CONTRIBUTION TO THE PHARMACOGNOSTICAL AND PHYTOBIOLOGICAL STUDY ON *ABUTILON THEOPHRASTI* MEDIK. (*MALVACEAE*)

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Abstract

Abutilon theophrasti Medik., velvet (*Malvaceae*) was pharmacognostically investigated aiming to establish its morpho-anatomical characteristics (root, stem, leaf, flower) and leaf chemical composition. A phytobiological test (*Triticum* test) assessed the activity of two extracts prepared from leaves (aqueous extract 5.00 – 0.033 % w/v and total extract of polysaccharides 1.00 – 0.006 w/v %) on plant cell division.

The following histo-anatomical elements were observed: secondary structure with dilated rays (root); incomplete secondary structure (stem); sclerenchymatous fibres (root, stem); dorsi-ventral (bifacial) structure with two-layered palisade tissue, druses of calcium oxalate, unicellular trichomes forming star-shaped groups, glandular hairs (leaf); and androspore with echinate exine and endothecium (flower). Carotenoids, sterols, triterpenes, rutin, polyphenolcarboxilic acids, condensed tannins, mono - and polysaccharides and reductive compounds were identified. Leaves contained 7.01 – 7.02 g % polysaccharides, 0.45 – 0.60 g % flavones (expressed as rutin) and 0.22 – 0.48 g % polyphenolcarboxilic acids (expressed as caffeic acid).

The aqueous extract (5.00 – 0.33% w/v) and polysaccharides fraction (1.00 – 0.5% w/v) inhibited the *Triticum* root growth (the aqueous extract more intensely); a mitoinhibitory activity was observed, with metaphases and telophases in tropokinesis, anaphases and telophases with bridges and bent membranes.

Rezumat

S-a cercetat farmacognostic *Abutilon theophrasti* Medik., pristolnic (*Malvaceae*) pentru a stabili caracterele morfo-anatomice ale rădăcinii, tulpinii, frunzei, elementelor florale şi compoziţia chimică a frunzelor. S-a realizat şi studiul fitobiologic (testul *Triticum*) care a reliefat acţiunea a două extracte obţinute din frunză (unul apos, 5,00 – 0,033% şi fracţiunea poliholozidică, 1,00 - 0,01%) asupra diviziunii celulei vegetale.

Au fost evidenţiate: structură secundară cu raze evazate (rădăcină), structură secundară incompletă (tulpină), fibre de sclerenchim (rădăcină, tulpină), structură heterogen-asimetrică, ţesut palisadic bistratificat, peri tectori unicelulari cu aspect stelat,

peri glandulari, druze de oxalat de calciu (frunză), polen cu exină echinulată şi endoteciu (floare). Au fost decelate carotenoide, steroli, triterpene, rutozidă, acizi polifenolcarboxilici, taninuri catehice, poliholozide, oze şi compuşi reducători. Produsul de tip *folium* conţine 7,01 – 7,02% poliholozide, 0,45 – 0,60 g % flavonozide (exprimate în rutozid) şi 0,22 – 0,48% acizi polifenolcarboxilici (exprimaţi în acid cafeic).

Extractul apos 5,00 – 0,33% şi fracţiunea poliholozidică 1,00 – 0,50% au inhibat creşterea radiculară (mai intens soluţia extractivă apoasă), producând o acţiune mitoinhibitoare (metafaze şi telofaze în tropocineză, anafaze şi telofaze cu punţi, membrane paraplasmatice ondulate).

Keywords: *Abutilon,* velvet, polysaccharides, flavonoids, mitoinhibitory action.

Introduction

Abutilon theophrasti Medik., velvet (*Malvaceae* family) is a common species of the spontaneous Romanian flora, widespread along hillside slopes, riverbanks, roadsides, in waste and sandy soil areas. Very resistent fibres extracted from its stem are similar with hemp or flax fibres and are known as Chinese hemp.

Limited information about the chemical composition and therapeutic properties of this species is available in the literature. It has been reported that the seeds contain 17.4 % proteins, 19.0 % siccative fatty oil and 33.8 % saccharides. Powdered seeds have been traditionally recommended internaly for their demulcent, diuretic, emolient, laxative and stomachic actions. The roots are empirically indicated for the treatment of dysentery and urinary incontinence, while the bark is said to be astringent and diuretic. Its leaves contain 0.01 % rutin [3] and are claimed to have demulcent, antiulcerous, antidiarrhoeal and febrifuge properties.

Another species of the same genus from tropical areas, *Abutilon indicum*, is also known for similar (but not identical) traditional uses. The leaves are used in the therapy of bronchitis, diarrhoea, inflammations, arthrites, fever; the root has diuretic, antihelmintical, antitussive properties, and the seeds are laxative and demulcent. Non-clinical data suggest that its parts or isolated components have hypoglycemic, hepatoprotective and analgesic activities [4, 11].

The aim of this research was to carry out a pharmacobotanical and pharmacognostical study of *Abutilon theophrasti* Medik. In order to establish its potential use in phytotherapy, considering that this species is widely spread in the South-Western parts of Romania and other species of the genus *Abutilon* seem to have interesting therapeutical properties.

Materials and Methods

Abutilon theophrasti (whole plant) was collected from Vânju Mare, Dolj county, Romania, in June-July 2008, during the blooming period. The identity of the species was checked by botanical examination and comparison with reference flora books [2, 6, 12]; besides, cross sections of root, stem, leaf and - for leaf and flower - surface preparations were carried out and analysed. Iodine green and carmine were used as stains for the cross sections, and sodium hydroxide 5% was used to clarify the surface preparations. A Labophot II Nikon research microscope was used for examination and microphotographs were taken with a specially adapted Nikon digital camera.

Qualitative chemical analysis (phytochemical screening) was performed using specific reactions [7]. Thin Layer Chromatography (TLC) for polyphenolcarboxilic acids of the etheric, alcoholic and aqueous extractive solutions obtained from leaves was carried out in the following experimental conditions:

- test solutions - etheric (SE), methanolic (SM) and aqueous (SA) extractive solutions from leaves;
- plate: Silica gel GF 25 U Merck;
- solvent system: ethyl acetate: formic acid: water (80: 8: 12);
- reference solutions: quercetin (Q), chlorogenic acid (Ch), rutin (Ru) (alcoholic solutions 0.01%);
- detection: UV (366 nm); diphenylboryloxiethylamine (alcoholic solution 1%) followed by polyethylene glycol (alcoholic solution 1%);
- 15 cm run.

Quantitative determinations were undertaken for polysaccharides by gravimetry (based on precipitation in alcohol) and flavones along with polyphenolcarboxilic acids by spectrophotometry (based on the chelating reaction with aluminium chloride, according to the Romanian Pharmacopoeia, 10th edition – *Cynarae folium* monograph, and on the formation of oxymes in the presence of sodium nitrite and sodium molybdate, respectively) [1]. A UV-VIS Cecil 2000 spectrophotometer was used for the measurements. All assays were performed in triplicate, the results being expressed as the interval between the minimum and maximum found values.

The activity of two leaf extracts on plant cell division was investigated using a *Triticum* bioassay (Constantinescu method). Embryonic wheat roots (*Triticum vulgare* Mill, 2008 from the Institute for Agricultural Research - Fundulea) germinated and treated in laboratory conditions were

used. An aqueous extractive solution A1 (5% w/v) was prepared by infusing the dried herbal leaves with water and from this initial solution five others were prepared through serial dilutions with water (A2 – 3.33%, A3 – 2.50 %, A4 – 1.66%, A5 – 0.33% and A6 – 0.03%). A colloidal solution was prepared by redispersing with water the precipitated polysaccharide fraction (B1 - 1% w/v); from the latter four others were prepared through serial dilutions with water (B2 – 0.5%, B3 0.33%, B4 – 0.06% and B5 – 0.01%). Microscopic analysis was performed on embryonic wheat roots stained with acetic orcein [5, 8-10]. Since the normality assumption was not met and there were differences among sample sizes, statistical comparisons of root lengths after 48 hours were carried out by Kruskal-Wallis test; for significant differences (p=0.05), post-hoc comparisons among medians were performed by means of Kruskal-Wallis z-test. For statistical purposes, comparisons of root lengths were performed after 48 hours (end of second day), in order to allow sufficient contact between extracts and wheat roots, while avoiding a too long time that might lead to artifacts (e.g. because of physico-chemical alterations of the extracts). Because acceptance of null hypothesis would suggest lack of genotoxicity, no correction for multiplicity was done. All data analyses were conducted using the NCSS statistical package software.

Results and Discussion

The morphological characteristics analysed through macroscopical examination corresponded to those reported in the scientific literature: herbaceous species; tap root; ramiferous, alternately-leaved stem, simple, cordiforme, tomentose leaves; axillary, hermaphrodite, actinomorphic, orange flowers, double, persistent calice, monadelfous androecium, unilocular anthers, plurilocular capsule, three black seeds in every locule [2, 7, 12].

The following specific histo-anatomical elements were seen in the microscopic assessment: secondary structure with dilated rays (root); incomplete secondary structure (stem); sclerenchymatous fibres (root, stem); collateral vascular bundles (stem, leaf); dorsi-ventral (bifacial) structure with two-layered palisade tissue, annular and spiral vessels, druses of calcium oxalate, anisocytic stomata, unicellular trichomes forming star-shaped groups and two types of glandular hairs – with mono- and pluricellular gland, respectively (leaf); and androspore with echinate exine and endothecium (flower) (figures 1 – 10).

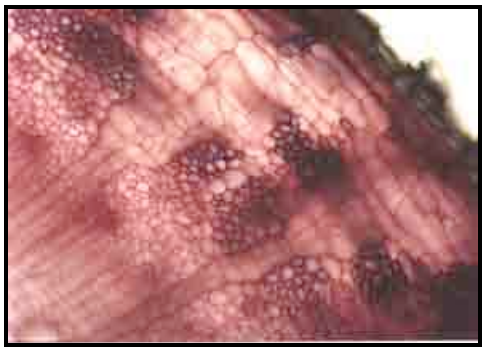

Figure 1
Root – secondary structure (ob.10x)

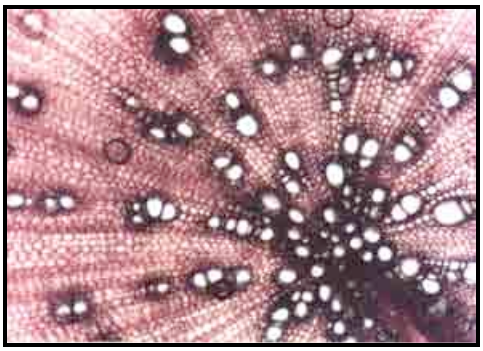

Figure 2
Xylem II with dilated rays, xylem I (ob. 4x)

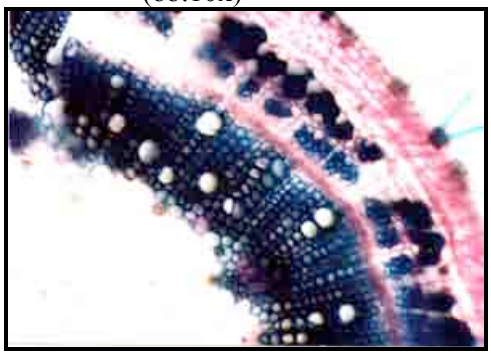

Figure 3
Stem – incomplete secondary structure (ob.4x)

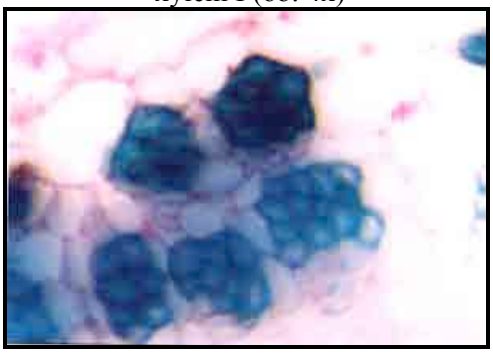

Figure 4
Stem - sclerenchymatous fibres (ob.4x)

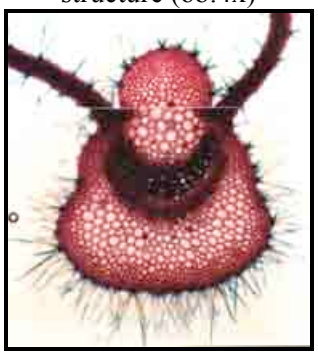

Figure 5
Leaf - heterogeneous-asymmetrical structure, collateral vascular bundle (ob.4x)

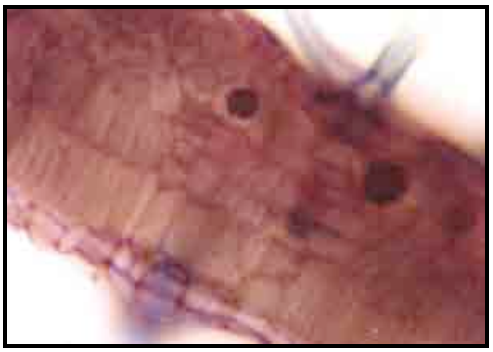

Figure 6
Leaf - bifacial structure (ob.10x)

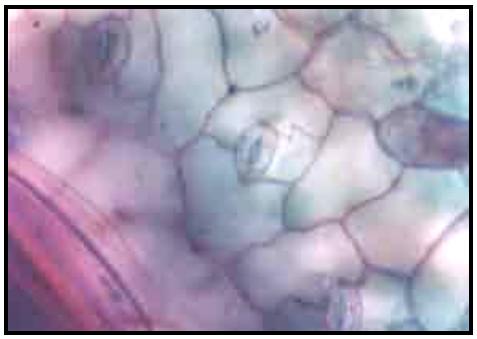

Figure 7
Anisocytic stomata (ob. 40x)

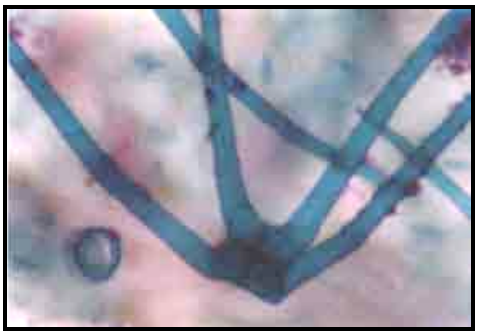

Figure 8
Unicellular trichomes forming star-shaped groups (ob.40x)

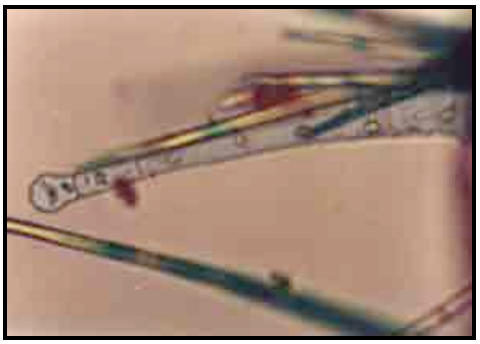

Figure 9
Monoglandular hairs with pluricellular foot (ob.40x)

Figure 10
Androspore with echinate exine; endothecium (ob.40x)

In leaves, the following substances were found: carotenoids, flavonoids, sterols, triterpenes, polyphenolcarboxilic acids, condensed tannins, polysaccharides, monosaccharides and reductive compounds (reaction signal intensity and the extracts wherein they were identified are shown in table I). All these compounds, except for polysaccharides and rutin, are not cited by the consulted scientific literature.

Table I

Results of phytochemical screening for the leaves of *Abutilon theophrasti*

No.	Phytochemical constituents	Etheric extract	Alcoholic extract	Aqueous extract
1.	carotenoids	+	-	-
2.	flavonoids	++	+	+
3.	sterols	+	-	-
4.	triterpenes	+	-	-
5.	polyphenolcarboxilic acids	-	+	++
6.	condensed tannins	-	+	+
7.	polysaccharides	-	-	++
8.	monosaccharides	-	-	+
9.	reductive compounds	-	+	+

” + +”: intensely positive reaction; “+ “: positive reaction; “-“: negative reaction

In leaves, the following compounds were identified by TLC: 4 flavonoid-like substances (Rf = 0.12 for rutin; 0.18; 0.70; 0.96) and 5 polyphenolcarboxilic acids (Rf = 0.04; 0.10; 0.15; 0.23; 0.97). Flavonoids were derived from quercetin (Rf = 0.93) and 3 other flavonoid aglycones (Rf = 0.47; 0.86; 0.89) (fig. 13, 14).

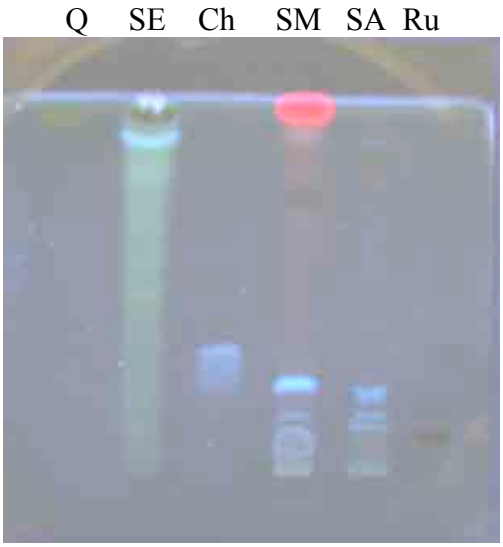

Figure 13
TLC chromatogram of – polyphenolcarboxilic acids and flavonoids (UV - 366 nm before revelation)

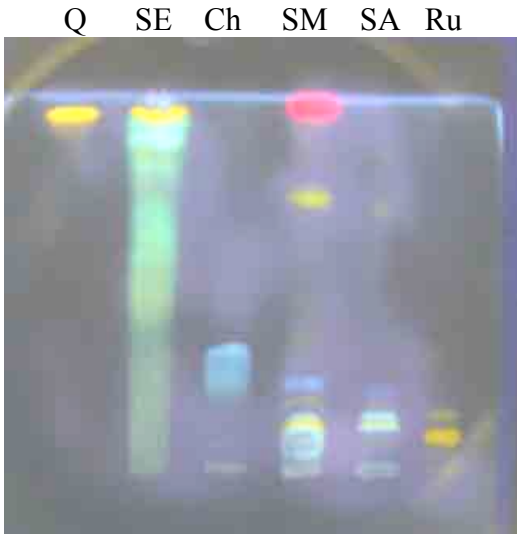

Figure 14
TLC chromatogram of – polyphenolcarboxilic acids and flavonoids (UV-366 nm after revelation)

where Q – quercetin reference solution, SE – etheric extractive solution, Ch – chlorogenic acid reference solution, SM – methanolic extractive solution, SA – aqueous extractive solution, Ru – rutin reference solution.

The contents of polysaccharides (mucilages), polyphenolcarboxilic acids and flavonoids are presented in table II. Our results show that the leaves of *Abutilon theophrasti* have a considerably higher content of flavonoids (0.45 – 0.60 g %) than previously reported rutin content (0.01%) in the literature. While our results cover the whole flavonoidic content *expressed as rutin*, the previously available data make reference to *rutin only*. Besides, several factors might still justify a discrepancy between the results reported here and those previously mentioned in the literature: the assay methods, the harvesting season for the herbal product, the environmental factors or biological variability of the species (the „Plants for a future" website cites an Indian book published in 1984 as a reference for this rutin content and we were not able to get additional information on the primary data source).

Table II
Results of the quantitative determinations

No.	Determination	Measure unit	Sample folium
1.	polysaccharides	g%	7.01 – 7.02
2.	flavonoids	g % (expressed as rutin)	0.45 – 0.60
3.	polyphenolcarboxilic acids	g % (expressed as caffeic acid)	0.22 – 0.48

The phytobiological test showed that the aqueous extract (5.00 – 1.66 %) had a concentration-dependent inhibitory effect on the radicular growth as compared with the control group ($p<0.05$, Kruskal-Wallis z test). For the 0.33% concentration the radicular elongation was comparable with that of the control sample; a slightly stimulating effect was seen at the lowest concentration, 0.03%, but it was not statistically significant ($p>0.05$) (fig. 15).

The microscopic examination of the embryonic wheat roots confirmed the mitoinhibitory effects observed at the high concentrations (5.00 – 1.66%): nuclei with 2 – 4 hypertrophied nucleoli, sinuous membranes, anaphases and telophases with delayed chromosomes were observed. Normal and altered mitoses (metaphases and telophases in tropokinesis) were observed at the lower concentrations (0.33 – 0.03%) (figures 16, 17).

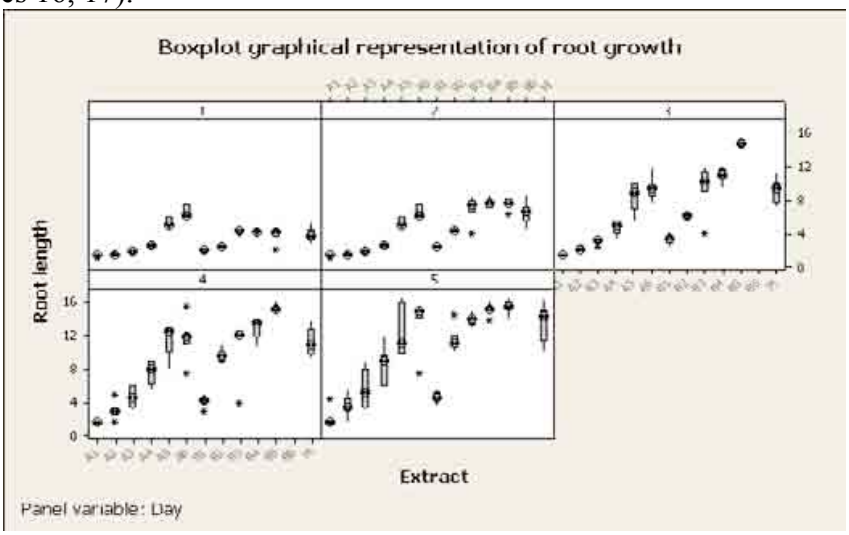

Figure 15
Boxplot representation of the effects of the aqueous extract and polysaccharide fraction on *Triticum* main root growth over 5 days. Medians, interquartile range and outliers (the latter shown as asterisks).

The polysaccharide fraction moderately inhibited the radicular elongation at the 1.00% concentration ($p<0.05$); at the lower concentrations (0.50%-0.01%) the effects of this fraction were not significantly different from the control ($p>0.05$). Frequent normal mitoses and rare minor alterations of the mitotic apparatus were observed. None of the tested extracts was otherwise cytotoxic.

Figure 16
Telophase with brigdes (ob.100x) (aqueous extract, 5%)

Figure 17
Nuclei, hypertrophic nucleoli (ob.100x) (aqueous extract 3.33%)

Conclusions

The leaves of *Abutilon theophrasti* contain carotenoids, flavonoids, sterols, triterpenes, polyphenolcarboxilic acids, condensed tannins, polysaccharides, monosaccharides and reductive compounds. They have a relatively high content of polysaccharides, flavonoids and polyphenolcarboxilic acids. Moderate genotoxicity of the aquous extract and of the polysaccharide fraction was only seen at high concentrations, suggesting that from this point of view the product is relatively safe.

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