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Study of Dry Extract of Tansy (*Tanacetum vulgare*) Using the Method of High-performance Liquid Chromatography

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ABSTRACT

For the first time the qualitative composition and quantitative maintenance of phenolic substances in tansy flowers dry extract was studied using the method of HPLC. 10 components of phenolic origin were identified. Components of phenolic compounds were represented by the following groups of substances: hydroxycinnamic acids, flavonols, flavan-3ol, flavones, catechins, coumarin, tannins and anthocyans. Among the identified substances dominated rutin, luteolin, ferulic acids 3,5-dichlorogenic and 4,5-dichlorogenic.

Keywords: Tansy, Flowers, Dry extract, Phenolic substances, HPLC

INTRODUCTION

Tansy (*Tanacetum vulgare* L.) is a herbaceous, perennial, wild-growing plant which is widely-spread on the territory of Ukraine and CIS countries. Chemical composition of tansy flowers is rather diversified. The main groups of biologically active substances (BAS) are phenolic compounds (luteolin, luteolin-7-glycoside, apigenin, kaempferol, chlorogenic and caffeic acids), volatile oil (α- and β-thujone, cineole, camphor, borneol), organic acids, alkaloids, polysaccharides, carotenoids, sesquiterpene lactones, tannins [1]. Tansy flowers have vermifugal, choleretic, hepatoprotective, anti-inflammatory, analgesic, antimicrobial, antipyretic pharmacological features [2-5]. Dry extract obtained from tansy flowers using the method of fractional maceration has hepatoprotective and choleretic action, which is provided by phenolic compounds. To study the qualitative composition of the extract the method of High-performance Liquid Chromatography (HPLC) was chosen [6-10].

MATERIALS AND METHODS

Plant material and chemicals

To obtain dry extract the flowers of tansy prepared in July 2014 in Kolomak district, Kharkiv region, Ukraine were used. Dried in an air shade way raw material was crushed in the quantity of 100.0 g and it was extracted with 70% ethanol at indoor temperature in proportion 1:5 for three times. The first extraction lasted for 12 h, the 2 subsequent lasted for 1 h each. Obtained extractions were infused, filtered and steamed under vacuum until they turned dry. The obtained extract is yellowish brown, bibulous with a peculiar odour. It is well dissolved in water, ethanol, badly dissolved in methanol.

To carry out the analysis the extract in the quantity of 0.202 g was dissolved in 10 ml of 20% ethanol and put into the column. The presence of phenolic compounds was identified through the comparison of time of keeping substances with Pharmacopoeial standardized samples of State Pharmacopoeia of Ukraine (PSS SPU) as well as with standardised extracts of plants. Models of luteolin, quercetin, quercemitrin, apigenin, apigenin-7-glycoside, rutin, hyperoside, catechin, gallic acid, chlorogenic acid, coffeic acid, ferulic acid, umbelliferone, scopoletin were used as PSS SPU. The purity of all samples made up over 98%. For identification of acacetin-7-glycoside, neochlorogenic, 3,5-dichlorogenic and 3-chlorogenic acid standardised extract of *Chrysanthemum morifolium* flowers, dry extract of globe artichoke (*Cynara scolymus*) leaves and caprifoil Japanese (*Lonicera japonica*) flowers were used.

Chromatographic conditions

While carrying out chromatographic researches of samples under investigation a liquid chromatograph equipped with diode matrix detector Shimadzu HPLC-system, ser.20 was used. The conditions of the analysis were as following: column Phenomenex Luna C18 (2), sized 250 × 4.6 mm, particle size: 5 mkm, temperature of column: 35°C, speed of movable phase flow: 1 ml/min, sample volume: 5 mkm. The system of solvents for elution: A: 0.01% Trifluroacetic Acid (TFA) in acetonitrile and B: 0.01% TFA in water (Table 1) [11].

Table 1: Program of gradient elution

Time (min)	Eluent A, (%)	Eluent B, (%)
0-5	95	5
5-35	95→75	5→25
35-40	75	25
40-60	75→50	25→50
60-65	50→20	50→80
65-70	20	80
70-85	95	5

While conducting HPLC analysis spectral measurements were made in the range of waves 180-800 nm with a pitch of 2 nm. UV-spectrums of absorption of analysed substances were recorded online. DAD software was used to check the purity of each peak. Quantitative maintenance of every identified compound was identified using the method of external standard, recalculated into dry extract accounting on the humidity. Identification of flavonoids was carried out at the wave length of (254 nm), of tannins (280 nm), of ferulic acids (330 nm). To calculate the results of quantitative maintenance the following formula was used:

$$\text{Assay (\%)} = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100}$$

Where, A_{pr}: Area of peak of the substance on the chromatogram of solution under investigation, A_{st}: Area of peak of the substance on the chromatogram of standard solution, m_{st}: Mass of standard sample of substance in standard solution (mg), m_{pr}: Mass of Georgina herb (mg), V_{pr}: Dilution of solution under investigation (ml), V_{st}: Dilution of standard solution (ml), P: Activity of the standard (%). Reproducibility of results was confirmed by carrying out the research for three times.

RESULTS AND DISCUSSION

As the consequence of studying dry extract using method of HPLC 10 substances including the compounds of the group of ferulic acids and flavonoids were identified (Figure 1).

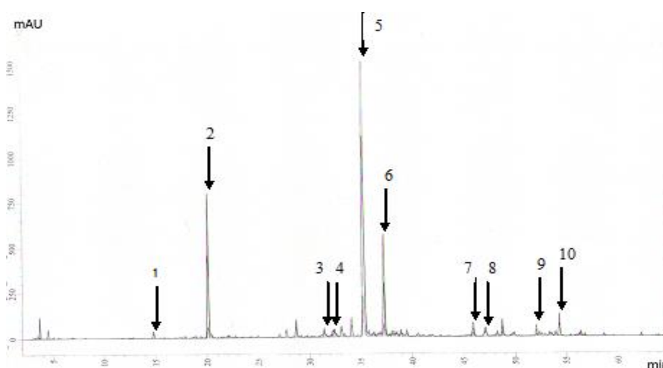

Figure 1: HPLC chromatogram of dry extract of tansy flowers

(1) neochlorogenic acid; (2) chlorogenic acid; (3) 3,5-dichlorogenic acid; (4) 4,5-dichlorogenic acid; (5) rutin; (6) hyperoside; (7) acacetin-7-glycoside; (8) luteolin; (9) apigenin; (10) kaempferol

Among the isolated compounds 10 were determined authentically (Table 2). Ferulic acids were represented with 4 compounds: chlorogenic, neochlorogenic, 3,5-dichlorogenic and 4,5-dichlorogenic acids. Flavonoids are represented with flavones (luteolin, apigenin, acacetin-7-glycoside), flavonols (rutin, hyperoside, kaempferol).

Table 2: Phenol substances of dry extract of tansy flowers

S. No.	Name of substance	Time of keeping (min)	Area of peak (mAU)	Length of wave (нм)	Substances maintenance (%)
			Ferulic acids		
1	neochlorogenic acid*	14,79	382396	330	0,128
2	chlorogenic acid	20,07	7197871	330	2,404
3	3,5-dichlorogenic acid**	35,21	21921084	330	7,323
4	4,5-dichlorogenic acid**	37,23	5477514	330	1,830
			Flavonoids		
5	rutin	31,39	578064	330	0,370
6	hyperoside	32,04	113281	370	0,161
7	acacetin-7-glycoside***	45,89	902590	330	0,018
8	luteolin	47,07	690173	370	0,340
9	apigenin	52,35	106806	370	0,055
10	kaempferol	53,35	146374	370	0,050

*standardised *Chrysanthemum morifolium* flower extract was used for identification; **standardised globe artichoke (*Cynara scolymus*) leaves dry extract was used for identification; ***standardised caprifoil Japanese (*Lonicera japonica*) flower dry extract was used for identification

Among the ferulic acids the following acids were accumulated: chlorogenic (2.4%), 3,5-dichlorogenic (7.3%) and 4,5-chlorogenic (1.8%) acids. Having analysed the contents of substances of flavonoid group, it was determined that in tansy flowers dry extract prevailing are rutin (0.37%) and luteolin (0.34%). In twice less quantity hyperoside (0.16%) accumulated. Moreover, ghost amounts of coumarin representatives such as umbelliferone, tannins–gallic acid, ferulic acids (coffeic and ferulic acids) and flavonoids (catechin, apigenin-7-glycoside) were determined.

UV-spectrums of primary compounds identified in tansy flowers dry extract are depicted in Figure 2.

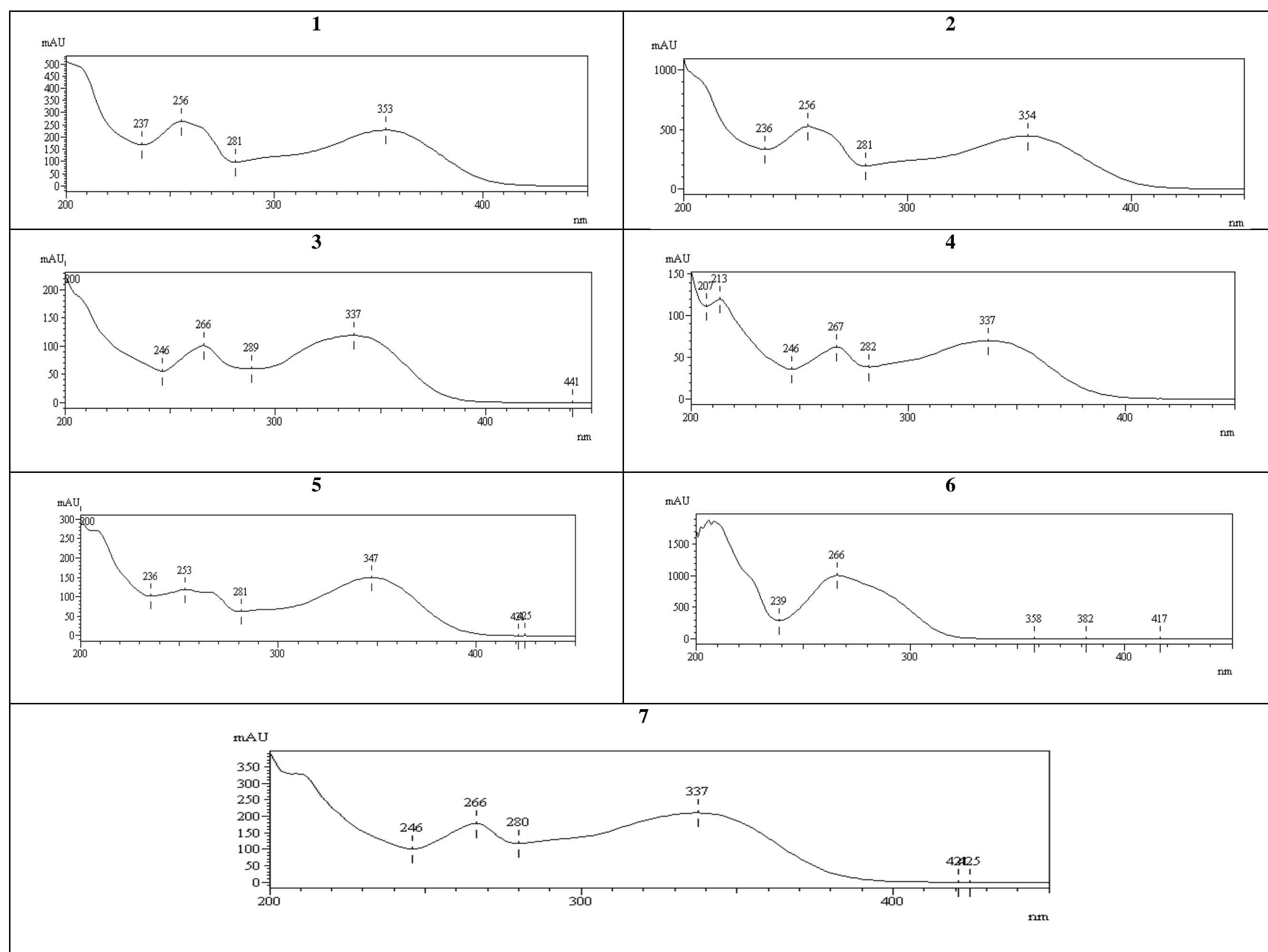

Figure 2: UV-spectrums of standard samples (SS) of basic phenolic substances

(1) UV-spectrum of rutin; (2) UV-spectrum of apigenin; (3) UV-spectrum of apigenin-7-glycoside; (4) UV-spectrum of hyperoside; (5) UV-spectrum of luteolin; (6) UV-spectrum of catechin; (7) UV-spectrum of gallic acid

For rutin and luteolin the peaks of absorption were observed in the shortwave (256 and 253 nm) and longwave (354 and 347 nm). Hyperoside maintenance was determined at the peaks of absorption in longwave region (354 nm). Flavonoids apigenin, apigenin-7-glycoside and catechine had common peaks of absorption (266 and 337 nm). Gallic acid was determined according to distinctive peak of absorption 266 nm.

CONCLUSION

For the first time the qualitative composition and quantitative maintenance of phenolic substances in tansy flowers dry extract was studied using the method of HPLC. 10 components of phenolic origin were identified. Among them ferulic acids 3,5-dichlorogenic and 4,5-dichlorogenic, which accumulated in the biggest quantity, were identified for the first time. Obtained data was used while standardization of tansy flowers dry extract.

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