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Comparative Study on *Hypericum triquetrifolium* Turra Fatty Acids

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Abstract: The leaves of nine populations of *Hypericum triquetrifolium* Turra growing wild in Tunisia were investigated for their fatty acids composition. Although, their low yields, total fatty acids composition showed an appreciable amount of α-linolenic acid (C18:3), linoleic acid (C18:2), oleic acid (C18:1) and palmitic acid (C16:0). Stearidonic acid (C18:4), an unusual plant fatty acid was also found. A one-way analysis of variance (ANOVA) revealed significant differences between studied populations. However, multivariate analysis showed that *H. triquetrifolium* samples were grouped according to their origin apart from three populations.

Key words: *Hypericum triquetrifolium* Turra, Cluciaceae, fatty acids, α-linolenic acid, stearidonic acid, multivariate analysis

INTRODUCTION

The genus *Hypericum* of the Cluciaceae family is a large group of shrubs or herbs consisting of approximately 450 species in 36 sections (Robson, 2001), widespread in warm temperate areas throughout the world and well represented in the Mediterranean area. One of the most important species of this genus is *Hypericum perforatum* L. (St. John's wort) which has been used in herbal medicine externally for the treatment of skin wounds, eczema and burns and internally, for diseases of the central nervous system, the alimentary tract and others (Bombardelli and Morazzoni, 1995; Barnes *et al*., 2001). Several studies showed that *H. perforatum* L. and other related species exhibit antidepressant, antiviral, antioxidant, antimicrobial, antifungal, anti-inflammatory, antitumor, anxiolytic, analgesic, anticonvulsive and wound healing actions (Sánchez-Mateo *et al*., 2002; Greeson *et al*., 2001). These actions were attributed to a large group of components, notably flavonoids, xanthones, tannins, phloroglucinols (hyperforin and adhyperforin) and naphtodianthrones: hypericin, protopseudohypericin, pseudohypericin and protohypericin (Allali *et al*., 2004; Kartnig *et al*., 1989; Kitanov, 2001). Among the 8 native *Hypericum* species from Tunisia, *H. triquetrifolium* Turra L (Peter's wort, wavyleaf St. John's wort or Tangled *Hypericum*) commonly known as Hamra was the most representative specie of the Cluciaceae family. It's ubiquitous in Northern, North-Eastern and North-Western areas. It appears from literature data that *H. triquetrifolium* Turra and *H. perforatum* are considered as the best source for hypericin (Allali *et al*., 2004). Numerous investigations have been directed at the bioactivity of these plants, but little is known about their FA composition and contents (Stojanovic *et al*., 2003). Since a survey of the literature revealed that no studies on FA composition of *H. triquetrifolium* Turra has been undertaken. Nevertheless, previous works reported that palmitic, linoleic and linolenic acids were found to be the major FA of some *Hypericum* species (Özen *et al*., 2004).

The major objective of this study is the phytochemical characterization of the aromatic and medicinal plants growing wild in Tunisia, the aim of this work was to highlight the FA composition of *H. triquetrifolium* Turra and compare it with those of other species of the genus.

MATERIALS AND METHODS

Plant material: Nine populations of *H. triquetrifolium* Turra were sampled during the summer 2005 at the flowering stage from different localities of various ecological conditions (Fig. 1). Plant samples were randomly collected in each population and identified by Prof. Dr. Mohammed El Hedi El Ouni (Department of biology, Faculty of Sciences, Bizerte, Tunisia) and according to the morphological description presented in Tunisian flora (Pottier-Alapetite, 1979). Plant materials were air dried at room temperature.

Total lipids extraction: Total lipids from the leaves were extracted by the modified method of Bligh and Dyer (1959). Thus, 2 g air dried leaves were extracted with

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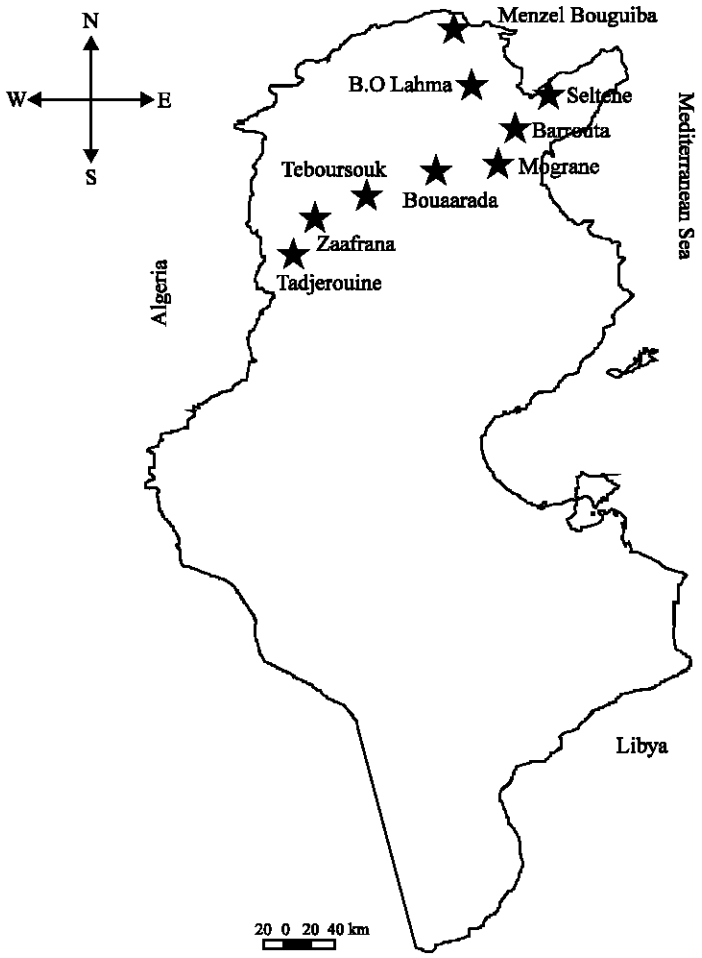

Fig.1: Sites of sampling of *H. triquetrifolium* Turra in different wild populations from Tunisia

chloroform/methanol (2:1, v/v) and using water for the washing stage during the extraction. After phase equilibration, the lower chloroform layer (total lipids) of each sample was evaporated to dryness under a stream of nitrogen, redissolved in toluene/ethanol (4:1, v/v) and stored at -20°C prior analysis.

Fatty acid methylation: Fatty acids were converted to Fatty Acids Methyl Esters (FAMEs) by using sodium methylate according to the method described by Cecchi *et al.* (1985). For quantification of FAMEs, a known quantity of Methyl heptadecanoate (C17:0) used as internal standard was added during methylation.

Chromatographic analysis: Fatty acids methyl esters from each sample were analysed by GC, using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) equipped with a Flame-ionization Detector (FID) and an Electronic Pressure Control (EPC) injector. A polyethylene glycol fused silica capillary column (HP-Innowax: 30 m ×0.25 mm ID, 0.25 μm film thickness) was used. The flow of the carrier gas (N_2) was 1.6 mL min^{-1}. The split ratio in the injector was 60:1. The detector and injector temperatures set at 275 and 250°C, respectively. The initial oven temperature was held at 150°C for 1 min, increased at a rate of 15°C min^{-1} to 200°C and then held there for 3 min and finally ramped at 2°C min^{-1} to 242°C. A blank was run after every two analyses. FAMEs were identified by comparison of their GC retention times with those of standard purified FAMEs.

Statistical analysis: Fatty acids composition is expressed as weight percentages of total FAMEs and also are means±SD of six different experiments from each population. The one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test using Statistica (Statsoft, 1998) and the differences between individual means were deemed to be significant at $p<0.05$.

In order to separate different studied populations on the basis of their FA composition, Principal Component Analysis (PCA) followed by hierarchical cluster analysis on the basis of Euclidean distance were employed.

RESULTS AND DISCUSSION

As shown in Fig. 2, Total Fatty Acids (TFA) content of *H. triquetrifolium* leaves was low and do not exceed 4.67 mg g^{-1} dry weight. Among the studied populations, there were wide variations in the TFA content. The GC analysis allowed the identification of 9 FA in all populations (Table 1). The percentage of Unsaturated Fatty Acids (UFA) was significantly ($p<0.05$) higher than saturated ones (SFA). Although the similarity in the FA composition in the 9 studied populations, there were remarkable differences in their percentages. Apart from Menzel Bourguiba, α-linolenic acid (C18:3) and Linoleic acid (C18:2) were the main UFA. Palmitic acid (C16:0) was the most abundant SFA while myristic acid (C14:0) was the less represented FA. Interestingly, the unusual FA, Stearidonic acid (C18:4) was also found, its percentages varied significantly ($p<0.05$) among all populations.

Fatty acid composition of related *Hypericum* species as *H. lysimachioides*, *H. scabrum*, *H scabroides*, *H. amblysepalum* (Özen *et al*., 2004) and *H. maculatum* (Stojanovic *et al*., 2003) has been previously studied. In these aforementioned species, FA profile was prevailed by the UFA and C18:3 was reported as the main FA. In contrast, in *H. perforatum* and *H. olympicum*, the FA composition was slightly dominated by SFA (Stojanovic *et al*., 2003). According to the latter author's, C18:3 was not detected in *H. perforatum*.

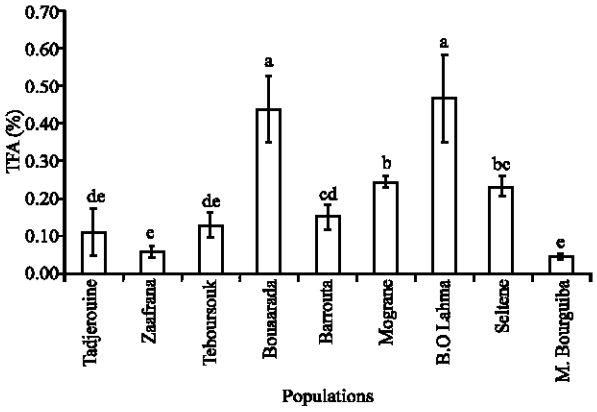

Fig. 2: Total fatty acids content (mg g^{-1} dry weight) in *H. triquetrifolium* leaves from the nine studied populations. *TFA values with different superscript (a-e) are significantly different at $p<0.05$

Stearidonic acid (C18:4), previously reported in leaves of *Boraginaceae* (Guil-Guerrero *et al*., 2003), *Vernonia galamensis* (Baye *et al*., 2005), *Oenothera biennis* (Pina *et al*., 1984), *Cynoglossum officinale*, *Anachus officinali*, *Anchus azuerea*, *Myosotis palustris*, *Echium vulgare*, *Pulmonaria officinalis*, *Symphytum officinale* (Griffiths *et al*., 1996) and *Ribes nigrum* (Dobson, 2000) was not found both in Turkish and Yugoslavian *Hypericum* species (Özen *et al*., 2004; Stojanovic *et al*., 2003). However, two unusual FA as 3-hydroxy-tetradecanoic acid (3-OH-C14:0) and 3-hydroxy-octadecanoic acid (3-OH-C18:0) were also reported in *H. lysimachioides* and *H. retusum* (Özen *et al*., 2004).

The ratios SFA/UFA (Saturated FA/Unsaturated FA), SFA/ω3 (Saturated FA/ Omega 3 FA) and ω3/ω6 (Omega 3 FA/ Omega 6 FA) are presented in Table 2. The ratio SFA/UFA was higher in Teboursouk (around 0.4) and significantly ($p<0.05$) lower in Seltène. These relative values of SFA/UFA ratio were much lower than those reported for *H. perforatum* (1.66), *H. maculatum* (0.83), *H. olympicum* (1.1) (Stojanovic *et al*., 2003), *H scabroides* (0.67) and *H. amblysepalum* (0.76) (Özen *et al*., 2004). The ratio SFA/ω3 showed significant ($p<0.05$) changes between all studied populations. Its highest value was obtained in Menzel Bourguiba (1.4) who showed a lower C18:3 content and was remarkably lower in Zaâfrana (0.39). As shown, ω3/ω6 ratio was noticeably higher in Zaâfrana (3.85) than in the other populations who present a slower but significant ($p<0.05$) changes.

Principal Component Analysis (PCA) was carried out in view to evaluate whether the FA composition reflected environmental relationship among all studied population. PCA results (Fig. 3) showed that the first axis explained about 28.52% of the total variance and the vertical axis a further 20.1%. Although these low percentages, the analysis provides a global statistical distinction between two groups. The first group included respectively Tadjerouine, Seltène, Mograne, Menzel Bourguiba and Barrouta. The four remaining population (Zaâfrana, Teboursouk, Bouaârada and B.O Lahma) were clustered in the second group. Atypical position of Tadjerouine and Menzel Bourguiba in the first group and Zaâfrana in the second group suggested that the latitude have negligible effect on the FA composition. The observed difference in the present repartition could be due to other environmental factors (soil, pH, altitude…). Furthermore, it is also possible that theses differences could have genetic explanation. However, with the exception of the three latter populations, others ones was grouped according to their provenance and showed a clear distinction (Fig. 4).

Table 1: Fatty acids composition of *H. triquetrifolium* leaves collected in nine wild populations from Tunisia

Population	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 α	C18:4	C20:0	SFA	UFA
Tadjerouine	1.29±0.27^{a}	17.15±0.46^{a}	3.9±0.85^{b}	8.53± 0.71^{a}	19.91±1.88^{e}	14.46±1.39^{d}	28.05±1.89^{d}	4.86±0.54^{e}	1.86±0.36^{e}	28.82±0.82^{a}	71.18± 2.74^{f}
Zaâfrana	1.87±0.31^{bc}	11.68±0.51^{de}	3.75±0.58^{b}	3.06±0.23^{hi}	15.27±0.43^{a}	12.52±1.17^{a}	47.47±2.26^{a}	2.62±0.27^{d}	1.76±0.32^{cd}	18.37±0.42^{fg}	81.63±3.42^{ab}
Teboursouk	2.0±0.35^{b}	16.5±0.61^{ab}	5.58±0.3^{a}	8.07±0.66a	18.12±2.17^{d}	15.21±0.54^{d}	27.46±1.29^{d}	4.71±0.27^{e}	2.35±0.39^{g}	28.92±1.29^{a}	71.08±1.23^{f}
Bouaârada	1.76±0.9^{bcd}	16.73±1.47^{ab}	1.9±0.24^{g}	2.45±0.21^{ef}	14.18±0.68^{a}	20.83±0.7^{b}	32.6±0.45^{g}	8.38±1.63^{a}	1.15±0.08^{g}	22.1±0.52^{c}	77.9± 2.8^{d}
Barrouta	1.53±0.32^{bcd}	14.64±0.98^{c}	3.61±0.29^{b}	2.89±0.23^{e}	21.64±2.45^{b}	20.63±0.62^{b}	27.14±1.09^{d}	6.16±0.65^{b}	1.75±0.44^{cd}	20.81±1.27^{d}	79.19± 3.3^{c}
Mograne	2.9±0.38^{a}	10.93±0.69^{ef}	2.55±0.24^{c}	3.65±0.42^{b}	10.16±0.64^{g}	30.09±1.17^{a}	33.11±1.77^{c}	4.65±0.48^{c}	1.96±0.30^{e}	19.45±0.64^{f}	80.55± 2.62^{b}
B.O. Lahma	1.49±0.26^{cd}	15.88±1.49^{b}	2.02±0.52^{g}	2.61±0.5^{ef}	11.51±0.55^{f}	21.87±0.68^{b}	36.09±1.15^{b}	2.95±0.61^{d}	4.78±0.17^{a}	24.76±0.68^{b}	75.24± 3.68^{e}
Seltène	1.75±0.46^{bcd}	12.1±0.49^{d}	3.39±0.39^{b}	1.93±0.38^{fg}	12.49±1.75^{f}	20.9±1.02^{b}	37.05±2.59^{b}	8.66±0.42^{a}	1.74±0.21^{cd}	17.51±0.65^{g}	82.49± 3.81^{a}
Menzel Bourguiba	1.63±0.16^{bcd}	10.29± 0.77^{g}	3.39±0.39^{b}	3.17±0.6^{hi}	42.87±3.56^{a}	17.12±0.89^{c}	13.19±0.63^{e}	5.02±0.44^{c}	3.31±0.94^{b}	18.41±2.26^{fg}	81.59±2.29^{ab}

* Means values in the same column with different superscripts (a-g) are significantly different at p<0.05

Table 2: SFA/UFA, SFA/ω3 and ω3/ω6 ratios in *H. triqetrifolium* leaves from the nine studied populations

	SFA/UFA	SFA/ω3	ω3/ω6
Tadjerouine	0.41±0.02^{a}	1.03±0.04^{b}	1.98±0.29^{b}
Zaâfrana	0.23±0.01^{e}	0.39±0.02^{g}	3.85±0.48^{a}
Teboursouk	0.41±0.02^{a}	1.06±0.09^{b}	1.81±0.11^{bc}
Bouaârada	0.28±0.01^{bc}	0.68±0.02^{cd}	1.57±0.05^{cd}
Barrouta	0.26±0.02^{cd}	0.77±0.06^{c}	1.32±0.05^{de}
Mograne	0.24±0.01^{de}	0.59±0.03^{ef}	1.10±0.04^{e}
B.O Lahma	0.33±0.01^{b}	0.67±0.04^{de}	1.69±0.11^{bc}
Seletène	0.21±0.01^{e}	0.47±0.01^{fg}	1.78±0.09^{bc}
Menzel Bourguiba	0.23±0.02^{e}	1.40±0.12^{a}	0.77±0.05^{f}

*Means values in the same column with different superscripts (a-g) are significantly different at p<0.05

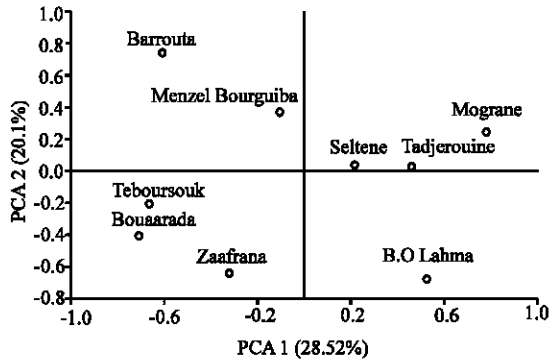

Fig. 3: Principal component analysis scatter plot of the different Tunisian populations of *H. triquetrifolium*

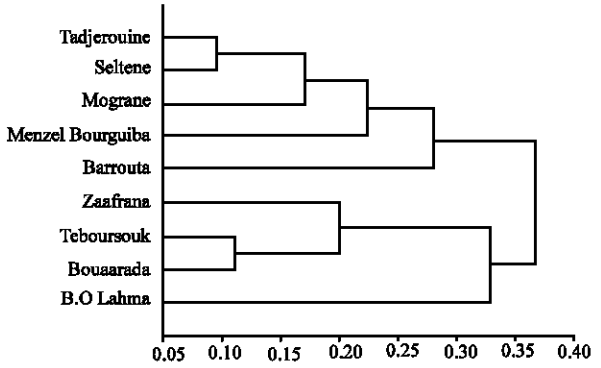

Fig. 4: Cluster analysis of the different Tunisian populations of *H. triquetrifolium*

In the light of the literature and from these results, it may be concluded that the leaves of *H. triquetrifolium* Turra were a good source of α-linolenic acid which have high healthy potential. The good quality of the oil of this specie was due to its high content on UFA. The use of this oil in human dietary and in the alimentary industry seems to be a promising alternative.

REFERENCES

Allali, F., K. Tawaha and T. Al-Eleimat, 2004. Determination of hypericin content in *Hypericum triquetrifolium* Turra (Hypericaceae) growing wild in Jordan. Nat. Prod. Res., 2: 147-151.

Barnes, J., L.A. Anderson and J.D. Phillipson, 2001. St John's wort (*Hypericum perforatum* L.): A review of its chemistry, pharmacology and clinical properties. J. Pharma. Pharmacol., 53: 583-600.

Baye, T., Heiko, Becker, C. Sabine and V. Witzke-Ehbrecht, 2005. *Vernonia galamensis*, a natural source of epoxy oil: Variation in fatty acid composition of seed and leaf lipids. Ind. Crops Prod., 21: 257-261.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bombardelli, E. and P. Morazzoni, 1995. *Hypericum perforatum*. Fitoterapia LXVI., 1: 43-58.

Cecchi, G., S. Blasini and J. Castano, 1985. Méthanolyse rapide des huiles en solvant Note de laboratoire. Rev. Fr. Corps Gras., 4: 163-164.

Dobson, G., 2000. Leaf lipids of *Rubes nigrum*: A plant containing 16:3, μ-18:3, β-18:3 and 18:4 fatty acids. Biochem. Soc. Trans., 28: 583-586.

Greeson, J.M., B. Sanford and D.A. Monti, 2001. St John's wort (*Hypericum perforatum*): A review of the current pharmacological, toxicological and clinical literature. Psychopharmacology, 153: 402-414.

Griffiths, G., E. Brechany, F.M. Jakson, W.W. Christie, S. Stymne and A.K. Stobart, 1996. Distribution and biosynthesis of stearidonic acid in leaves of *Borago officinalis*. Phytochemistry, 43: 381-386.

Guil-Guerrero, J.L., F. García-Maroto, M.A. Vilches-Ferròn and D. López-Alonso, 2003. Gamma-linolenic acid from fourteen boraginaceae species. Ind. Crops Prod., 18: 85-89.

Kartnig, T., A. Gruber and H. Sauer, 1989. Comparative phytochemical investigations of *Hypericum* species. Planta Med., 55: 215.

Kitanov, M.K., 2001. Hypericin and pseudohypericin in some *Hypericum* species. Biochem. Syst. Ecol., 29: 171-178.

Özen, H.Ç., M. Bashan, C. Keskin and Z. Toker, 2004. Fatty acid and 3-hydroxy fatty acid composition of two *Hypericum* species from Turkey. Eur. J. Lipid Sci. Technol., 160: 68-70.

Pina, M., J. Graille, P. Grignac, A. Lacombe, O. Quenot and P. Garnier, 1984. Research on *Oenothera* rich in gamma linolenic acid. Oléagineux, 39: 593-596.

Pottier-Alapetite, G., 1979. Flore de la Tunisie., pp: 506-509.

Robson, N.K.B., 2001. Studies in the genus *Hypericum* L. (Guttiferae). 4 (1). Sections 7. Roscyna to 9. *Hypericum sensu* lato (part 1). Bull. Br. Mus. (Nat. Hist.) Bot., 31: 37-88.

Sánchez-Mateo, C.C., B. Prado and R.M. Rabanal, 2002. Antidepressant effects of the methanol extract of several *Hypericum* species from the Canary Islands. J. Ethnopharmacol., 79: 119-127.

Statsoft, 1998. STATISTICA for Windows (Computer program electronic manual). StatSoft Inc.,Tulsa,OK.

Stojanovic, G., R. Palic, C.H. Tarr, C. Reddy and O. Marinkovic, 2003. *n*-Alkanes and fatty acids of *Hypericum perforatum*, *Hypericum maculatum* and *Hypericum olympicum*. Biochem. Syst. Ecol., 31: 223-226.