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Fatty acid Profiles of soxhlet and Ultrasonic obtained oils from the promising medicinal legume *Cytisus triflorus* L'Hérit.

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ABSTRACT
Cytisus triflorus L'Hérit. (syn. C. villosus Pourr.) is a perennial shurb used in algerian remedies for medicinal purposes. The plant is supposed to contain potentially active substances but its constituents have only been rarely studied. The oils of leave and stem of C. triflorus L'Hérit. were isolated by conventional soxhlet method and by ultrasonic extraction. The oils were analysed by GC and GC-MS. The analyzed leave and stem, in spite of their low fat content, lower than 2%, presented high proportions of unsaturated fatty acids. α-Linolenic acid (48,24% - 47,32%, respectively) was the most prominent fatty acid in soxhlet and ultrasonic obtained leave oils, whereas 8,11-octadecadienoic acid and linoleic acid (54,22% - 25,06%, respectively) were the major constituents of the soxhlet and ultrasonic isolated stem oils. Both soxhlet and ultrasonic leave oils contain lesser than 30% saturated fatty acids with palmitic acid as their main contributor (22,69%-11,51%, respectively) whereas stearic and palmitic acid (19,25%-24,4%, respectively) were the major saturated fatty acids of stem soxhlet and ultrasonic obtained oils determined at 38,15% and 45,77%,
Keywords: *Cytisus triflorus; fatty acids; soxhlet; ultrasound extraction; α-linolenic acid; 8,11-octadecadienoic acid .*

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INTRODUCTION

The genus *Cytisus* belongs to the tribe *Genisteae* of the *Fabaceae* family, the second largest family of flowering plants. It comprises about 60 species spread in the spontaneous flora of many countries [1]. Previous studies reported on quinolizidine and dipiperidine alkaloids (sparteine, lupanine, ammodendrine) [2,3], flavonoids (rutin, quercetin, kaempferol, genesteine) [4,5], and essential oils [6-8] of *Cytisus* species. In addition, several pharmacological effects of flavonoids isolated from these plants have been demonstrated [4,8]. However, there are few papers about the total fatty acids composition of these leguminous shrubs, which reported exclusively on the oil content of their flowers [6-8].

Lipid composition of plants has lately received particular attention, especially in their content in the essential fatty acids EFAs. These are polyunsaturated fatty acids and are similar to vitamins in their importance to one's overall health [10]. In addition to their nutritional, industrial and pharmaceutical uses, patterns of fatty acids variation in plants have proven to be useful tools in taxonomic and phylogenetic studies [11,12]. The usual analytical method of oils and fats recovery from solid plant materials is the soxhlet system. Howerver, ultrasoun assisted extraction (UAE) has recently been recognized for application in the eddible oil industry to improve efficiency and reduce extraction time [13,14]. This novel extraction procedure has also been combined with other classical methods such as aqueous enzymatic oil extraction (AEOE) [15] and more recently soxhlet system [16].

Cytisus triflorus L'Hérit. (syn. *C. villosus* Pourr.) is a lesser known leguminous shrub. It is the most widespread species among the 8 species growing naturally throughout the north of Algeria. It is a perennial shurb of 1-2m height with hairy branches. Leaves are arranged in three, with densely silky hairs, the medial is almost twice longer than the laterals, darkening when dryed.1-3 yellow flowers armpit the superior leaves. The fruit is a flattened hairy pod [17]. The leaves of the plant have been

regarded as powerful remedy for treating abdominal pain, wounds healing and as haemostatic, antifungal and hypotensor, traditionally recognized by healers and consumers.

While a great number of chemical and pharmacological studies have been carried on many *Cytisus* species native of Europe, Africa and Asia, especially the broom *Cytisus scoparius*, some species are almost unexplored and it's the case of *C. triflorus*. Moreover, the taxonomic status of *C. triflorus* is critical in view of various taxonomic markers [1] and few reports have been traced concerning its phytochemistry and pharmacological potential [9, 18-20].

Since it was previously shown that the fatty acid composition of photosynthetic tissues improve human and livestock health and growth performance and can be a good tool for chemotaxonomic studies, and as a continuation of our research on the shrub *C. triflorus*, we report on the total fatty acids isolated by soxhlet and ultrasonic processes from its leaves and stems.

MATERIALS AND METHODS

Plant material

The vegetative aerial parts of *Cytisus triflorus* L'Hérit. were collected in May 2014 in the region of Azazga (North of Algeria) and authenticated by Dr M. Zaoui, Department of Biology, Normal Superior School, Algiers (Algeria). Voucher specimen (CTA 125/03/11) was deposited at the herbarium of the Reseach unity VARENBIOMOL of the University of Constantine 1.

Soxhlet extraction of oils

Lipids from the air-dried powders stems (2g) and leaves (3g) were refluxed in hexane for 3 hours by a soxhlet extractor. The solvent was evaporated under vacuum at 30 °C and lipids were obtained in similar percentages of 2,33% and 2,1% yields from leave and stem, respectively.

Ultrasonic extraction

About 3g and 1g of the powdered leave and stem were extracted separately with n-hexan using a Bandelin ultrasonic bath for 30 mn, at a fixed ultrasound power of 150 W and frequencies of 35 kHz. The solvent was evaporated under reduced pressure at 37 °C. The total lipids were isolated according to Hawas et al. 2012 [21] with slight modification and were found to be 2,51% and 1,64% yields for leave and stem, respectively.

Preparation of fatty acid methyl esters (FAMEs)

The lipidic fractions containing free fatty acids of each plant material were transesterified to methyl esters by refluxing, separately, the total lipid fractions in acidified anhydrous methanol (2%) for 40 min at 100 °C. The FAMEs were extracted from the reactional mixtures with hexane.

Gas chromatography analysis (GC)

The GC analysis was carried out with a Chromopack CP 9002 chromatograph equipped with a flame ionization detector (FID) and a split/splitless injection port, used in the split mode (1/100). The separation of FAMEs was made on a DB 23 (50% cyanopropyl) column (30 m x 0.25 mm x 0.25 µm). The injector and detector were both set at 250°C. The oven temperature was kept at 200°C. The carrier gas was azote.

Gas chromatography-mass spectrometry analysis (GC-MS)

The gas chromatography coupled with the mass spectrometry was performed with a Hewlett-Packard 6890 gas chromatograph combined with an Agilent 5973 mass spectrometer and equipped with an HP-5 MS (5% phenyl-polymethylsiloxane) capillary column (30m x 0.25mm x 0.25µm). The column temperature was programmed from 150°C (4 min) to 230 °C at a ramp of 2 °C per minute with holding time 36 min. The injector temperature with split ratio (1/100) was kept at 250 °C. Helium was used as carried gas at a flow rate of 1ml/min. The mass spectrometer was operated in electron impact ionization (70 eV).

Identification of the fatty acids was achieved by comparing the retention times of the peaks with those of the pure available standards and their mass fragmentations with those of mass spectra database search (Nist 2002). The relative fatty acid methyl ester percentages were calculated from GC-FD integration

RESULTS AND DISCUSSION

Identified fatty acids of leave and stem oils obtained by soxhlet extraction with their abundance (%) are listed in table1.

9 FAMEs were identified by GC and GC-MS analysis. The GC semi-quantification revealed that 100% of the composition of the stem oil was fatty acids, similarly to the leave oil (99,94%). Both oils shown a high relative percentage of the unsaturated fatty acids methyl esters representing 72,51 % and 61,95 % of the total leaves and stems oils, respectively; with α-linolenic acid (48,24%) and linoleic acid (13,29%) versus 8,11-octadecadienoic (54,22%) as their main constituents, respectively. Inversely to the unsaturated fatty acids methyl esters, the relative percentage of the saturated lipid fraction was higher in the stems oil

(38,05%) than in the leaves one (27,43%), with as major compounds stearic acid (19,25%) and palmitic acid (22,69%), respectively. Myristic acid was present in trace amounts in the stem oil, while arachidic acid was not identified in the soxhlet leave oil.
Table 2 show a comparative composition of soxhlet extracted oils and ultrasonic obtained oils from leave and stem of *C. triflorus*.

TABLE1: FATTY ACID COMPOSITION OF *C. TRIFLORUS* OILS OBTAINED BY SOXHLET METHOD.

RT[a]	Fatty acids	Stem	Leave
4.97	Tetradecanoic acid (myristic acid)	trace	1,38
5.97	Hexadecanoic acid (palmitic acid)	13,6	22,69
6.26	9-hexadecenoic acid (palmitoleic acid)	trace	2,15
8.02	Octadecanoic acid (stearic acid)	19,25	3,36
8.42	9-octadecenoic acid (oleic acid)	3,36	6,29
9.30	9,12-octadecadienoic acid (Z,Z) (linoleic acid)	1,73	13,73
9.87	8,11-octadecadienoic acid	54,22	2,56
10.5	9,12,15-octadecatrienoic acid (Z,Z,Z) (α-linolenic acid)	2,64	48,24
11.5	Eicosanoic acid (arachidic acid)	5,2	-
	∑SFAs[d]	38,05	27,43
	∑UFAs[e]	61,95	72,51
	∑Fas[f]	100	99,94

[a]RT: retention time
[b]SE: soxhlet extraction
[c]UE: ultrasound extraction
[d]SFA: Saturated fatty acids
[e]UFA: Unsaturated fatty acids
[f]FA: Fatty acids

TABLE 2: COMPARATIVE COMPOSITION OF OILS OBTAINED BY SOXHLET AND ULTRASOUND EXTRACTION

RT[a]	Fatty acids	Stem		Leave	
		SE[b]	UE[c]	SE	UE
4,71	Dodecanoic acid (lauric acid)	-	-	-	0,79
4.97	Tetradecanoic acid (myristic acid)	trace	trace	1,38	1,39
5.97	Hexadecanoic acid (palmitic acid)	13,6	24,4	22,69	11,51
6.26	9-hexadecenoic acid (palmitoleic acid)	Trace	1,55	2,15	1,44
8.02	Octadecanoic acid (stearic acid)	19,25	7,84	3,36	3,38
8.42	9-octadecenoic acid (oleic acid)	3,36	13,94	6,29	6,71
9.30	9,12-octadecadienoic acid (Z,Z) (linoleic acid)	1,73	25,06	13,27	14,38
9.87	8,11-octadecadienoic acid	54,22	-	2,56	-
10.5	9,12,15-octadecatrienoic acid (Z,Z,Z) (α-linolenic acid)	2,64	13,66	48,24	47,32
11.5	Eicosanoic acid (arachidic acid)	5,2	13,53	-	10,11
	∑SFAs[d]	38,05	45,77	27,43	27,63
	∑UFAs[e]	61,95	54,21	72,51	69,85
	∑Fas[f]	100	99,98	99,94	97,48

[a]RT: retention time
[b]SE: soxhlet extraction
[c]UE: ultrasound extraction
[d]SFA: Saturated fatty acids
[e]UFA: Unsaturated fatty acids
[f]FA: Fatty acids

The unsaturated fatty acids were found to be the major constituents of both samples obtained by the two methods, with no major differences between the main components of Soxhlet and ultrasonic leave oils (oleic acid: 6,29%- 6,71% ; Linoleic acid: 13,27%-14,38% and α-linolenic acid: 48,24%-47,32% ; respectively). Whereas, the ultrasonic stem oil compared to the soxhlet oil provided the highest

concentrations of oleic (13,94%-3,36%), linoleic acid (25,06%-1,73%) and α-linolenic acid (13,66%-2,64%). However, the unsaturated 8,11-octadecadienoic fatty acid being the main unsaturated fatty acid of the soxhlet stem oil (54,22%) has not been identified in both stem and leave oils obtained by ultrasonic extraction. This fatty acid is one of the 28 possible isomers derived from linoleic acid. This may be due to its detorioration by ultrasound treatment (35 KHz, 150 W, 30 mn). In fact, high intensity ultrasounds can lead to oxidation and modify the chemical structure and hence the functionality of all major nutrients such as proteins, polysaccharides and lipids [22].

The saturated fatty acid palmitic acid was dominated in both soxhlet and ultrasonic obtained leave oils (22,69% - 11,51% ; respectively), whereas lauric acid (0,79%) and arachidic acid (10,11%) were only identified in the ultrasonic oils. Inversely to leave oils, palmitic acid was the main constituent of the saturated fatty acid fraction of the ultrasonic isolated stem oil (24,4%), whereas the soxhlet one was majored by stearic acid (19,25%). Arachidic acid was identified with higher relative percentage in ultrasonic isolated oil (13,53%) than in soxhlet obtained one (5,2%).

The ratio of unsaturated/saturated fatty acids (U/S) were 1,62 , 1,18 and 2,64 , 2,52 for stem and leave oils obtained by soxhlet and ultrasonic extraction, respectively. As the taxonomic position of the species *C. triflorus* is still clear enough, the fatty acid composition and the U/S index of its oils could be useful as a chemiotaxonomic indicator.

The efficiency of soxhlet and ultrasonic method on lipid extraction from plant materials could be related to their histological structure as well as to their cellular membrane ultrastructure. As reported by Babaei et al., 2006 [13], the rheological nature of the seed structure (hardeness, compactness) may have a direct impact on the capacity of ultrasound to improve extraction of lipids compounds from plant cells.

To the best of our knowledge, this is the first report on isolation and identification of oils from vegetative parts of *Cytisus* species, *Cytisus triflorus* L'Hérit. Previous report on the lipid composition of flower from *Cytisus multiflorus* found that α-linolenic acid followed by linoleic acid contributed to the prevalence of the unsaturated fatty acids, while palmitic acid was the main constituent of the saturated fatty acids [8]. Similarly, α-linolenic acid and palmitic acid were found to be the major compounds of flower essential oil from *Cytisus sessifolius* [7]. Essential oil of fresh flower of *Cytisus scoparius* was identified to contain methyl palmitate, methyl arachidate, methyl caproate, methyl enanthate, methyl pelargonate, methyl caprate, methyl laurate, methyl myristate and metrhyl stearate [6]. These are the only data about the fatty acid composition of *Cytisus* species.

CONCLUSION

The results obtained from this study show that regardless the extraction, the essential unsaturated fatty acids were found in both vegetative organs at the highest level especially in the leaves. *Cytisus triflorus* stem oil is of the type 8,11-octadecadienoic and stearic acids whereas the leave oil is of the type α-linolenic and palmitic acids. The high content of α-linoleic acid and palmitic acid of leaves may be partially responsible for significant anti-inflammatory activity exhibited by the leave hydroalcoholic extract, in our previous study, beside the flavonoids of the plant [9]. Further studies on the oil content of other aerial parts of this plant may be of great interest to broaden the knowledge on its chemotaxonomy and pharmacology. Furthermore, the high content of unsaturated fatty acids reinforce the interest of including *C. triflorus* plant particularly its leaves as a nutrient and as a medicine to improve health and growth performance in human and livestock.

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