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Comparative analysis of leaf exudate flavonoids in genus Tanacetum

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Abstract:

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The tribe *Anthemideae* of the *Asteraceae* comprises a large number of species that have been still are used as medicinal plants, particularly in folk medicine. It has recently shown flavonoid aglycones are accumulated in several medicinal plants, especially on their leaf and stem surfaces.

The genus *Tanacetum* (*Asteraceae*) comprises of about 145 species, complied mainly from the Floras of Europe, Turkey and Russia. In completion of our studies on *Tanacetum* exudate flavonoids, 6 species of Tanacetum distributed in Bulgaria were analyzed for their aglicone profiles. Thirteen flavonoid aglycones have been indentified from 6 plants covering the both sections- sect. T.tanacetum *(Tanacetum vulgare, Tanacetum balsamita, Tanacetum millefolium)* and sect. T. pyrethrum *(Tanacetum parthenium, Tanacetum corymbosum, Tanacetum macrophyllum*) of genus Tanacetum that were collected in Bulgaria. The flavonoids were indentified by co-TLC with anthentic markers. The major accumulation trend is represented by the presence of 6- hydroxyflavones, 6- hydroxyflavonols and their methyl derivatives in the leaf exudates. The most common aglycones were luteolin, 6- hydroxyluteolin 6-methyl ether, 6-hydroxyluteolin 6, 3'-dimethyl ether and quercetagetin 3, 6- dimethyl ether. The ecological significance of external aglycone formation in xerophytes is discussed.

Key words: genus *Tanacetum*, tribe Anthemideae, *Asteraceae,* flavonoid aglycones, 6- hydroxyflavones, 6- hydroxyflavonols

Introduction

The tribe Anthemideae of the Asteraceae comprises a large number of species that have been still are used as medicinal plants, particularly in folk medicine. It has recently shown (Wollenweber, 1990) flavonoid aglycones are accumulated in several medicinal plants, especially on their leaf and stem surfaces.

The genus Tanacetum (Asteraceae) comprises of about 145 species, complied mainly from the Floras of Europe, Turkey and Russia. The chemotaxonomy of Tanacetum has suffered great neglect in comparison with that of its "sister" genera like Artemisia. At the stated in previous papers, chemical investigation of species of the genus Tanacetum - *Tanacetum serotinum* (Stefanovic et al., 1985), *Tanacetum parthenium (*Stefanovic et al., 1985; Williams et al., 1995*), Tanacetum macrophyllum* (Stefanovic et al., 1982), *Tanacetum vulgare* (Stefanovic et al., 1985), *Tanacetum corymbosum* (Thomas, 1988), *Tanacetum cilicium* (Thomas, 1989) on the contents of sesquiterpene lactones and flavonoids has reveled. These compounds are capable of reacting inhibitory effects of *Tanacetum parthenium* L.(feverfew) on platelet and leukocyte secretion and be responsible for their anti-

inflammatory and cytotoxic properties (Williams et al., 1995). However such compounds are likely to be present since several flavonoid methyl ethers have been characterized from Tanacetum species. Thus, 6-hydroxyluteolin 6, 3'-dimethyl ether (jaceosidin, **7**) and quercetagetin 3, 6, 3'-trimethyl ether (jaceidin, **13**) have been reported from the flowers of *T. vulgare*, while 6-hydroxyluteolin 6, 7, 3'-trimethyl ether (cirsilineol, **8**), 6-hydroxyluteolin 6, 7, 3', 4'-tetramethyl ether (**9**), quercetagetin 3, 6, 7, 4'-tetramethyl ether (casticin, **15**) have been found in aerial parts of *T. santolinoides* (Wollenweber, Mann, 1988). More recently a unique flavonoid structure with a carboxylic acid substituent in the 7-position, 3, 5, 3'-trihydroxy 4'-methoxy- 7-carbomethoxyflavone has been characterized from *Tanacetum macrophyllum* together with quercetagetin 3, 6, 4'-trimethyl ether (**14**) (Ivancheva, Stancheva, 1997). The present paper reports the lipophilic flavonoids identified from the leaves of Tanacetum species growing in Bulgaria.

Materials and Methods

Dried leaves and steams from wild populations and cultivated plants were used for analysis.

Voucher specimens are deposited at the Herbaria of the Institute of Botany, Sofia.

List of plant samples

Section *Tanacetum*

T. vulgare L.: (1). Central Stara planina Mt., Trojan; (2). Sofia region, Tzarigradsko schosse; (3). Sredna gora Mt., Pirdop; (4). Rila Mt., Borovetz; *T. balsamita*: (5). Experimental field of Institute of Botany, BAS, Sofia; *T. millefolium*: (6). Region of Kavarna, Kaliakra

Section *Pyrethrum*

T. parthenium: (7). West Stara planina Mt., Barsia; *T. corymbosum* L.: (8). Sofia region, Kokaljane monastery; (9). Rodopes Mt., Chudni mostove; (10). Sofia regoin, Lozenska planina; *T. macrophyllum* L.: (11). Sofia region, Kokaljane monastery.

Aerial parts (leaves and stems) were briefly rinsed with acetone to dissolve the exudate materials which was concentrated by evaporation of the solvent. The exudate was chromatographed over Sephadex LH 20 to separate the flavonoids from the dominating terpenoid consistuents. Concentrated flavonoid fractions were subjected to comparative TLC on Silica and Polyamide using several solvent systems (Wollenweber et al., 1987). Individual compounds were identified by co-chromatography with markers. In some cases the structures were confirmed by UV-Spectroscopy.

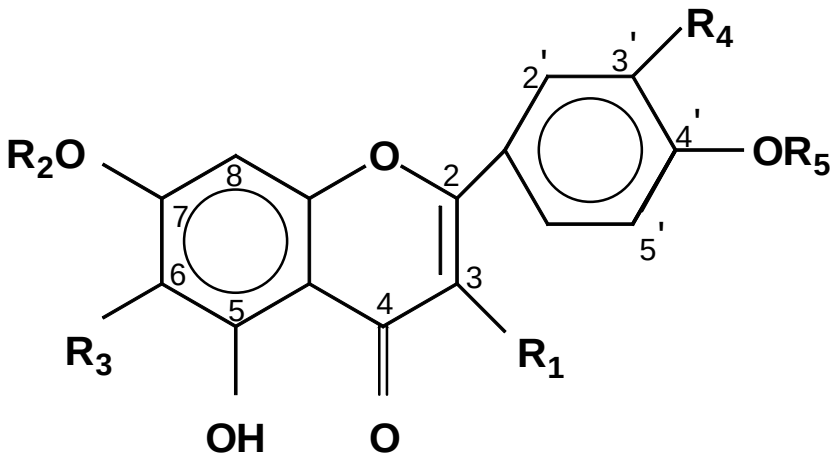
Results and Discussion

The *Tanacetum* species analysed were belong to different systematic group. Their arrangement in Table 1 follow principally the taxonomic concept to Kuzmanov (Kuzmanov, 1984). The main distinction is made between *T. balsamita, T. millefolium* and *T. vulgare* L., sect. Tanacetum (yellow flowering) and *T. parthenium* (L.) Schultz. Bip., *T. corymbosum* (L.) Schultz. Bip., *T. macrophyllum* (Waldst. et Kit.) Schultz. Bip. in sect. Pyrethrum (white flowering).*T. balsamita* L. is cultivated in various parts of the country as a medicinal and decorative plant, and as a flavouring plant. It originates from Central Asia (Soreng, Cope, 1991).

The major accululation trend is represented by the presence of 6- hydroxyflavones, 6-hydroxyflavonols and their derivatives in the leaf exudates (Table 1 and for formule see Fig. 1). The flavones scutellarin and 6- hydroxyluteolin are represented by a series of methyl derivatives, while the derivatives of the flavonols 6-hydroxykaempferol and quercetagetin are observed with a higher degree of methylation. Thus, within the both sections the most common aglycones were luteolin (**4**), 6- hydroxyluteolin 6- methyl ether (nepetin, **6**), 6- hydroxyluteolin 6, 3'- dimethyl ether (jaceosidin, **7**) and quercetagetin 3, 6-dimethoxy ether (axillarin, **12**). Further derivatives of flavones and flavonols co-occurred in varied amounds. Within sect. Pyrethrum, luteolin (**4**), nepetin (**6**) and jaceosidin (**7**) were encountered in one taxon each. Flavonoids lacking 6- substitution rarely accumulated in the leaf exudates, but luteolin (**4**) and quercetin 3- methyl ether (**11**) are found in both sections. Quercetagetin 3, 6, 4'- trimethyl ether (centaureidin, **14**) and quercetagetin 3, 6, 3', 4'- tetramethoxy ether (**16**) were detected only in *T. corymbosum*. In case of *T. balsamita* (cultivated) were detected only one derivative of quercetagetin, another flavonols lack. 7- substitution derivatives lack in investigated species.

Pure apigenin (**1**) is found for the first time in genus Tanacetum. We have investigated external flavonoid aglycones by HPLC (unpublished data) and these results were confirmed. It is assumed that external flavonoids might act as UV-screen and antivirus activity, thus being ecologically signi-

Figure 1.



	R_1	R_2	R_3	R_4	R_5
(1). Apigenin	H	H	H	H	H
(2). Scutellarein 6- Me (hispidulin)	H	H	OCH_3	H	H
(3). Scutellarein 6, 4'- diMe (pectolinarigenin)	H	H	OCH_3	H	CH_3
(4). Luteolin	H	H	H	OH	H
(5). Luteolin 3'- Me (chrysoeriol)	H	H	H	OCH_3	H
(6). 6- Hydroxyluteolin 6- Me (nepetin)	H	H	OCH_3	OH	H
(7). 6- Hydroxyluteolin 6, 3'- diMe (jaceosidin)	H	H	OCH_3	OCH_3	H
(8). 6- Hydroxyluteolin 6, 7, 3'- triMe (cirsilineol)	H	CH_3	OCH_3	OCH_3	H
(9). 6- Hydroxyluteolin 6, 7, 3', 4'- tetraMe	H	CH_3	OCH_3	OCH_3	CH_3
(10). 6- Hydroxykaempferol 3, 6, 4'- triMe (methylbetuletol)	OCH_3	H	OCH_3	H	CH_3
(11). Quercetin 3- Me	OCH_3	H	H	OH	H
(12). Quercetagetin 3, 6- diMe (axillarin)	OCH_3	H	OCH_3	OH	H
(13). Quercetagetin 3, 6, 3'- triMe (jaceidin)	OCH_3	H	OCH_3	OCH_3	H
(14). Quercetagetin 3, 6, 4'- triMe (centaureidin)	OCH_3	H	OCH_3	OH	CH_3
(15). Quercetagetin 3, 6, 7, 4'- tetraMe (casticin)	OCH_3	CH_3	OCH_3	OH	CH_3
(16). Quercetagetin 3, 6, 3', 4'- tetraMe	OCH_3	H	OCH_3	OCH_3	CH_3

ficant (Marby et al., 1970). This is also indicated by their occurrence on xerophytes species, whereas those restricted to mesic habitats were devoid of lipophilic aglycones. Their functional role, however, has to be kept in mind if this phenomenon is to be interpreted systematically.

However, it should be noted that aglycone profiles observed within genus Tanacetum proved to be of more chemotaxonomic significance. The diversity in terms of flavonoid substitution trends is high and formation of exudate flavonoid aglycones is related to the habitat since most of the taxa analysed are either meso or prefer xeric habitats (Wollenweber, 1990).

Conclusion

The major accumulation trend is represented by the presence of 6- hydroxyflavones, 6-hydroxyflavonols and their methyl derivatives in the leaf exudates. The most common aglycones

Table 1. Flavonoid aglycones distributed in genus *Tanacetum sp*.

Compounds	Flavones							Flavonols					
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Sect. Tanacetum													
Tanacetum vulgare - (1)		+	+	(+)		+	++		(+)	+	++		
(2)		+	++			+	++			++	+		
(3)		+				+	++			+	+		
(4)				+	+	(+)	++		(+)	+	++		
Tanacetum balsamita -(5)		+	++	+		+	+			+			
Tanacetum millefolium - (6)	+	+	+					+			+	+	
Sect. Pyrethrum													
Tanacetum parthenium -(7)				(+)		(+)		+		(+)	(+)		
Tanacetum corymbosum - (8)		++		(+)		++	(+)		(+)	++	(+)		
Tanacetum corymbosum - (9)				(+)		(+)		+	(+)	++	++		
Tanacetum corymbosum - (10)				(+)		(+)				(+)		++	++
Tanacetum macrophyllum - (11)				(+)		+			(+)	+			

Legend:

(1). Central Stara Planina Mt.,Trojan
(2). Sofia region, Tzarigradsko schosse
(3). Sredna gora Mt., Pirdop
(4). Rila Mt., Borovetz
(5). Experimental field of Inst. of Botany, BAS, Sofia
(6). Region of Kavarna, Kaliakra
(7). West Stara Planina Mt., Barzia
(8). Sofia region, Kokaljane monastery
(9). Rodopes Mt., Chudni mostove
(10). Sofia region, Lozenska planina
(11). Sofia region, Kokaljane monastery

Flavones
1. Apigenin
2. Scut- 6- Me (hispidulin)
3. Scut- 6, 4'- diMe (pectolinarigenin)
4. Luteolin
5. Lut- 3'- Me (chrysoeriol)
6. 6- OH Lut- 6-Me (nepetin)
7. 6- OH Lut- 6, 3'-diMe (jaceosidin)
Flavonols
8. 6- OH Kae 3, 6, 4'- triMe (methylbetuletol)
9. Qu- 3- Me
10. Queg- 3, 6- diMe (axillarin)
11. Queg- 3, 6, 3'- triMe (jaceidin)
12. Queg- 3, 6, 4'- triMe (centaureidin)
13. Queg-3, 6, 3' 4'- tetraMe

were luteolin (**4**), 6- hydroxyluteolin 6-methyl ether (nepetin, **6**), 6-hydroxyluteolin 6, 3'-dimethyl ether (jaceosidin, **7**) and quercetagetin 3, 6- dimethoxy ether (axillarin, **12**).

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Резюме

Сравнителен анализ на повърхностно разположени флавоноидни агликони в род *Tanacetum*

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Триб *Anthemideae*, сем. *Asteraceae* обхваща голям брой видове, които намират приложение като лекарствени растения. Известно е, че флавоноидните агликони са разположени по повърхността на листата и стъблата на много медицински видове.

Род *Tanacetum* (*Asteraceae*) обхваща около 145 вида, разпространени главно в Европа, Турция и Русия. Изследвани са повърхностно разположените флавоноидни агликони в 6 вида *Tanacetum,* растящи в България. Тринадесет флавоноидни агликони са изолирани и идентифицирани от шесте вида от двете секции на род *Tanacetum*- секция *T. tanacetum* (*Tanacetum vulgare, Tanacetum balsamita, Tanacetum millefolium)* и секция T. pyrethrum *(Tanacetum parthenium, Tanacetum corymbosum, Tanacetum macrophyllum*) разпространени в различни райони на България. Идентификацията е извършена чрез ТСХ и е извършено сравнение с автентични проби (свидетели). Главната акумулираща тенденция в род Tanacetum е към синтез на 6-хидроксифлавони и 6-хидроксифлавоноли и техните метилирани производни в листните ексудати. Общите агликони са лутеолин, 6 -хидроксилутеолин 6 -метилов етер (непетин), 6-хидроксилутеолин 6, 3' -диметилов етер (яцеозидин) и кверцетагетин 3, 6 -диметил етер (аксилярин). Екологичното значение на екстерналните агликони е накратко дискутирано.