Proximate Composition and Some Physico-chemical Properties of *Abutilon theophrasti* (velvetleaf) Seed Oil

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The qualitative chemical study of the Abutilon theophrasti Medik. seed, harvested from the spontaneous flora of Romania revealed the presence of the following classes of biologically active compounds: oil, sterols, free and glycosylated triterpenes, sugars and mixed polysaccharides (mucilages). Oleanolic acid, a triterpenoid sapogenin with biological activity was also identified in the seed. Proximate composition and physicochemical analyses were carried out in the extracted seed oil of Abutilon theophrasti Medik. The average oil content of the seeds was about 10.5 % by weight. The HPLC analysis shows that the obtained oil contains 3.4 % free fatty acids, 11.4% diacilglycerols and 74.5% triacilglycerols. The fatty acids composition determined as methyl esters by GC-MS analysis indicates that the seed oil is rich in linoleic acid (38.96 %), an essential fatty acid. Other saturated and unsaturated fatty acids (palmitic, stearic and oleic) were also identified. The results suggest that the indigenous seeds of Abutilon theophrasti Medik. may be used as a source of linoleic acid.

Key words: velvetleaf seed oil, linoleic acid, HPLC analysis, GC-MS analysis

The genus *Abutilon,* originating from China, contains approximately 160 species worldwide, primarily in tropical and subtropical areas. *Abutilon* species are cultivated for medicinal and ornamental purposes as well as a fiber source [1]. *Abutilon theophrasti* Medik. (velvetleaf, China jute), is a herbaceous annual plant, common in the spontaneous flora of Romania [2]. Seeds are kidney-shaped, flattened, dull greyish brown. Each flower is capable of producing over 200 seeds [3].

The powered seed is demulcent, diuretic, emollient, laxative and stomachic [4]. The fresh or dried seeds of this species are used as food and traditionally in the treatment of some dermatological conditions (due to its contents in fatty acids and mucilages), in constipation (due to its mucilages) [5], as well in the in treatment of dysentery, stomach-aches etc [6]. The previous chemical studies on non-indigenous *Abutilon* seeds showed the presence of proteins (17.4%), lipids (16%) and carbohydrates (33.8%) [7].

The literature does not contain any data on the chemical composition of the *Abutilon theophrasti* species from Romania. The aim of this study was to identify the main classes of bioactive compounds and to analyse by chromatographic methods the extracted seed oil (glyceride esters profile by HPLC and organic acids as methyl-esters by GC-MS). The compounds of special interest were linoleic acid and oleanolic acid.

Linoleic acid (*cis, cis*-octadeca-9, 12-dienoic acid (18:2)) is a member of the group of essential fatty acids called omega-6-fatty acids. This fatty acid has been identified as an essential one because it cannot be made by mammalian tissues and has to be obtained from plant or marine dietary sources. It is abundant in many plant oils, especially safflower, sunflower and cottonseed oil [8]. Linoleic acid (LA) is a component of cell membranes, precursor of eicosanoids (prostaglandins, prostacyclins, thromboxanes, leukotrienes) involved in the processes of platelets aggregation. Its deficit has been incriminated as a cause for some dermatological conditions (erythema, eczematous lesions, impetigo), arterial hypertension and platelets hypo-aggregation [9]. Linoleic acid at a relatively low level seems to cause decrease in serum HDL cholesterol correlated with the reduction in serum zinc level [10] and the prevention of cardiovascular disease [11]. The LA intake is associated with a significant decrease in risk for coronary heart disease [12]. Essential fatty acid deficiency has been increasingly reported in patients with cystic fibrosis. Linoleic acid supplementation has a positive effect on the growth of infants [13] and was associated with reversal of refractory eczematous dermatitis [14]. A number of studies suggest that diabetic patients require higher than normal intakes of linoleic acid. LA supplementation was shown to attenuate diabetic complications [15]. In vitro data have suggested that conjugated linoleic acid might play a significant role in cancer prevention or even treatment, at least for some tumour lines[16]. Its oxidation by lipoxidase was reported to increase tumour cell death [17]. An LA anti-inflammatory effect has also been described [18].

Oleanolic acid is a naturally triterpenoid, widely occurring in food and medicinal plants. Oleanolic acid was reported to demonstrate anti-inflammatory, and antihyperlipidemic properties [19]. It has been recognized to have hepatoprotective effects [20, 21], being marketed in China as an oral drug for human liver disorders. In several non-clinical studies oleanolic acid was shown to inhibit tumour initiation and promotion, as well as to induce cell differentiation and apoptosis [19].

Experimental part

Materials and methods

Herbal product

Seeds of *Abutilon theophrasti* Medik. species were harvested when fully mature, in September 2007, from the spontaneous flora (Vânju Mare, Mehedinţi county). They were manually selected and naturally dried in laboratory conditions.

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Overall qualitative chemical examination

The herbal product was successively extracted by reflux with solvents of different polarities (ethyl-ether, methanol and water). In each of the resulting fractions, chemical reactions specific to the various groups of active principles were performed, according to the working techniques provided in the scientific literature [22].

Histochemical examination

Abutilon seed powder was treated with hematoxylin (to identify cells containing mucilage) and an alcoholic solution 20g/L of Sudan III, to put into evidence the oil [23]. The preparations thus obtained were examined with a light microscope Labophot 2 Nikon.

Thin layer chromatography (TLC) condition for identification of oleanolic acid

The alcoholic extract obtained by refluxing 0.5 g of seeds with 5 mL methanol for 30 min. The extractive solution was concentrated on a sand bath. As reference substances oleanolic and ursolic acids (Sigma) and methanolic solutions (0.1%) were used.

TLC was performed on SIL GEL plates (Merck), the elution system being chlorophorm/acetone (8:2, v/v). The chromatographic plates thus obtained were sprayed successively with acetic anhydride and H_2SO_4/ethanol, heated for 5 min. at 100°C and examined in UV (366 nm, *Camag* UV lamp) and visible light [24].

Cold extraction of seed oil

2 g of dried, powdered (sieve no. V – *Romanian Pharmacopoeia*) herbal material was extracted at room temperature with 20 mL petroleum ether, by mechanical stirring for 4 hours. After filtration and drying on anhydrous Na_2SO_4, the ether was removed by room temperature evaporation.

Infrared spectrum

FTIR-ATR spectrum of seed oil (from 4000 to 400 cm^{-1}) was recorded on a Vertex 70 (Bruker) spectrometer equipped with attenuated total reflectance cell provided with a diamond crystal. Spectrum has been processed by the instrument software OPUS.

High Performance Liquid Chromatography (HPLC) analysis

For the HPLC analysis of seed oil, a modified HPLC method [25] was used. The equipment included a Varian Prostar High Performance Liquid Chromatograph, equipped with a Prostar 240 quaternary pump, a Prostar 330 DAD detector, and a Inertsil 5 ODS-2 (250 x 4.6 mm IDx5 mm) column with precolumn C18. The flow rate of the binary solvent mixture (methanol, solvent A, and 2-propanol/n-hexane 5:4 by volume, solvent B) was 1 mL/min with a linear gradient (from 100% A to 40% A to 40% A + 60% B in 32 min). The injection volume was 20 µL. The column temperature was held constant at 26-28°C. The components were detected at 205 nm. The acquisition channels were 205, 254 nm with complete registration of the spectra in the 190-350 nm range). The sample (2 mg oil) has been dissolved in 10 mL solvent B and filtered through 0.45 mm Millipore filters. Mono- and triacylglycerols were identified by comparison of the retention times with those of standards.

Fatty acids composition by Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Fatty acids composition of seed oil was performed after conversion of fatty acyl groups to their methyl esters with sodium methoxide. Over 50 mg of seed oil introduced into a vial with Teflon septum, 2 mL of freshly prepared sodium methoxide in anhydrous methanol, were added. The vial was maintained for 10 min at 50°C in an ultrasound bath. After cooling, 0.1 mL glacial acetic acid and 5 mL of water were added. The converted methyl esters were extracted in hexane (2x5 mL) using a separatory funnel. The pooled extracts were introduced in a vial containing anhydrous Na_2SO_4. After 20 min, from this solution 0.5 µL were sampled and injected using a split system [26, 27].

A gas chromatograph coupled with a mass spectrometer (GC 8000-MS 800 Fision Instruments) was used. Separation was performed on a capillary column DB-5MS with a length of 30m, inner diameter of 0.32 mm and thickness of the stationary phase layer of 0.25 µm. The column was operated in a programmed temperature regimen: 2 min at 40°C, 40-160°C at 5°C/min, where it was maintained for 2 min , followed by 160- 280°C at 10°C/min, the column being maintained for 20 min at 280°C. An injection split-splitless system in the split variant was used, with a flow split ratio of 1:50. The temperature was 250°C in injector. Helium with a flow rate of 2 mL/min was used as a carrier gas.

The detector MD800 model is a mass spectrometer of quadrupole type using impact ionization, the energy of ionization electrons being of 70 eV. The source and interface temperatures were of 200°C and 280°C, respectively. The mass spectrum was acquired on the interval 30-600 a.m.u., with a scan rate of 1 scan/s, using MassLab software.

Compound identification was done by comparing the mass spectra obtained experimentally with those of some data libraries: Adams, NIST (National Institute of Standardisation, UAS) Wiley 1996 Ed.

Results and discussion

The qualitative chemical study of *Abutilon theophrasti* Medik. show the presence of the following classes of biologically active chemical compounds: oil, sterols, free and glycosylated triterpenes, sugars and mixed polysaccharides (mucilages). The mucilages were also histochemically identified. By histochemical examination, oil droplets stained in orange-red were revealed (fig. 1).

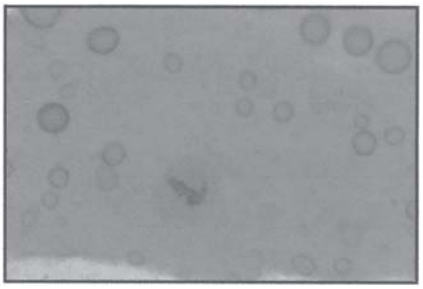

Fig. 1. Microphotograph showing oil droplets seen in *Abutilon theophrasti* seeds (magnification x 100)

Oleanolic acid was identified by TLC (Rf=0.82; yellow fluorescence in UV at λ =366 nm; purple colour in visible, after pulveration with acetic anhydride and H_2SO_4/ethanol, fig. 2). The literature does not mention the presence of this triterpenic aglycon in the seeds of *Abutilon theophrasti* species.

By cold extraction of 2 g of seed powder with ethyl ether, 0.21 g of oil were obtained (corresponding to a contents of 10.5% based on the dry seed), yellowish, odourless. By comparing this result with the data from the literature consulted, it may be stated that the indigenous product has a lower contents in oil than the seeds of North-American (15-17%) [28] or Asian (18.5%) [29] origin.

The quality of the oil has been evaluated based on the IR profile. Having in view the value 133 for the iodine number, higher than 100, the oil has a high degree of unsaturation, being susceptible to thermal and/or oxidative polymerization [30, 31]

Fig. 2. TLC chromatogram (viewed with a UV lamp at 366 nm: 1. methanolic solution of *Abutilon theophrasti* seeds; 2. oleanolic acid (methanolic solution, 0.1%); 3. ursolic acid (methanolic solution 0.1%)

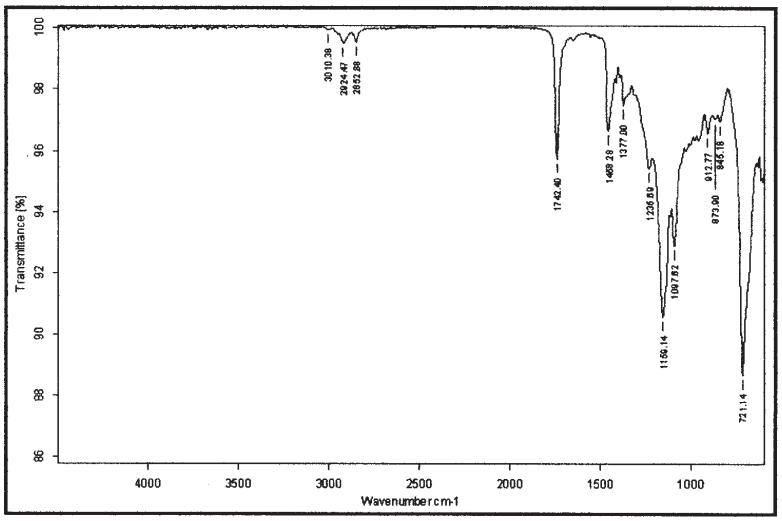

Fig. 3. ATR–FTIR spectrum of *Abutilon theophrasti* seed oil

The IR spectrum of *Abutilon theophrasti* seed oil displays characteristic bands of aliphatic hydrocarbonate chains affected to stretching, bending and rocking vibrations in the 3000-2850 cm^{-1} range, and in the finger print region, respectively. Stretching vibrations corresponding to C-H bonds from methylene groups appear at 2925 (ν_{as}) and 2853 cm^{-1} (ν_s). Bands that appear in the finger print region at 1458 cm^{-1} and 721 cm^{-1}(fi) are assigned to the rocking vibrations γ and δ respectively of the C-H bonds from methylene groups. Band at 912 cm^{-1} are assigned to γ_{Csp2-H}. Bands specific to esters at 1742 ($\nu_{C=O}$), 1159 and 1098 cm^{-1} (ν_{C-O}) are also present. The valence vibration band C_{sp2}-H at 3010 cm^{-1} was assigned to the *cis* double bond. If an oxidative process occurs, the intensity of *cis* C_{sp2}-H band, usually situated between 3050 and 3010 cm^{-1} decreases, and the broadening of the band at 1742 cm^{-1} attributed to C=O group is observed [32].

The HPLC analysis (fig. 4) shows that the extracted oil contains 74.5% of triacylglycerols, 11.4% of diacilglycerols and 3.4% of free fatty acids. The content of the components was determined by measuring the peaks areas in the 2.615-4.385 min for free fatty acids, peak areas in the 13.585-19.385, and 20.462-32.923 min range for di-, and triacylglycerols, respectively.

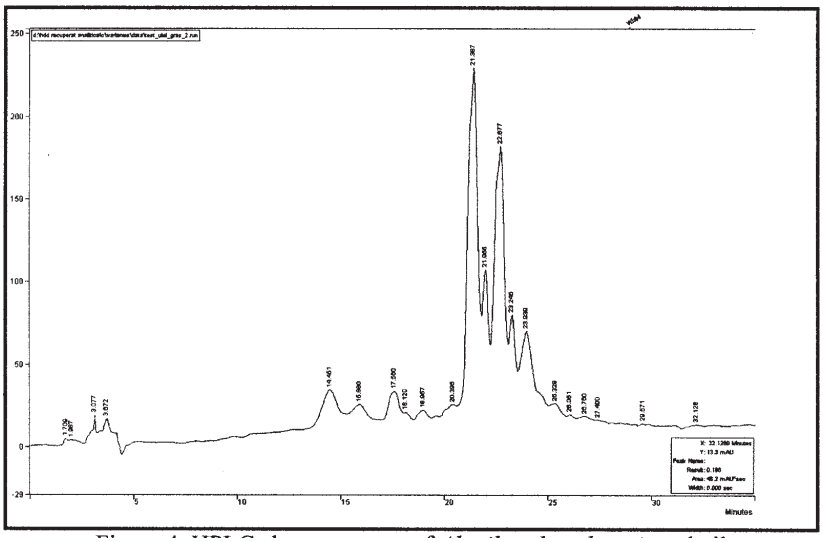

Fig. 4. HPLC chromatogram of *Abutilon theophrasti* seed oil

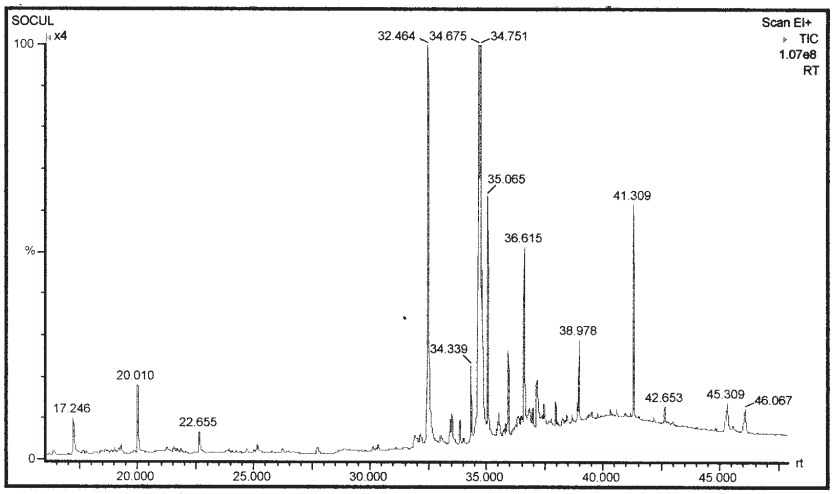

Fig. 5. GC separation of the A*butilon theophrasti* seed oil (after derivatization)

Table 1
CHEMICAL COMPOUNDS IDENTIFIED IN THE LIPOPHILIC FRACTION OF *ABUTILON THEOPHRASTI* SEEDS, AFTER DERIVATIZATION

No.	Compound	t_R	Area (%)
1.	alkane	17.246	1.12
2.	alkane	20.010	1.70
3.	alkane	22.655	0.64
4.	palmitic acid	32.464	16.59
5.	7-methyl-8-hexadecenoic acid	33.515	0.71
6.	heptadecanoic acid	33.862	0.47
7.	linoleic acid	34.675	38.96
8.	oleic acid	34.751	14.19
9.	stearic acid	35.065	4.23
10.	2-octyl-cyclopropanoctanoic acid	35.943	1.45

The chromatographic separation of the components of the *Abutilon theophrasti* seed lipophilic extract, after derivatisation, is shown in figure 5, and the results of the GC/MS (identified compounds, peak area and retention time t_R) are presented in table 1.

Through the GC/MS analysis of the oil after derivatization, 24 compounds where separated, among which 10 were identified, corresponding to 80.06% of the total area. Among the latter, the fatty acids represent 76.6%, prevailing the linoleic (38.96%), palmitic (16.59%) and oleic acids (14.19%). In smaller amounts there are also presented the stearic (4.23%) and 2-octyl-cyclopropanocatnoic (1.45%). In very small amounts the 7-methyl-8- hexadecenoic (0.71%) and heptadecanoic (0.47%) acid were identified.

The high contents in linoleic acid is comparable with that of peanut (*Arachis hypogea*), sesame (*Sesamum indicum*) and common sea-buckthorn oil (*Hippophäe rhamnoides*), but lower than that of evening primrose (*Oenothera biennis*) [9] or of *Abutilon theophrasti* of Italian origin (66.5%) [33].

Conclusions

Abutilon theophrasti Medik. seeds were analyzed. The qualitative chemical analysis allowed the identification of the main classes of bioactive principles. The obtained oil has a high degree of esterification containing 74.5% of triacylglycerols, 11.4% of diacilglycerols and 3.4% of free fatty acids, content determined by HPLC analysis. The GC-MS analysis of the fatty acids was performed after derivatization, by transforming the glycerol esters in metilic esters, showing a high amount of linoleic acid (38.96%). By TLC the oleanolic acid was identified for the first time in this species, its presence not being mentioned by the scientific literature consulted by the authors. Oleanolic acid and linoleic acid may explain scientifically the traditional use of this product for its anti-inflammatory effects. Results show that *Abutilon theophrasti* Medik. seed oil can be used as a source of linoleic acid.

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