

PHYTOCHEMICAL AND PHARMACOLOGICAL RESEARCH ON SOME EXTRACTS OBTAINED FROM *SERPYPILLI HERBA*

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Abstract

A 1:10 tincture and an 1:2 hydro-alcoholic fluid extract were obtained from the vegetal product *Serpylli herba* originating from the spontaneous species *Thymus pulegioides* L. The active principles identified in the two extracts by thin layer chromatography were: luteolin, apigenin, caffeic acid, rosmarinic acid, triterpenic acids.

The tincture had a content (m/m, g %) of 0.024 - 0.027 flavonoids (expressed as rutin) and 0.336 - 0.356 polyphenolic acids (expressed as chlorogenic acid), while the fluid extract had a content (m/m, g %) of 0.104 - 0.142 flavonoids (expressed as rutin) and 1.78 - 1.84 polyphenolic acids (expressed as chlorogenic acid).

Both extracts presented an anti-inflammatory effect demonstrated by the plethysmometric technique (rat paw edema induced by kaolin 10 %), effect more intense for the tincture, compared to the control substance (diclofenac) 4 hours after the administration and to the fluid extract.

Rezumat

Din produsul vegetal *Serpylli herba* provenit de la specia spontană *Thymus pulegioides* L. s-au obținut o tinctură 1:10 și un extract fluid hidro-alcoolic 1:2. Principiile active identificate prin cromatografie în strat subțire în cele două extracte sunt: luteol, apigenol, acid cafeic, acid rozmarinic, acizi triterpenici.

Tinctura conține 0,024 - 0,027 g % flavonozide (exprimate în rutozidă) și 0,336 - 0,356 g % acizi polifenolici (exprimați în acid clorogenic), iar extractul fluid 0,104 - 0,142 g % flavonozide (exprimate în rutozidă) și 1,78 - 1,84 g % acizi polifenolici (exprimați în acid clorogenic).

Ambele extracte manifestă acțiune antiinflamatoare, evidențiată prin metoda pletismometrică (edem indus cu caolin 10 %). În cazul tincturii, efectul antiinflamator este mai intens față de cel al substanței de referință (diclofenac), la 4 ore de la administrare și față de cel al extractului fluid.

Keywords: *Serpylli herba*, flavonoids, polyphenolic acids, anti-inflammatory effect

Introduction

The herbal drug *Serpylli herba* mentioned in the European Pharmacopoeia 6th edition [21], can be obtained from several spontaneous species of *Thymus* (*Lamiaceae*) and is considered an important source of active principles: essential oils, flavonoids, polyphenolic acids, triterpenes [10,20]. The importance of this herbal drug for phytotherapy is demonstrated by several recent studies which confirmed the traditional uses and brought new data regarding the possibilities of therapeutic valorification [15,17,18]. *Serpylli herba* is well-known for its therapeutic effects: cough-suppressant, antiseptic, antioxidative and spasmolytic [10,18,20]. A hypotensive effect and inhibitory properties upon *Helicobacter pylori* were also demonstrated [6].

Recent published data showed that methanolic and chloroformic extracts from *Thymus* species have anti-inflammatory properties, more evident for the chloroformic extracts in which the concentration of ursolic acid is higher [8,9,13].

Also, according to references, flavonoid compounds, among which luteolin and apigenin (specific to the *Thymus* genus) may provide anti-inflammatory properties for the extracts in which they are present [1].

The aim of this study was to obtain and chemically characterize some extracts from *Serpylli herba* and also to test their anti-inflammatory properties.

Materials and methods

The herbal drug was obtained from the flowering aerial part (*herba*) of *Thymus pulegioides* L., a spontaneous species harvested from Bucegi Mountain's area. From the dried (at 25°C, for 3 weeks) herbal drug, two hydro-alcoholic extracts were obtained:

- A) a 1:10 tincture prepared by the maceration technique according to the Romanian Pharmacopoeia Xth edition, using ethanol 40% as solvent [22];
- B) a 1:2 fluid extract prepared by the percolation technique of Squibb using ethanol 70% [7].

The two extracts were phytochemically analyzed (identification and assay of the main groups of active principles). Flavonoids were qualitatively determined by thin layer chromatography (TLC) on silicagel 60 F254 (Merck) using as mobile phase the solvent system ethyl acetate-water-formic acid-acetic acid (72:14:7:7) and NEU/PEG reagent (1% methanolic solution of diphenylborate of aminoethanol and 5% methanolic solution of

polyethylenglicol 400) (Fluka) for identification; for the analysis of flavonoid aglycones the mobile phase was toluene-ethyl acetate-formic acid (5:3:1) [4,5,12]. The assay was performed spectrophotometrically with AlCl_3 using the method indicated in the Romanian Pharmacopoeia Xth edition for *Cynarae folium*, the flavonoid content being expressed in rutin g% [22].

Polyphenolcarboxylic acids such as caffeic acid were qualitatively analyzed by TLC using the technique mentioned at the flavonoid analysis [12]. The assay of these compounds was performed according to the method presented in the European Pharmacopoea 6th edition for *Fraxini folium* monograph, the content being expressed in chlorogenic acid g% [21].

Triterpenic compounds were qualitatively analysed by TLC on silica gel 60 F254 using as mobile phase chloroform-acetone (8:2) and Liebermann-Burchard reagent as revelator [3].

All reference substances (rutin, hyperoside, isoquercitrin, apigenin-7-neohesperoside, luteolin, apigenin, caffeic acid, rosmarinic acid, chlorogenic acid, ursolic acid, oleanolic acid) were purchased from Merck.

The anti-inflammatory activity of the two extracts was determined using the rat paw edema test induced by kaolin 10% (plethysmometric technique) and diclofenac (Merck) as control substance [11,14,16,19].

For this experimental model, four groups of eight male Wistar rats, weighing around 150 g, were used. The animals were kept in standard conditions, the access to food and water being prohibited 24 h before the experiment.

On the experiment day, the following substances were administered intraperitoneally to the animals:

Group I (control): 1 mL/200g b.w. distilled water;

Group II: 1 mL/200g b.w. tincture;

Group III: 1 mL/200g b.w. extract (prior diluted to 1:10);

Group IV (reference): 10mg/kg b.w. diclofenac [16].

One hour after the substance administration, the initial left hind paw volume was determined with an Ugo Basile digital plethysmometer model 7140, followed by an intraplantar administration in the same paw of 0.1 mL kaolin 10% suspension.

After two hours, four hours and 24 hours following the kaolin administration, the volume of the injected hind paw was determined again by the same method. The inflammatory edema (expressed in mL) was calculated as a difference between the volume of the injected member at 2 h, 4 h and 24 h and the initial paw volume.

The percentages of the inflammation inhibition were calculated using the formula:

$$\% \text{ inflammation inhibition} = (1 - X \text{ substance} / X \text{ control}) \times 100,$$

where:

X substance = the mean value of inflammatory edema in the treated animals;

X control = the mean value of inflammatory edema in untreated animals (control).

The results were expressed as mean values $\pm$ standard deviation, the statistical interpretation being performed with the Student's t test.

Results and discussion

The two hydro-alcoholic extracts were yellow-brown liquids with specific taste and odour.

From the TLC analysis, multiple flavonoids fractions were observed in both extracts. The most important (considering the intensity of the spots) were the fractions with $R_f = 0.36$ - yellow-orange fluorescence; $R_f = 0.44$ - yellow fluorescence and $R_f = 0.52$ - yellow fluorescence. The separated spots did not match with the heterosides used as reference substances.

The flavonoid aglycones identified in both extracts were luteolin ($R_f = 0.34$ - yellow fluorescence) and apigenin ($R_f = 0.50$ - yellow-green fluorescence).

Among the polyphenolic acids, caffeic acid ($R_f = 0.37$ - blue fluorescence) and rosmarinic acid ($R_f = 0.13$ - blue fluorescence) were identified in both the tincture and the extract.

Triterpenic compounds (ursolic acid, oleanolic acid) were identified in both extracts. The two substances used as reference (ursolic acid, oleanolic acid) presented the same behavior on the TLC ($R_f = 0.77$ - yellow fluorescence), so that we could not specify which of the two compounds is present in the analyzed samples. It was observed that the spot correspondent to ursolic/oleanolic acid is more intense in the tincture, which can indicate a higher concentration of these compounds in the tincture compared to the fluid extract.

The identification by TLC of the mentioned compounds in the two extracts from *Serpylli herba* is predictable. The available scientific data show that luteolin, apigenin, caffeic and rosmarinic acids and also ursolic and oleanolic acids are characteristic for *Thymus* species [2,18].

The level of active principles (flavonoids, polyphenolic acids) determined for the two extracts is presented in table I. Also the extraction rates of these types of active principles are presented in the same table.

Table I

The level of flavonoids and polyphenolic acids from the tincture and the fluid extract from *Serpylli herba*

Active substance	Tincture	Fluid extract
Flavonoids (g % rutin)	0.024 – 0.027	0.104 – 0.142
Polyphenolic acids (g % chlorogenic acid)	0.336 – 0.356	1.78 – 1.84
Extraction rate	43.10 % (flavonoids) 88.95 % (polyphenolic acids)	45.80 % (flavonoids) 63.88 % (polyphenolic acids)

The level of flavonoids and polyphenolic acids is higher in the fluid extract, compared to the tincture, as expected, because of the higher ratio herbal drug: solvent in the fluid extract and because of the higher concentration of solvent used for extraction. The extraction rate of polyphenolic acids is nevertheless higher in the case of the tincture which indicates that a higher amount of polyphenolic acids from plant material could be extracted by maceration (procedure used for the tincture).

In order to perform the pharmacological testing, the fluid extract was diluted to 1:10 to achieve a similar ratio vegetal product: solvent as for the tincture. The amount of active principles in the diluted fluid extract (0.021 - 0.028 g % flavonoids and 0.356 - 0.368 g % polyphenolic acids) is very similar to the 1:10 tincture (see table I).

The rat paw edema test induced by kaolin 10% demonstrated the presence of a statistically significant anti-inflammatory effect for the two extracts. The results are presented in table II and figure 1.

Table II

The anti-inflammatory activity of the tincture and fluid extract from *Serpylli herba* in the experimental model of the rat paw edema induced by kaolin 10%

Group	Dose	Edema			Inhibition %		
		2 h X ± e.s.	4 h X ± e.s.	24 h X ± e.s.	2 h	4 h	24 h
I: Control (distilled water)	1mL/200g b.w.	0.708± 0.170	0.977± 0.350	1.917± 0.152	-	-	-
II: Tincture	1mL/200g b.w.	0.463± 0.146*	0.476± 0.202*	1.411± 0.224*	34.60	51.27	26.39
III: Fluid extract	1mL/200g b.w.	0.471± 0.167*	0.783± 0.295	1.573± 0.367*	33.47	19.85	17.94
IV: Diclofenac (Reference)	10 mg/kg b.w.	0.382± 0.08*	0.49± 0.14*	1.07± 0.12*	46.04	49.84	44.18

*Statistically significant ($p < 0.05$); X = mean value of edema in the group of animals; e.s. = standard deviation

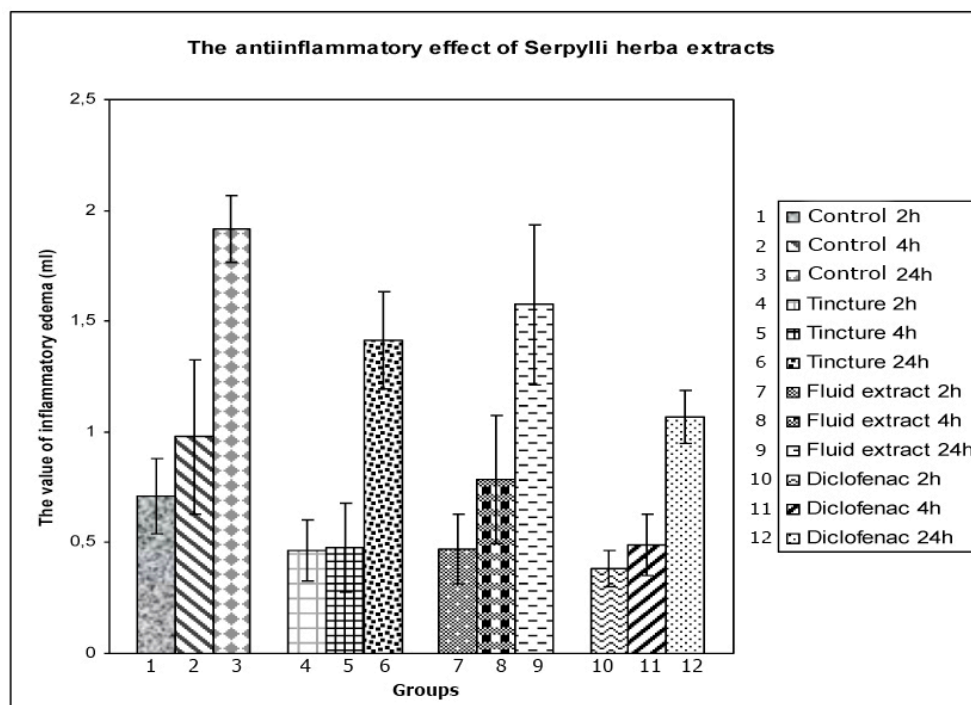


Figure 1

The anti-inflammatory effect of the tincture and fluid extract determined after 2, 4 and 24 hours following administration, by comparison to control and reference (diclofenac)

In the experimental model of rat paw edema, percentages of inflammatory edema inhibition are variable, depending on the administered substance and the moment of evaluation.

The tincture presented a statistically significant anti-inflammatory effect (at all time intervals of evaluation), even superior to diclofenac 4 hours after the induction of inflammation (51.27% compared to 49.84%).

For the fluid extract, the anti-inflammatory effect was statistically significant 2 hours and 24 hours after the induction of inflammation. The effect was inferior to diclofenac (reference substance) and also to the tincture.

The anti-inflammatory effect of the two tested extracts is probably due to triterpenic compounds (ursolic and oleanolic acids) but also to some flavonoids (luteolin, apigenin and derivatives).

The total amount of flavonoids being very similar for the two extracts, the stronger anti-inflammatory effect of the tincture could be explained by a possible greater amount of triterpenic acids.

Conclusions

The compounds identified by TLC in the two herbal extracts (tincture 1:10 and fluid extract 1:2) obtained from *Serpylli herba* were: luteolin, apigenin, caffeic acid, rosmarinic acid, ursolic/oleanolic acid.

The amount of active principles was higher in the fluid extract (0.12% flavonoids, 1.81% polyphenolic acids) compared to the tincture (0.025% flavonoids, 0.346% polyphenolic acids).

The two extracts presented an anti-inflammatory effect superior to the control group, more intense for the 1:10 tincture compared to the fluid extract. Four hours after the induction of the inflammation, the anti-inflammatory effect of the tincture was also superior to the reference group (diclofenac).

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