BOTANICAL AND PHYTOCHEMICAL STUDIES ON TANACETUM VULGARE L. FROM TRANSYLVANIA

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Abstract: *The aim of this study is to bring new contributions to pharmacognostical knowledge of Tanacetum vulgare L. (Asteraceae), common tansy, harvested from the spontaneous flora of Transylvania. In this study, head flowers of tansy and of Tanacetum balsamita var. tanacetoides harvested from around Cluj- Napoca were used. It was carried out a microscopically and a gas-chromatography and a mass-spectrometry study. Head flowers of tansy presented morphological and histo-anatomical features related to those described in the scientific research. The differences to the head flowers of Tanacetum balsamita var. tanacetoides were in the involucres and pollen grains. Two compounds, selina- 3,7(11)- diene and 2-acetyl-3,4,6- trimethylpyrazine were for the first time identified in the essential oil of tansy. Thujen-2- one was the major compound. The knowledge of botanical and phytochemical characteristics of Tanacetum vulgare allows a correct identification of the plant material important for medical purpose.*

Cuvinte cheie: *Tanacetum vulgare L., inflorescenţe, microscopie, gas-cromatografie, spectrometrie de masă*

Rezumat: *Studiul urmăreşte să aducă noi contribuţii la cunoaşterea farmacognostică a speciei Tanacetum vulgare L. (Asteraceae), vetrice, recoltată din flora spontană a României, din Transilvania. În cadrul studiului s-au utilizat inflorescenţele recoltate de la specia Tanacetum vulgare L. şi de la specia Tanacetum balsamita var tanacetoides din zona Cluj- Napoca. S-a realizat un studiu microscopic şi unul gaz-cromatografic cuplat cu spectrometrie de masă. Inflorescenţele speciei Tanacetum vulgare prezintă caracteristici morfologice şi histo-anatomice asemănătoare celor descrise în literatura de specialitate. Diferenţele faţă de inflorescenţele speciei Tanacetum balsamita var tanacetoides apar la nivelul involucrului şi granulelor de polen. Compusul majoritar identificat a fost tuion-2-onă. Doi compuşi, selina- 3,7(11)- dienă şi 2- acetil- 3,4,6-trimetilpirazin au fost pentru prima dată identificaţi în uleiul volatil de vetrice. Cunoaşterea caracteristicilor botanice şi fitochimice ale speciei T. vulgare, permite identificarea corectă a materialului vegetal în vederea utilizării sale în scop medicinal.*

INTRODUCTION

Tanacetum vulgare L., (*Asteraceae*), common tansy, is well known for the anthelmintic action. Because of its toxicity, the tansy products should be taken with some caution. According to scientific research, thujone is the compound of the essential oil responsible for the anthelmintic action, but also for its toxicity.(1-6)

In our country, tansy is widespread, but there are few published studies on the morphological, histo-anatomical and phytochemical features. This information is important for a correct identification of the plant material, but also for a correct capitalization in therapy.

PURPOSE

The main objective of this study was to highlight the main morphological and histo-anatomical features and to analyze the essential oil isolated from the head flowers of *Tanacetum vulgare* harvested from the spontaneous flora of Transylvania.

METHODS

The analysis was made on dry head flowers from *Tanacetum vulgare* harvested from the spontaneous flora of Transylvania, from around Cluj- Napoca during the flowering stage (June), in 2013. For comparison, one has also made a botanical analysis of *Tanacetum balsamita var. tanacetoides*, which may easily be confused by tansy. A specimen of each of the two species may be found stored at the department of Pharmacognosy, faculty of Pharmacy Cluj- Napoca (Voucher nr. 26, 27).

For the botanical analysis, the Motic stereomicroscope for the morphological elements and the Olympus microscope CX31 for the histo-anatomical features of the two species were used.

For the microscopical analysis, one used the examination technique of the powder products of *Tanaceti vulgare herba* and *Tanaceti balsamitae herba*, after the clarification with chloral hydrate solution 80%. The microscopic preparations were made according to the European Pharmacopoeia 7th edition, monograph of *Tanaceti parthenii herba*.(7)

The isolation of the essential oil was made by hydro-distillation, using a Clevenger-type apparatus in accordance with the Romanian Pharmacopoeia 10th Edition.(8)

The essential oil was analyzed using a gas-chromatograph coupled with mass-spectrometer apparatus. GC-MS analysis was performed using an Hewlett- Packard 5872 gas-cromatograph, II series 5972 MS, Agilent 6890, a capillary

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column HP- MS 5 (30m x 0,26 mm i.d.; film thickness 0,25 µm). The injector and detector temperatures were kept at 250°C and 280°C, respectively. Helium was used as carrier gas, a flow rate of 1 mL/ min; oven temperature programme was 60- 240° C at rate of 3° C/ min. MS analysis was performed in the same conditions, with ionization voltage of 70eV, mass range was from 41 to 300 m/ z.

The constituents of the essential oils were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley).(13)

RESULTS

After the microscopic analysis, there were determined the following features of *Tanacetum vulgare*:

- The head flowers are small, tube-shaped, arranged into calathids on branching corymbtype. These calathids are protected by many overlapping bracts which make the involucre. The involucre is hemispherical, flattened, with green bracts, brown margins, hard and obtuse at the top. The internal bed of the bracts is membranous. The central flowers are tube-shaped with 5 teeth and have 5 stamens inserted in the corolla (figure no. 1).

Examined under a microscope using chloral hydrate solution R., the powder shows the following diagnostic characters:

- Fragments of disc florets containing small rosette crystals of calcium oxalate, spherical pollen grains, with 3 pores and a spiny exine may be present (figure no. 2).
- There were identified numerous large, multicellular, uniseriate covering trichomes consisting of a rhomboidal basal cell, 3-5 smaller, thick- walled rectangular cells and a very long, flat, slender terminal cell, curved at a right angle to the axis of the basal cell (T- form) (figure no. 3).

In comparison to tansy, *Tanacetum balsamita var tanacetoides* shows differences in involucre. It consists two rows of bracts, each of them has rounded bracts on the top, and the external one are short and sharp on the top (figure no. 4). The pollen grains have spherical simple 1 pore form and not 3 pores as tansy has .

Figure. no. 1. Headflowers of Tanacetum vulgare L

Figure no. 2. Pollen grains of Tanacetum vulgare L

The concentration of the essential oil of the head flowers of *Tanacetum vulgare* (dried material) determined by hydrodistillation, was 0,89% (v/m%). In the literature, the reported levels of essential oil in *Tanacetum vulgare* head flowers are between 0,4% - 1,1%.(14)

Gas-chromatography coupled with mass-spectrometry analysis:

The identification was made by using the retention time and the mass spectrum of the compounds from the library date. 160 compounds were separated and 105 were identified. 14 compounds had a concentration higher than 1%, representing 56,2% of the essential oil composition. In table 1, are shown the compounds with a higher concentration than 0,1%. The results represent the average of 3 determinations.

Figure no. 3. Trichomes of Tanacetum balsamita L

Figure no. 4. The involucre of Tanacetum vulgare L

Table no. 1. Identified compounds of the essential oil isolated from the head flowers of Tanacetum vulgare

No.	Compound	Retention time Rt	RI	Concentration %
1	Thujen- 2-one	13,66	1176	13,66
2	Crysanthenyl acetate	16,19	1238	13,18
3	Artemisia ketone	9,23	1061	6,66
4	Camphor	12,37	1145	4,75
5	Alpha- thujone	10,86	1107	3,07
6	Germacrene- D	26,22	1480	2,99
7	Selina- 3,7(11)- diene	32,96	1655	2,69
8	Beta- thujone	11,22	1116	1,54
9	2(1H)- Pyridinone	13,18	1165	1,43
10	1,8- Cineole	8,18	1031	1,42
11	Beta- Patchoulene	26,31	1482	1,31
12	l-4-Terpineol	13,75	1179	1,23
13	Thymol	18,48	1292	1,14
14	2- Acetyl- 3,4,6- trimethylpyrazine	31,91	1627	1,13
15	Beta- caryophyllene	23,71	1418	0,7
16	Naphtalene	26,98	1499	0,68
17	Yomogi- alcohol	7,11	1000	0,49
18	Geranyl isovalerate	30,28	1584	0,48
19	Artemisia alcohol	9,99	1083	0,47
20	Alpha- Terpineol	14,23	1191	0,47
21	p- Cymen	7,95	1024	0,47
22	Viridiflorol	32,82	1651	0,41
23	Borneol L	13,25	1167	0,42
24	Chromolaenin	43,68	1896	0,42
25	Bicyclogermacrene	26,81	1495	0,34
26	(-)- Caryophyllene-oxid	30,14	1580	0,34
27	gamma- Terpinene	9,12	1058	0,34
28	(+) spathulenol	29,93	1575	0,33
29	delta- cadinene	27,9	1523	0,29
30	trans- Chrysanthenol	12,02	1136	0,26
31	Neryl Acetate	21,53	1366	0,24

32	Myrtenol	14,47	1197	0,24
33	Bornyl acetate	18,21	1286	0,23
34	7- epi- alpha- selinene	27,62	1515	0,23
35	Isopinocarveol	12,13	1138	0,21
36	Trans-beta- Farnesene	25,3	1458	0,2
37	Germacrene B	31,53	1617	0,19
38	Izolongifolene	21,93	1375	0,19
39	Chrysanthenone	11,6	1125	0,18
40	Izocitronellol	29,38	1561	0,17
41	alpha- Guaiene	30,9	1599	0,16
42	alpha - terpipene	7,7	1017	0,16
43	Nerolidol	29,47	1563	0,16
44	Farnesene	28,88	1548	0,15
45	cis- Jasmone	22,9	1398	0,14
46	Methyl biphenyl- 4-carboxylate	33,61	1672	0,14
47	Cuminal	16,31	1241	0,13
48	Limonene	8,09	1024	0,12
49	(+)- beta- Guaiene	32,53	1643	0,12
50	alpha- Terpinene	11,43	1121	0,11
51	Naphthalene	32,37	1639	0,11
52	Camphene	14,89	1207	0,11

DISCUSSIONS

Head flowers of *Tanacetum vulgare* showed morphological and histo-anatomical features related to those described in the scientific research.

The major compound is represented by thujen-2-one (13,66%). The essential oil contain 2 isomers of thujone, alpha- and beta- thujone, monoterpenes ketones, responsible for the toxicity of the essential oils of tansy.(3)

Chrysanthenyl acetate is also present in high concentration (13,18%) and it is a compound often identified in the essential oils of *Tanacetum vulgare* harvested from different geographical regions.(15-17)

Two compounds, selina- 3,7(11)- diene and 2-acetyl-3,4,6- trimethylpyrazine were for the first time identified in the essential oil of tansy.

CONCLUSIONS

In this study, there were set down main morphological and histo-anatomical features of the head flowers of *Tanacetum vulgare* L. harvested from around Cluj-Napoca. In comparison to the head flowers of *Tanacetum balsamita var tanacetoides* the differences are in the involucre and pollen grains.

105 compounds were identified by gas-cromatography coupled with mass spectrometry, such as: thujen-2–one, chrysanthenil acetate, artemisia ketone, camphor, alfa- and beta-thujone, germacrene D, 1,8- cineol, in concentration higher than 1%. Selina- 3,7 (11)- diene and 2-acetyl-3,4,6-trimethylpyrazine were identified for the first time in the essential oil of tansy harvested in Transilvania.

The knowledge of botanical and phytochemical characteristics of *Tanacetum vulgare*, allows a correct identification of the plant material important for medical purpose.

REFERENCES

1. Álverez AL, Habtemariam S, Badaturuge MJ, Parra CF. In vitro anti HSV 1 and HSV-2 activity of Tanacetum vulgare extracts and isolated compounds: An approach to their mechanisms of action. Phytotherapy Research 2011;25(1):296- 301.
2. Godinho LS, Carvalho LSA, Castro CCB, Dias MM, Faria Pinto P, Miller Crotti EA, Pinto PLS, Moraes J, Da Silva Filho AA. Antihelmintic Activity of Crude Extract and Essential Oil of Tanacetum vulgare (Asteraceae) against Adult Worms of Schistosoma mansoni. Hindawi Publishing Corporation, Scientific World Journal 2014;2014:1-9.
3. Abass OPK, Wiesner J. Thujone and thujone containing herbal medicinal and botanical products: Toxicological assessment. Regulatory Toxicology and Pharmacology. 2013;65:100-107.
4. Sanaa L, Yafar H, Israili, Badiaa L. Acute and chronic toxicity of izophilised aqueous extract of Tanacetum vulgare leaves in rodents. Journal of Ethnopharmacology 2008;14:221-227.
5. Asta J, Danute M. The inflorescence and leaf essential oils of Tanacetum vulgare L. var. vulgare growing wild in Lithuania. Biochemical Systematics and Ecology 2005;33:487-498.
6. Gang X, Schepetkin AI. Quinn MT. Immunomodulatory activity of acidic polysaccharides isolated from Tanacetum vulgare L. Int Immunopharmacol 2007;7(13):1639- 1650.
7. European Directorate for Quality Medicines, European Pharmacopoeia, 7th ed. European Directorate for Quality Medicines, Strasbourg; 2010.
8. Farmacopeea Română, ediţia a X-a, Ed. Medicală, Bucureşti 2008:1046-1064.
9. Bojiţă M, Roman L, Săndulescu R. Oprean R. Analiza şi controlul medicamentelor. Ed. Intelcredo 2003(2):296.
10. Rohfeld B. Mikroskopisches Farbatlas pflanzlicher Drogen, Spektrum Akademischer Verlag 2009:104.
11. Bonnier G, Darvin R, Porinset I. La grande flore, 3rd ed.. Belim Press 1990:545- 546.
12. Ciocârlan V. Flora ilustrată a Romaniei, Pteridophyta et Spermatophyta. Ed. Ceres 2000:800-804.
13. Oprean R, Tămaş M, Săndulescu R, Roman L. Essential oil analysis. I. Evaluation of essential oils composition using both GC and MS fingerprints. Journal of Pharmaceutical and Biomedical Analysis 1998;18:651- 657.
14. Tétényi P, Kaposi P, Héthelyi E. Variations in the essential oils of Tanacetum vulgare. Phytochemistry 1975;14(7):1539-1544.
15. Neszmelyi A, Milne GWA, Podanyi B, Kocza I, Hethelyi E.. Composition of the essential oil of clone 409 of Tanacetum vulgare and 2D NMR investigation of trans-chrysanthenil acetate. Journal of Essential oil Research 1992;4(3):243- 250.
16. De Pooter HL, Verneesch J, Schamp N. The Essential oils of Tanacetum vulgare L. and Tanacetum parthenium (L.) Schultz – Bip, Journal of Essential oil Research 1989;1(1):9-13.
17. Keskitalo M, Pelm E, Simon EJ. Variation in volatile compounds from tansy (Tanacetum vulgare L.) related to genetic and morphological differences of genotypes. Biochemical Systematics and Ecology 2001;29(3):267-285.