

**CONTRIBUTIONS TO THE
PHARMACOGNOSTICAL AND
PHYTOBIOLOGICAL STUDY OF *FALLOPIA
AUBERTII* (L. HENRY) HOLUB. (*POLYGONACEAE*)**

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Abstract

In order to evaluate the therapeutic potential of the *Fallopia aubertii* (L.Henry) Holub (Russian vine) species for the use of the flowered aerial parts as raw material in the preparation of an active pharmacological extract, we decided to establish the microscopic characters, to identify the chemical constituents and to verify the cytotoxicity of this herbal product.

The root and the stem are characterized by secondary structure with a well developed secondary xylem and sclerenchyma; the leaf blade - dorsi-ventral structure with two vascular bundles and calcium oxalate druses at the main vein, pluricellular glandular trichoma and anomocytic stomata within the epidermis; druses of calcium oxalate are present in all the organs examined.

The following classes of phytochemicals were identified: carotenoids, flavonoids, sterols/triterpenes, polyphenol carboxylic acids, condensed tannins, polysaccharides, proanthocyanidins and reductive compounds. Through TLC (thin layer chromatography) analysis quercetin, kaempferol, myricetin, hyperoside, isoquercitrin, rutin and chlorogenic acid were identified.

The 50% (w/v) aqueous and ethanolic solutions, with concentrations between 3.33% and 0.33%, presented cytotoxic activity (Constantinescu phytobiological method – *Triticum* bioassay).

Rezumat

Pentru evaluarea potențialului terapeutic al speciei *Fallopia aubertii* (L.Henry) Holub, în vederea utilizării părții aeriene înflorite ca materie primă la prepararea unui extract farmacologic activ, ne-am propus stabilirea caracterelor microscopice, identificarea constituenților chimici și verificarea citotoxicității.

Rădăcina și tulpina se caracterizează prin structură secundară cu lemnul secundar bine dezvoltat și fibre sclerificate; limbul foliar - prin structură heterogen asimetrică, cu peri glandulari tetracelulari sesili, stomate de tip anomocitic, iar nervura principală prin două fascicule libero-lemnoase; druze de oxalat de calciu sunt prezente în toate organele analizate.

Au fost decelate: carotenoide, flavonozide, steroli/triterpene, acizi polifenolcarboxilici, taninuri condensate, polizaharide, proantociani și compuși reducători. Prin cromatografie în strat subțire s-au identificat: quercetolul, kaempferolul, miricetolul, hiperozida, izoquercitrozida, rutozida și acidul clorogenic.

Soluțiile apoase și cele hidroalcoolice cu etanol 50% de concentrații 3,33%-0,33% au prezentat acțiune citotoxică (metoda fitobiologică Constantinescu- testul *Triticum*).

Keywords: *Fallopia aubertii*, microscopy, phenolic compounds, cytotoxicity.

Introduction

Fallopia baldschuanica (Regel) Holub syn. *Fallopia aubertii* (L.Henry) Holub from the *Polygonaceae* family is a woody species with a voluble stem that can reach up to 10 meters [1, 2]. The species is native from Baldshuan Khanate (Turkestan) and is cultivated in Europe as an ornamental garden plant [2].

In the consulted literature, *F. aubertii* is not listed as having therapeutic uses. The chemical composition has been scarcely investigated and only the flavonoid fraction has been characterized from a qualitative point of view, for taxonomic purposes; the aerial parts contain heterosides of quercetin (3-O-galactoside, 3-O-glucoside, 3-O-arabinoside, 3-O-(acyl) glucoside), apigenin (6-C- glucoside, 8-C-glucoside) and luteolin (6-C-glucoside, 8-C-glucoside, 7-O-glucoside) [6].

The aim of this research was to establish the main characters of the species through microscopic examination, to identify the major compounds (phenolic compounds) in the flowered herbal product through chemical screening and thin layer chromatography (TLC) and to assess its cytotoxic activity through a pharmacobotanical study intended to evaluate the potential use of this species in the preparation of an active pharmacological extract.

Materials and Methods

The plant material was harvested in Bucharest (private garden), in September 2010, during the blooming period. The identity of the species was confirmed by macroscopic examination and comparison with the morphological characters described in the reference flora books [1, 2].

The microscopic examination was performed on cross sections through the root, stem and leaf (clarified with Javel water and double staining with iodine green and alum-carmin) and from leaf and flower surface preparations (clarified with NaOH 5%)[7]. The microscopic preparations were analyzed using a Labophot 2-Nikon microscope (ocular

10x, ob. 4x, 10x, 40x) and microphotographs were taken with a specially adapted Nikon digital camera.

The qualitative analysis and cytotoxicity assessment were performed on the flowered *herba* (stems, leaves and flowers). The qualitative analysis was performed according to the methods listed in the literature, through characteristic chemical reactions for each class of phytochemicals (phytochemical screening) [4] and through TLC for flavonoids and phenolic acids [8, 12, 13].

The sample preparation for the phytochemical screening and for TLC: 5,00 g of dry product were extracted with diethyl ether (25 mL, 24 h maceration), then with methanol and finally with distilled water (refluxed for 30 minutes with 50 mL of each solvent). All extractive solutions were coded as PA_{Eth} (the etheric solution), PA_M (the methanolic solution) and PA_A (the aqueous solution). 25 mL of the PA_M and the PA_A solutions were subjected to acid hydrolysis with HCl 1M, 1:1 (v/v), then extracted with diethyl ether (3 times with 10 mL). The combined etheric solutions, dehydrated on anhydrous Na₂SO₄ and filtered, were concentrated on the water bath, up to 5 mL. The respective solutions were marked as PA_{MH} and PA_{AH}.

The TLC analysis:

- stationary phase: silica gel 60 F₂₅₄ on an Al/plastic support (Merck);
- mobile phase: chloroform – acetone, 80:20 (v/v) (Mph. 1) [8], for the identification of the sterols; ethyl acetate - formic acid - acetic acid – water, 72:7:7:14 (v/v) (Mph. 2) for the identification of heterosides [12]; petroleum ether – ethyl acetate – formic acid, 40:60:1 (v/v) (Mph. 3), for the identification of the aglycones [13];
- samples to be analyzed: PA_{Eth}, PA_M, PA_A, PA_{MH} and PA_{AH};
- reference substances: (dissolved in ethanol 96%): β -sitosterol, stigmasterol, caffeic acid, chlorogenic acid, quercetine, kaempferol, myricetin, rutin trihydrate (1 mg/mL), hyperoside and isoquercitroside (0.2 mg/mL) (Sigma Aldrich);
- reagents: acetic anhydride, sulfuric acid – ethanol, 1:1 (v/v) (reagent 1) – for the sterols; Natural Reagent Product (diphenylboric acid aminoethyl ester) = PEG (Neu/Peg. No.28) (reagent 2) – for the flavonoids.

The order of applying the solutions to be analyzed and the reference substances on the chromatographic plates is presented in figures 9 and 10.

The assessment of cytotoxicity through the Constantinescu method (*Triticum* test) was carried out by determining the maximal dilution of the

extractive solutions which, depending on the time of action, influences on the radicular elongation and the karyokinetic film [3, 9, 10]. The solutions to be analyzed were obtained by refluxing 2.5 g of herbal product with 50 mL of distilled water – PA_A and ethylic alcohol 50% - PA_E (w/v) for 30 minutes.

Embryonic wheat roots (*Triticum vulgare* Mill, Dropia cultivar, acquired from the Fundulea Agricultural Research Institute) of 1 cm length have been maintained for 5 days in contact with the solutions to be analyzed PA_A and PA_E in concentrations of 3.33%, 2.5%, 1.66%, 0.33% and 0.03% (w/v) in Petri dishes (90 mm diameter), in a Sanyo MLR- 351H germination room, at 25 °C temperature, 75% relative humidity and in the absence of light. A control sample was prepared by replacing the extractive solutions with distilled water - M. The modifications of the karyokinetic film were observed after 24 hours on embryonic roots stained with diluted acetic orcein solution [5], through microscopic examination using a Labophot 2 Nikon microscope (ocular 10x, ob. 40x, 100x).

The inhibitory effect (Ef_i) of the two extractive solutions was calculated (using Excel 2003 software) in comparison to the control using the following formula [3]:

$$Ef_i = 100 - \frac{P-1}{M-1}100$$

P – sample average of the root elongation (cm)
M – control sample average of the root elongation (cm)
1 – initial value of the embryonic roots (cm)
 100 – the results are expressed as a percentage

Due to abnormal distribution of the values of the radicular elongation, for the statistical analysis of the results we used the Kruskal-Wallis test and the Dunn post-test, with a confidence interval of 95% (CI 95%) (GraphPadPrism software, v. 5.0).

Results and Discussion

The microscopic examination of the cross sections of the root revealed secondary structure with sclerenchyma fibers (Figure 1). The stem presents secondary structure (suber, phellogen, phelloderm, cortical parenchyma with calcium oxalate druses and sclerenchyma, as well as secondary phloem and xylem) (Figure 2).

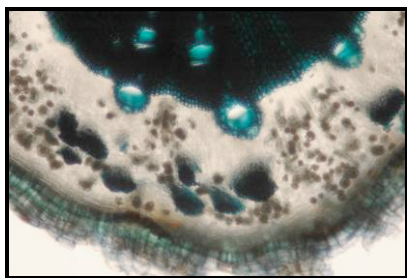


Figure 1.
Root cross-section (ob. 4x)



Figure 2.
Stem cross-section (ob. 10x)



Figure 3.
Leaf cross-section (ob. 10x)

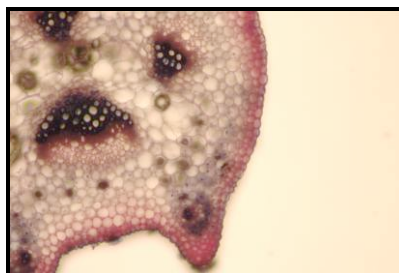


Figure 4.
Petiole cross-section (ob. 10x)

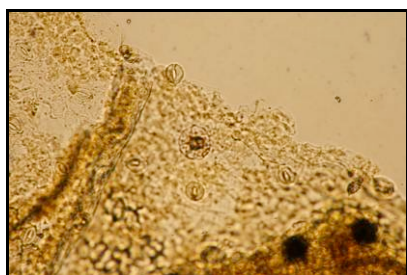


Figure 5.
Leaf surface preparation: calcium oxalate
druses, sessile glandular hairs (ob. 10x)

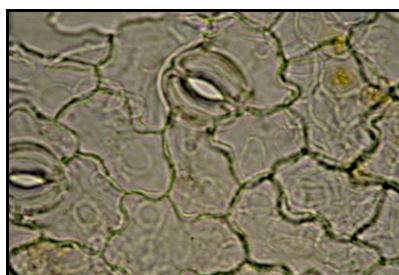


Figure 6.
Leaf surface preparation: anomocytic type
stomata (ob. 40x)



Figure 7.
Flower surface preparation: pollen with
smooth exine (ob. 40x)

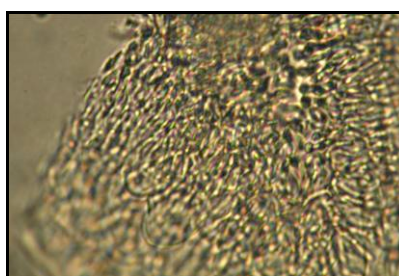


Figure 8.
Flower surface preparation: endothecium
(ob. 40x)

The leaf blade has a dorsi-ventral structure with two collateral vascular bundles and unicellular trichomes on the surface of the epidermis (figure 3). The petiole presents a semicircular structure with two obvious margins, angular collenchyma and 6 vascular bundles (figure 4). In the leaf surface preparation there were observed: calcium oxalate druses, pluricellular sessile glandular trichomes, anomocytic stomata (in the leaf, figures 5 – 6), pollen grains with smooth exine and anther endothecium (in the flower, figures 7-8).

Except the pollen grains, the microscopic characters that we identified are not mentioned in the literature [11].

The *phytochemical screening* has revealed: carotenoids, flavonoids, sterols/triterpenes, catechin tannins, proanthocyanidins, polysaccharides, polyphenolcarboxylic acids, monosaccharides and reducing compounds (table I).

Table I.
Phytochemical screening of *Fallopia aubertii* (herba)

Nr. crt.	Active principles	Etheric solution	Methanolic solution	Aqueous solution
1	carotenoids	+	NA	NA
2	alkaloids	-	-	-
3	flavonoids	-	++	++
4	Sterols/ triterpenes	+	+	-
5	coumarins	-	-	-
6	anthracenosides	-	-	-
7	condensed tannins	NA	+	+
8	polysaccharides	NA	NA	+
9.	polyphenolicarboxylic acids	NA	++	++
10.	anthocyanins	NA	-	-
11.	proanthocyanidins	NA	+	++
12.	monosaccharides	NA	NA	+
13.	reductive compounds	NA	++	++

” + +”: intensely positive reaction; “+ “: positive reaction; “-“: negative reaction, NA – not applicable

Through TLC, on the basis of the R_f values and of the behavior towards the reagents, identical to those of the reference substances, the following were identified: β -sitosterol/stigmasterol ($R_f=0.75-0.78$), rutin ($R_f=0.20$), hyperoside ($R_f=0.43$), isoquercitroside ($R_f=0.46$), chlorogenic acid ($R_f=0.32-0.33$), quercetin ($R_f=0.69-0.73$), myricetin ($R_f=0.62$), kaempferin ($R_f=0.77-0.79$). The other spots remained unidentified due to the lack of reference substances.

Except the quercetine and heterosides rutin, isoquercitrin and hyperoside, all other compounds that we identified are not mentioned in literature.

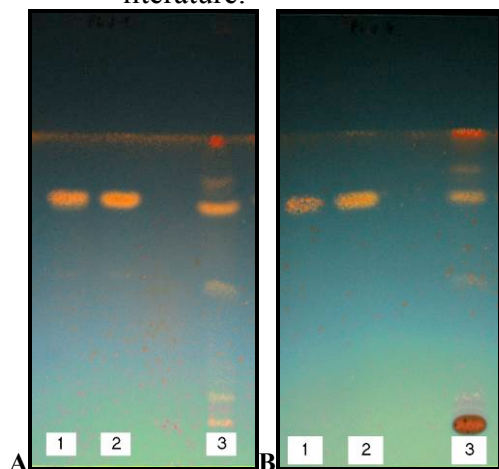


Figure 9.

Fig. 9. TLC chromatograms of sterols: A- 1 - β -sitosterol, 2 - stigmasterol, 3 - PA_E ; B- 1 - β -sitosterol, 2 - stigmasterol, 3 - PA_{MH} .

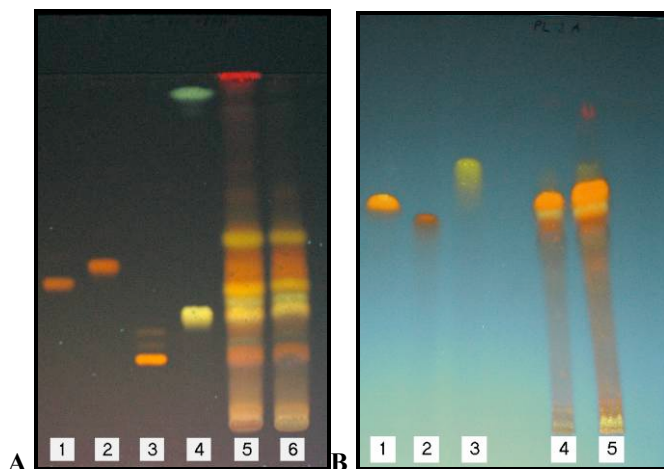


Figure 10.

Fig. 10. TLC chromatograms of flavonoids and polyphenolcarboxylic acids (A) and flavonoid aglycones (B): A: 1 - hyperoside, 2 - isoquercitrin, 3 - rutin, 4 - chlorogenic acid (down), caffeic acid (up); B: 1 - quercetin, 2 - miricetol, 3 - kaempferol, 4 - PA_M , 5 - PA_A .

Table II.

The inhibitory effect (expressed as percentage) on the radicular elongation as compared to the control and the statistical significance for the solutions to be analyzed (PA and PE) during the 5 testing days.

Sample	Effi P_{Aqu} (%)					Effi P_{Eth} (%)				
	24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h
conc. sample/ statistic test										
3.33%	98.15	98.77	99.19	99.23	99.23	98.61	99.26	99.51	99.54	99.54
Dunn's Test	*	**	***	***	***	*	**	***	***	***
2.50%	96.76	98.28	98.86	98.92	98.92	97.22	98.52	99.03	99.07	99.08
Dunn's Test	si	**	***	***	***	si	**	***	***	***
1.66%	97.22	98.52	99.03	99.07	99.08	97.22	98.52	99.03	98.76	98.77
Dunn's Test	*	**	***	***	***	*	**	***	***	***
0.33%	96.30	97.78	98.38	98.45	98.46	95.83	97.54	98.38	98.45	98.46
Dunn's Test	si	**	***	***	***	si	**	***	***	***
0.033%	12.96	1.48	19.32	11.30	-1.08	11.57	-3.45	18.67	13.47	0.31
Dunn's Test	si	si	si	si	si	si	si	si	si	si
Kruskal-Wallis test	***					***				

si – statistically insignificant ($p > 0.05$), *, **, *** - statistically significant (* $p = 0.01-0.05$, ** $p = 0.001-0.01$, *** $p < 0.001$).

The results of the phytobiological test (table II) show that the aqueous solution and the 50% ethanolic one inhibit the radicular elongation at concentrations of 3.33% - 0.33%; at 0.03% the test solutions are statistically similar with the control sample. The results are supported by the microscopic examination: for the 3.33% - 0.33% concentrations a cytotoxic effect was observed (absence of cell divisions, disorganized nuclear material), while at 0.33%, frequent, normal cell divisions were seen.

The flowered aerial parts harvested from *Fallopia aubertii* is cytotoxic at high (3.33 – 1.66%), and also at average concentrations (0.33%). At a low concentration (0.03%) the plant material is not toxic.

Conclusions

The main histo-anatomical elements of the *Fallopia aubertii* L. species are: secondary structures for root and stem, with sclerenchymatous fibers and druses of calcium oxalate; sessile, pluricellular glandular trichomes and anomocytic stomata in the leaf; pollen grains with smooth exine in the flower. The flowered aerial parts of *F. aubertii* contain flavonoids, carotenoids, sterols/triterpenes, polyphenol carboxylic acids, condensed tannins, polysaccharides, proanthocyanidins and have cytotoxic properties (conferred by compounds soluble in water and ethanol). The cytotoxicity observed in this study justifies a more in-depth analysis of the various chemical fractions obtained from the aerial parts of *F. aubertii*, if the standardized extract is to be administrated chronically.

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