-	+	-	+	-	+++	++	++	-
CMG21	-	-	+++	+	++	-	-	-	-	-
CMG22	-	-	+++	+	++	-	-	-	-	-
CMG11	-	-	+++	++	-	-	+++	±	++	
Rumex									+	
*CMR2	+	-	+	+	-	-	+	+		
CMR4	-	-	-	±	-	++	++	+	-	-
CMR6	-	-	±	++	-	±	±	+	+	-
CMR7	++	-	±	-	-	±	±	++	-	-
CMR9	+	-	+	+	-	-	++	+	+	-
CMR12	-	-	+	-	-	±	+++	-	-	-
CMR14	+	-	-	±	±	±	++	+	-	-
Scirpus										
*CNM4	++	+	-	+	-	+++	+	+	+	+
CMN23	-	±	-	+	±	±	+++	+	++	+++
CMN8	++	+	-	+	-	+++	-	+++	+	+
CMN6	-	+	-	+	-	++	-	++	+	+
CMN18	++	+	-	++	-	+++	++	++	+	++

*C=Collection number

-(non flavonoid), ± (non or a few flavonoid), + (few flavonoid), ++ (middle concentration of flavonoid), +++ (high concentration of flavonoid)

Table 1. Continued

3. Results

All studied plant species exceptional two subspecies *Sophora alopecuroides* and *Medicago sativa* contained flavonoid compounds in their leaves. Their flavonoid profiles show a wide variety between the species. Data in Table 1 shows the sampling and also two-dimensional paper and thin layer chromatographical data of 48 studied *plant samples* from Markazi Province, Iran.

4. Discussion

Studies of leaf flavonoids showed some phytochemical characters such as total number of flavonoids, flavonoid group such as aglycone, flavones *C*- and *C-/O* glycoside and flavonoid sulphate and kind of flavonoids such as kaempherol, quercetin, myricetin are valuable for chemotaxonomy and their usage.

Chemical study of two *Chrozophora* species using two dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed both *Chrozophora* species contain flavonoid sulphates, flavone *C* and *C-/O*-glycosides and aglycon. Also all of studied species have apigenin and quercetin while rutin was just found in *C. tinctoria* species that is recorded first time for Markazi Province. All of studied species have flavonoid compounds that have variation in their flavonoid type and number (Table 1).

Phytochemical studies of the Euphorbiaceae have been extremely useful in clarifying systematic relationships within the family (Simpson and Levin 1994). Flavonoids occur widely in plants and are a biologically major and chemically diverse group of secondary metabolites that are popular compounds for chemotaxonomic surveys of plant genera and families [Harborne 1994]. There are some studies in this connection. Mues and Zinsmeister (1988) have discussed about variation of occurrence phenolic compounds in mosses and liverworts. Also they showed there is a clear flavonoid distinction between the subclasses Marchantiidae and Jungermanniidae. Another important chemotaxonomic programme has concerned the ferns and fern allies [Harborne 1986]. The phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e. g. at the species and genus level. Turning to the angiosperms, a chemotaxonomic survey of 255 species of the family Iridaceae has been carried out by Williams et al (1986), who found that flavone *C*-glycosides were present in 66% of the samples [Harborne 1986]. Another family survey has been carried out in the *Polygonaceae*, in which 28 species were analysed for their flavonoid pattern [Harborne 1986]. Studying flavonoid pattern can be used for chemosystematic and lower taxonomic levels. 25 *Avena* species (Poaceae) were investigated for the flavonoid content of leaf tissue [Saleh et al 1988]. Diploid *triticum* species could be divided into two groups depending on the presence or absence of two major di-*C*-glycosylflavones (Harborne et al 1986). Flavonoid data of the genus *Vitis* indicate three chemical groups [Moore and Giannasi 1994]. Several studies indicated that flavonoids occurred in various species of *Euphorbia*. They may be useful taxonomic markers within the genus. Also *Euphorbia* flavonoids are very important for their toxicity and some different potential clinical applications such as their antiatherosclerotic, antiinflammatory, antitumor, antithrombogenic, antiosteoporotic and antiviral effects [Nijveldt et al 2001]. Papp et al (2005) showed populations of *Euphorbia cyparissias* can be separated clearly from each other according to their morphology and flavonoid pattern.Our results showed all studied

Euphorbia species contained flavonoid compounds in their leaves that their flavonoid profiles show a wide variety between the taxa. There are flavonoid sulphate and flavone *C* and *C*-/*O*-glycosides in all species, but *E. bungei*, *E. heteradena* and *E. microsciadea* in addition these two flavonoid types have dihydroflavonol 3-*O*-monoglycosides. *E. cordifoila*, *E. heteradena*, *E. microsciadeae* and *E. petiolata* have the highest number of total flavonoid compounds (9) and *E. bungei* and *E. helioscopia* have the lowest number of flavonoid compounds (2) in their leaves (Table 1). Identification of flavonoids by standards showed all of studied *Euphorbia* species contain rutin with the exception of *E. macroclada* and *E. petiolata*. Also all taxa studied, except 2 species (*E. cordifolia* and *E. tehranica*) have quercetin. Harborne and Baxter (1999) reported that quercetin is widely distributed in various plant families. Kaempferol found in 8 species and myricetin was found just in *E. macroclada* and *E. petiolata* (Table 1). As Volobueva (1970) showed two-dimentional paper chromatography and acid hydrolysis of *E. helioscopia* alcoholic extract yielded quercetin and kaempferol. Also Gautam and Mukhraya (1981) isolated quercetin 3-*O* glucoside and kaempferol 3-*O* glucoside from *E. larica*, *E. virgata*, *E. chamaesyce* and *E. magalanta* and rutin obtained with the exception *E. chamaesyce*. 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 [Stafford 1991]. Chemical study of 17 *Euphorbia* species using two dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed rutin, quercetin and kaempferol are the most representative compounds for the genus and the presence of myricetin is a taxonomic character for separation of some *Euphorbia* species. It is believed that *Euphorbia* species can be separated from each other according to their flavonoid pattern.

Application of plant flavonoides data can revealed similarity and relationship between plants and inferring phylogeny and used in their taxonomy. 2-dimentional paper chromatography (2-D PC), on leaves of *Alhagi camelorum* Fisch, *Cersis silliquastrum* L., *Coronilla varia* L., *Glycirhiza glarba* L. and *Robnia peseudoacia* from Markazi Province showed all of five named species contain aglycones. *A. camelorum* and *G. glarba* had the most flavonoid variation and concentration having flavon glycoside and two subspecies of *Sophora alopecuroides* and *Medicago sativa* had not or had the least. The most flavonoid compounds similarity was between *C. silliquastrum* and *R. peseudoacia* (Table 1).

Phytochemical examination of the studied *polygonum* species showed all of *Polygonum* species contain flavon *C*- and *C*-/*O*-glycosides. *P. hyrcanicum* had the most flavonoid variation and concentration and *P. convolvolus* species with having just one flavonoid had the least. Flavonoid sulphates was found just in *P. persicaria* species (Table 1).

Chemical study of four *Reseda* species using two dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed all studied *Reseda* species contained flavonoid compounds in their leaves and kempferol is the most representative compound for the genus (Table 1). 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 antiatherosclerotic, antiinflammatory, antitumor, antithrombogenic, antiosteoporotic and antiviral effects [Nijveldt et al 2001]. 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 [Treutter 2006].

Chemical studies of seven *Rumex* species using two dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed all of studied *Rumex* species contain flavonoid compounds with wide variation. *R. chalpensis*, *R. obtosifolius* and *R. tuberusos* species had the most flavonoid number and *R. crispus* species had the least. Identified flavonoid compounds in all of studied species with the exception *R. crispus* (lack flavonoid sulphate) are flavones *C* and *C-/O* glucoside. *R. acetosella* and *R. conglomerates* had aglycon. Rutin and luteolin found in all of studied species exceptional *R. chalpensis*, *R. obtosifolius* and *R. pulcher*. All of studied species showed wide variation in existing and concentration of myricetin, apigenin, narengenin, rhamnetin, quercetin and kaempferol. All of studied species with the exception *R. chalpensis* and *R. tuberusos* had quercetin and also kaempferol found in 3 species (*R. chalpensis*, *R. pulcher* and *R. acetosella*) (Table 1).

Phytochemical studies on five species of *Scirpus* (*S. holoschenus* L., *S. lacustris* L., *S. littoralis* Kuntze, *S. maritimus* L. and *S. multicaule*) from different parts of Markazi Province, Iran area using two-dimentional paper chromatography (2-DPC) and thin layer chromatography (TLC) showed all of studied taxa contain vitexin, luteolin, rutin and rhamnetin. There were chrysin and naringenin in all of populations with the exception of *S. lucustris* and apigenin was found in 3 species weheras others lack. Quercetin was not found in *S. maritimus* and *S. littoralis* where as three other species had (Table 1).

Our studies showed the most of collected plant species 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 [Chalker-Scott 1999]. Finally, 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 species.

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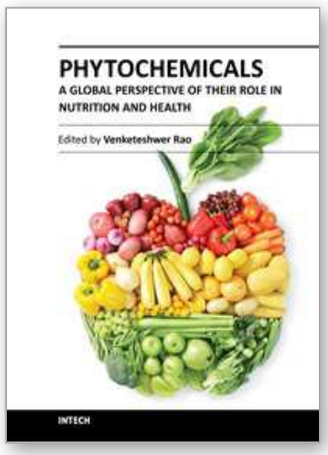
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Phytochemicals - A Global Perspective of Their Role in Nutrition and Health

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Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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