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FLAVONOIDS DISTRIBUTION IN SELECTED MEDICINAL PLANTS OF MARGALLA HILLS AND SURROUNDINGS

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Abstract

The present studies comprise the distribution of important flavonoids amongst the selected 13 medicinal plants *viz*., *Woodfordia fruticosa*, *Adhatoda vasica*, *Chenopodium ambrosoides*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo*, *Peganum harmala*, *Broussonetia papyrifera*, *Taraxacum officinale*, *Urtica dioica*, *Verbascum thapsus*, *Caryopteris grata* and *Mimosa rubicaulis* collected from Margalla Hills for their authentication. Kaemferol was only detected in *Verbascum thapsus*. Myrcetin was detected in *Woodfordia fruticosa*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo* and *Broussonetia papyrifera*. Catechin was detected in *Woodfordia fruticosa* , *Chenopodium ambrosoides* and *Caryopteris grata*. Vitexin was found *absent in all the plants under study except Adhatoda vasica, Chenopodium ambrosoides and Peganum harmala*. Orientin was detected in *Woodfordia fruticosa*, *Adhatoda vasica*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo*, *Peganum harmala*, *Urtica dioica* and *Caryopteris grata*. Rutin and Kaempferol-7-neohesperoside were detected only in *Broussonetia papyrifera*. Quercitin was detected in *Euphorbia hirta*, *Verbascum thapsus*, *Caryopteris grata* and *Mimosa rubicaulis*. Luteolin was common among all the species.

Introduction

Flavonoids are the best known group of polyphenols comprising of about 4000 structures. Most classes of phenols occur naturally as glycosides (Harborne, 1986). The total number of phenols both flavonoids and non-flavonoids is more than 8000. One of the important properties of phenols is their ability to ionize in the presence of base (Thomson, 1986).

Polyphenols constitute the main bioactive phytochemicals that have been proven to be effective in the prevention of certain chronic diseases such as coronary heart diseases, cancers and diabetes (Asami *et al*., 2003). Tannins are fairly frequently encountered in food products of plant vegetable origin such as tea and many fruits. The oxidation inhibition activity of tannins have been known for a long time and it is assumed to be due to the presence of gallic and digallic (Ihekoronye & Ngody, 1985). These bioactive principles present in medicinal plants are the causing agents to cure many diseases such as colic in man, dressing on sores for maturate tumours, whitlow, inflammatory, cancer, mental illness, fatigue, lumbago, gonorrhea, dysentery and anti-microbial effects (Edeoga *et al.*, 2006).

Plant phenolics are secondary metabolites with diverse chemical nature and potential including: phenolic acids, flavonoids, tannins, coumarins, lignans, xanthones and stilbenes (Liu, 2004; Harborne, 1980). Flavonoids play important role in imparting bright colours to flowers, fruits and berries that make the biosphere beautiful (Brouillard & Dangles, 1993). In addition to their biological, nutraceutical and clinical effects (Maimoona *et al*., 2011), flavonoids including proanthocyanidins are implicated in various plant defense mechanisms (Stafford, 1988). Flavonoids may act as phytoalexins which are produced in response to the attack of microorganisms (Laks & Pruner, 1989; Synder & Nicholson, 1990; Dixon, 1986). These bioactive defense compounds are also responsible for plant responses to environmental hazards, such as temperature fluctuations, air pollution (Gietych & Karolewski, 1993) and UV radiation (Tegelberg *et al*., 2004). Phenolic compounds vary greatly after heat damage to stems and crown, proving their worth as bioindicators of thermal stress. Accidental fire or prescribed burning activates secondary metabolism to produce phenolic compounds (Alonso *et al*., 2002; Cannac *et al*., 2007).

Flavonoids are found in many plants and have a wide range of actions. They are found almost in any part of the plants like phenylpropanoids and hydroxybenzoic acids. Flavonoids are a class of phenolic compounds ubiquitously found in plant parts like leaves, seeds, fruits, bark and flowers. They are called plant secondary metabolites (Buluck, 1965) having several pharmacological effects and other health benefits in humans due to their different properties like antioxidant, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic and antithrombolic (Middleton *et al*., 2000).

A major focus in the analysis of natural products is the characterization of compounds with the minimum amount of sample preparation. The separation of compounds can be achieved rapidly and in routin by chromatography. There are a multitude of chromatographic techniques but they all have in common separation of compounds through the use of variations in mobile and stationary phases (Robards *et al.*, 1994).

The current investigation was aimed to determine the quantification of flavonoids and their distribution in selected medicinal plants collected from Margalla Hills, Islamabad Pakistan.

Materials and Methods

Margalla Hills Islamabad are rich in medicinal flora. The selected medicinal plants listed below in Table 1 have been analysed for antimicrobial, Phytotoxicity and other biological activities as well as for their phytochemical analysis. The present article focuses on the distribution of some important flavonoids in these species.

Table 1. List of selected medicinal plants collected from Margalla Hills for the study.

S.No.	Family	Botanical name	Vernacular name	Part of the plant used
1.	Lytheraceae	*Woodfordia fruticosa* (L.) S.Kurz	Dhawi	Leaves
2.	Acanthaceae	*Adhatoda vasica* Nees in wall	Bhekkar	Leaves and twigs
3.	Chenopodiaceae	*Chenopodium ambrosioides* Linn	Chandan bathwa	Aerial parts
4.	Caprifoliaceae	*Viburnum cotinifolium* D. Don	Taliana	Leaves
5.	Euphorbiaceae	*Euphorbia hirta* Linn.	Dudhi	Aerial parts
6.	Verbenaceae	*Vitex negundo* Linn	Banna	Leaves & twigs
7.	Zygophyllaceae	*Peganum harmala* Linn	Harmal	Aerial parts
8.	Moraceae	*Broussonetia papyrifera* Vent.	Jangli Shahtoot/toot	leaves
9.	Asteraceae	*Taraxacum officinale* Weber	Dudal	Flowers
10.	Urticaceae	*Urtica dioica* Linn.	Bichu booti	Aerial parts
11.	Scrophulariaceae	*Verbascum thapsus* Linn.	Gidar tambaku	Aerial parts
12.	Verbenaceae	*Caryopteris grata* Benth & Hook.f.	-	Leaves
13.	Mimosaceae	*Mimosa rubicaulis* Lam	Ral	Stem

Thin layer chromatography: This technique was used for the qualitative analysis of flavonoids among the medicinal plants in comparison to the standard flavonoids with the help of detection flavonoids reagents . Different flavonoids have also been quantitatively analyzed on the basis of their proportionate occurrence by Bate-Smith (1977) method among the test samples.

Reference flavonoids compounds: The commercially available (99.97% pure) flavonoids compounds were used as standard for matching their colour under UV illuminator and their respective Rf values with those present in the samples. This information is reliable for the qualitative assessment as well as quantitative assessment of the respective flavonoids compounds present in the test samples on the basis of their spot sizes. The standard flavonoids used were Kaempferol, Myricetin, catechin, quercitin, vitexin, luteolin, orientin, isoquercitin, hyperside, isovitexin, luteolin-7-glucoside, rutin, kaempferol-7-neohesperidoside, apigenin**.**

Flavonoids reagents: 1% ethanolic 2-Amino ethyl diphenyl borinate solution (reagent A) and 5% ethanolic solution of polyethylene glycol-400 (reagent B) were prepared for the detection purpose.

Spotting and development of the TLC plates: 0.1gm of the methanolic extract of each plant and the same amount of each plant extract extracted with the extracting flavonoid solvent (140:50:10 MeOH-H_2O-CH_3COOH) dissolved in 1 ml respective solvent was used for the spotting in order to visualize the different chemical constituents and the flavonoids respectively. Silica gel 60 F 254 TLC plates (20 x 20 cm) were marked at 1 cm from each side and were heated for silica activation at 110 ^{0}C for 40 minutes. 5-10 µl of the sample was spotted with the help of jet pointed capillary on the line marked both circular as well as horizontal (in case of flavonoids) at one corner of the plate. 4-5 applied at equal distances on each plate.

Mobile phases: Flavonoid mobile phase WAB (Water: Act. Acid: Butanol; 5:1:4)

The spotted TLC plates were poured in TLC tank for vapours saturation. After 20 minutes plates were kept and allowed to develop. When the mobile phase reached at upper end (just below 1 cm), the plates were removed and solvent front was marked. Plates were allowed to air dried and were visualised for simple TLC. The TLC plates for flavonoids detection were developed in flavonoids mobile phase (Water: Act. Acid: Butanol; 5:1: 4) and after dryness they were sprayed with flavonoid reagent A (1% ethanolic 2-Aminoethyl diphenyl borinate solution followed by reagent B ethanolic solution of polyethylene glycol- 400).

Visualization of the TLC plates: TLC plates were visualized in UV light (254 nm) and (365 nm) and marked the visualized spots for the determination of Rf values**.**

Measurement of Rf value: The Rf value is defined as the distance traveled by the compound (from the origin) divided by the distance traveled by the solvent (from the origin) or both distances covered by mobile phase and substances were measured and Rf value was calculated as:

$$Rf = \frac{\text{Distance traveled by visualized spot}}{\text{Distance traveled by solvent front}}$$

Rf value is actually mobility of any one compound in a particular solvent. Rf values are always less than 1. The Rf values of the authentic markers in comparison with those of the unknown provide the basic informations about the structure of those compounds. Flavonoids were detected under illumination 365 nm for their characteristic colours and retention factors.

Results and Discussion

Tables 3 a, b, c show the qualitative distribution of flavonoids among different plant species whereas Table 2 shows the appearance of standard flavonoids with their Rf values and color appearance as a comparison tool for the samples studied. Kaempferol was absent in *W. fruticosa*, *A. vasica*, C. *ambrosoides*, *V. cotinifolium*, *E. hirta*, *V. negundo*, *P. harmala*, *B. papyrifera*, *T. officinale*, *U.*

dioica, *C. grata* and *M. rubicaulis* but only present in *V. thapsus*. Myrcetin was present in *W. fruticosa*, *V.Cotinifolium*, *E.hirta*, *V. negundo* and *B. papyrifera* while absent in *A. vasica*, *C. ambrosoides*, *P. harmala*, *T. officinale*, *U .dioica*, *V. thapsus*, *C.grata* and *M. rubicaulis*. Catechin was present in *W. fruticosa*, *C. ambrosoides* and *C. grata* while absent in all the others. Vitexin was present in *A. vasica*, *C. ambrosoides* and *P. harmala* while absent in all the others. Orientin was present in *W. fruticosa*, *A. vasica*, *V. cotinifolium*, *E. hirta*, *V. negundo*, *P. harmala*, *U. dioica* and *C. grata* while absent in all the others. Isoquercitin was present only in *W. fruticosa*, *A. vasica* and *C. grata* but absent in all the others. Hyperside was only present in *V. cotinifolium* and *P. harmala* while absent in the remaining species. Isovitexin was only present in *C. ambrosoides* and *V.negundo* while absent in all the other species. Luteolin-7 - Glucoside was absent in all the species studied. Rutin was absent in all the species except *B. papyrifera*. Kaempferol-7 - neohesperoside was absent in all the species except in *B. papyrifera*. Quercitin was also absent in all the species except *E. hirta*, *V. thapsus*, *C. grata* and *M. rubicaulis*. Luteolin was present in all the species studied. Apigenin was only present in *C. ambrosoides* and *V. negundo* while absent in all the other species.

Table 2. Appearance of standards (Flavonoids) under UV illuminator (365 nm).

S. No	Flavonoids	Rf value	Color reagent (A)	Color reagent (B)
1.	Kaempferol	0.81	Light green	Light green
2.	Myricetin	0.73	Orange	Dark green
3.	Catechin	0.74	Dark green	Dark brown
4.	Vitexin	0.65	Light green	Light green
5.	Orientin	0.48	Light green	Light green
6.	Isoquercitin	0.52	Orange	Orange
7.	Hyperside	0.54	Orange	Dark brown
8.	Isovitexin	0.57	Light green	Light green
9.	Luteolin-7-glucoside	0.57	Flourescent yellow	Orange
10.	Rutin	0.42	Flourescent yellow	Orange
11.	Kampferol-7-neohesp- eridoside	0.55	Light Olive green	Light Olive green
12.	Quercitin	0.87	Yellow	Orange
13.	Luteolin	0.90	Flourescent yellow	Flourescent yellow
14.	Apigenin	0.86	Light green	Light green

Maximum (70.83%) Kaemferol was recorded in *V. thapsus* followed by Luteolin (17%) and Quercitin (16.69%) in the same plant species. Higher Luteolin (20%) content was observed in *W. fruticosa*, whereas: maximum myricetin (15.75%) was recorded in *V. negundo*. Maximum percentage of Quercitin (65.38%) was observed in *M. rubicaulis*. Vitexin and Catechin were maximum (6.15 % and 19 %) in *P. harmala* and *C. grata* respectively (Tables 3a, 3b, 3c).

Most classes of phenols occur as glycosides. Myrcetin is a natural flavonoid having both antioxidative and peroxidative properties and also serve as potent antimutagen and anticarcinogen (Ong & Khoo, 1997). Since Myrcetin was present in *Woodfordia fruticosa, Viburnum cotinifolium, Euphorbia hirta, Vitex negundo* and *Broussonetia papyrifera*, so they may possess antimutagen and anticarcinogen activities. Due to the presence of Quercitin in *Euphorbia hirta, Verbascum thapsus, Caryopteris grata* and *Mimosa rubicaulis*, they may possess protective effects against the hydrogen peroxide induced DNA damage in human lymphocytes since both Myrcetin and Quercitin have protective effects against the hydrogen peroxide induced DNA damage in human lymphocytes (Duthie *et al*., 1997). Quercitin shows antifungal activity (Roy *et al*., 1996). Due to the presence of Quercitin in *Euphorbia hirta, Verbascum thapsus, Caryopteris grata* and *Mimosa rubicaulis*, they may possess antifungal, DPPH scavenging and antimalarial activity. According to Braca *et al*., (2002), Quercitin exhibited DPPH scavenging activity. Quercitin also shows antimalarial activity against *Plasmodium falciparum* (Khalid *et al*., 1996). Luteolin - 7 - glycoside acted as antiulcer drug in rats (Rainova *et al*., 1988). Apigenin and Quercitin both possess anti-inflammatory effects. Due to the presence of Apigenin in *Chenopodium ambrosoides* and *Vitex negundo* they may possess ant-inflammatory activity. Due to the presence of Quercitin in *Euphorbia hirta, Verbascum thapsus, Caryopteris grata* and *Mimosa rubicaulis* they may posess anti-inflammatory effects. Vitexin due to its specific electrophysiological effects in anaesthetized dogs resulted in decreased arotic pressure, pulmonary capillary pressure and heart rate (Occbiuto *et al*., 1990). Due to the presence of Vitexin in *Adhatoda vasica, Chenopodium ambrosoides* and *Peganum harmala*, they may possess electrophysiological effects to decrease arotic pressure, pulmonary capillary pressure and heart rate. Rutin exhibited analgesic activity in response to acetic acid writhing test in mice (Sambantham *et al*., 1999). Due to the presence of Rutin in *Broussonetia papyrifera*, it may possess antioxidant and analgesic activity. According to Radi *et al*., (2004), both rutin and catechin showed antioxidant activity. Due to the presence of Catechin in *Woodfordia fruticosa, Chenopodium ambrosoides* and *Caryopteris grata*, these plants may possess antioxidant activity. Qureshi *et al*. (2007) reported that Quercitin is responsible for antioxidant activity in certain plants.

Table 3a. Flavonoid distribution (in %) among selected medicinal plants.

S. No.	Flavonoids	Plant species				
		W. fruticosa	***A. vasica***	***C. ambrosoides***	***V. Cotinifolium***	***E. hirta***
1.	Kaempferol	-	-	-	-	-
2.	Myricetin	9.18	-	-	1.87	6.4
3.	Catechin	1.6	-	0.87	-	-
4.	Vitexin	-	6.66	2.4	-	-
5.	Orientin	2.20	5.38	-	3.84	6.41
6.	Isoquercitin	3.07	7.69	-	-	-
7.	Hyperside	-	-	-	6.15	-
8.	Isovitexin	-	-	2.57	-	-
9.	Luteolin-7- Glucoside	-	-	-	-	-
10.	Rutin	-	-	-	-	-
11.	Kaempferol-7-Neohesperidoside	-	-	-	-	-
12.	Quercitin	-	-	-	-	3.83
13.	Luteolin	20.00	3.38	2.85	1.71	1.47
14.	Apigenin	-	-	2.11	-	-

Table 3b. Flavonoid distribution (in %) among selected medicinal plants.

S. No	Flavonoids	Plant species				
		V. negundo	***P. harmala***	***B. papyrifera***	***T. officinale***	***U. dioica***
1.	Kaempferol	-	-	-	-	-
2.	Myricetin	15.75	-	2.66	-	-
3.	Catechin	-	-	-	-	-
4.	Vitexin	-	6.15	-	-	-
5.	Orientin	3.07	4.86	-	-	-
6.	Isoquercitin	-	-	-	-	-
7.	Hyperside	-	4.62	-	-	-
8.	Isovitexin	3.51	-	-	-	-
9.	Luteolin-7- Glucoside	-	-	-	-	-
10.	Rutin	-	-	6.2	-	-
11.	Kaempferol-7-Neohesperidoside	-	-	5.6	-	-
12.	Quercitin	-	-	-	-	3.83
13.	Luteolin	3.14	1.00	0.81	2.5	3.6
14.	Apigenin	3.68	-	-	-	-

Table 3c. Flavonoid distribution (in %) among selected medicinal plants.

S.No.	Flavonoids	Plant species		
		V. thapsus	***C. grata***	***M. rubicaulis***
1.	Kaempferol	70.83	-	-
2.	Myricetin	-	-	-
3.	Catechin	-	19.2	-
4.	Vitexin	-	-	-
5.	Orientin	-	3.84	-
6.	Isoquercitin	-	4.00	-
7.	Hyperside	-	-	-
8.	Isovitexin	-	-	-
9.	Luteolin-7- Glucoside	-	-	-
10.	Rutin	-	-	-
11.	Kaempferol-7- Neohesperidoside	-	-	-
12.	Quercitin	16.69	6.13	65.38
13.	Luteolin	17.00	3.5	2.3
14.	Apigenin	-	-	-

Conclusion

It is inferred that the plants showing the presence of different flavonoids can be potential sources of these natural compounds exhibiting different activities. Further research in this regard is recommended to isolate these bioactive principles.

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