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Composition of Fatty Acids in TribulusTerrestris L. Collected from Tunisia flora: Its Richness in health beneficial Omegas

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Abstract—*Tribulusterrestris L*. have been used for a long time in many cultures around the world for the prevention and treatment of various disorders. In this study, *T. terrestris* plant grown in the North east of Tunisian flora (Forét Dar Chichou; Birhalloufa) was collected during the ripening stage (June-September). The harvested samples from, roots, leaves and seeds have been dried and extracted. The fixed oil content of the extracts was examined by GC-MS analysis. The main fatty acid components of plant extracts are linoleic acid, oleic acid. Seeds have the highest levels of linoleic acid (66.26 %).

Keywords— Fatty acids, Omega6s, Preventive medecine, Seeds, *Tribulus terrestris.*

I. INTRODUCTION

Secondary metabolites and bioactive compounds from medicinal plants are expected to be more specific, biodegradable and have fewer side effects than synthetic ones. They can be a very good source for developing new and more effective drugs [1], [2]. Natural antimicrobials and antioxidants have many advantages over synthetic ones for the environment and human health. Using derived medicinal plants offer effective therapeutic benefits, and are relatively safer than synthetic alternatives [1], [3].

Therefore compounds derived from these plants can be developed as health supplements or potential medicinal drugs in order to preserve the health of the aging population [4]. Actually many plants that were previously used as natural products, will need to be more analyzed and transformed to meet the demands of consumers.

Tribulus terrestris L. also seems to be one of these plants [1]. *T. terrestris*, known as Gokshur (Sanskrit), Caltrops (English); Gokhru (in Hindi); and Khan-e-khusakkhurd (Urdu), Qutib to (Bedouin language) in several countries [5], [6].

There is only one *Tribulus* species in Tunisia; [2] while 20 species are identified in the world. Extensive research studies have been conducted to prove their biological activity and the pharmacology of its extracts Anticancer [7], antimicrobial [8], antioxidant [9], anti-inflammatory [10], antidiabetic, [5] and immune modulator [6].

In the Tunisian flora, only our last studies had established the anti cancer [11], antioxidant [12] potential and anti leishmanial effect of the *Tribulus* [13]. But, until now we haven't studied its content in fatty acids which exhibit health-promoting benefits in preventing cancer, causing a reduction in body fat, anti inflammatory properties, reducing obesity, and eliminating the severity of atherosclerosis and diabetes [14].

Overall only few reports studied *Tribulus's* fatty acids [10]. Thus, the aim of this study is to carry out the analysis of the fatty acid content in different parts of the plant in particular: seeds, roots and leaves.

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II. MATERIAL AND METHODS

Reagents and standards

All solvents used in our experiments: Tert-Butyl-Methyl Ether (TBME), cyclohexane; KOH; N-methyl-N-trimethylethylsilyl-heptafluorobutyramide (MSHFBA); dihydrocholesterol; chloroform; the homologous fatty acids and sterols rutin and apegenin standards were purchased from Sigma Aldrich (Steinheim, Germany).

Description of plant material

Called "Zorzlaazouza" in the Boukrim region (Cap Bon), this plant begins to grow in April spreading on the ground that is why it is called *Tribulusterrestris,* in June the plant begins to develop small yellow flowers transforming in mid September-October into a seed with 5 very painful thorns when touched, especially during collection.

Collection of Plant Material

Samples of *T.terrestris* (Figure1) were collected during ripening stage from plants cultivated in the experimental station of "Dar Chichou" North east Tunisia;37° 03′ 00″ N 11° 00′ 51″ E altitude 28 m. Plant materiel was identified as mentioned in our last publication [11]. Driedroots, seeds and leaves, were grounded by a mill equipped with a grid whose holes are 1.00 mm in diameter and stocked in plastic bags until chemical analysis that were done in ENSCIACET laboratory.

Fig1.(T.terrestris Grown in the North east of Tunisia)

Lipid extraction

The specimen of leaves dried and powdered were Soxhlet-extracted with cylohexane for 6 h. The extract, concentrated under reduced pressure using a rotary evaporator at 60 °C, was kept in obscurity at 4°C until analysis.

Fatty acids extraction

The FAs extractions, realized according the procedure of Macherey Nagel, by dissolving of 20 mg of oils in 1 ml TBME solvent. Then, 50 µL reagents were added to 100 µL of this solution. This methylation with TMSH was recommended for free acids, chlorophenoxycarboxylic acids, their salts and derivatives as well as for phenols and chlorophenols [(Butte, 1983)] [15] in order to simplify of the sample preparation. Lipids or triglycerides can be converted to the corresponding fatty acid methylesters (FAMEs) by a simple trans esterification. This reaction was very elegant and convenient, because it was just necessary to add the reagent (0.2 M in methanol) to the sample solution. Removal of excess reagent was not required, since in the injector of the gas chromatograph at 250°C pyrolysis to volatile methanol and dimethylsulfide will occur. Due to the high reactivity, complete derivatization was often obtained at ambient temperature. However, heating (10 min at 100°C) in a closed sample vial may be necessary.

III. RESULTS AND DISCUSSION

We plan in this chapter to study and compare fatty acids synthesized by the different parts of the plant (leaves, seeds and roots).

Seeds fatty acids:Analysis of fatty acids composition indicate three groups (table), the major cluster is characterized by linoleic acid (C18: 2n6c), the second group is moderately represented by palmitic (C16: 0), oleic (C18:1n9c) and stearic (C18:0), and the last group is weakly by represented myristic acids (C14: 0), pentadecanoic (C15:0), arachidic (C20:0).

This composition seems to be predominated by the presence of unsaturated fatty acids and especially polyunsaturated fatty acids which represent 66.6% of TFAs.

Two fatty acids characterize the *Tribulus* seed; these are the oleic acids, (C18:1n 9c; omega6) and linoleic (C18: 2n 6c), present with respective levels of 66.26 and 11.21% of AGT. **(Fig2 Chromatogram).**

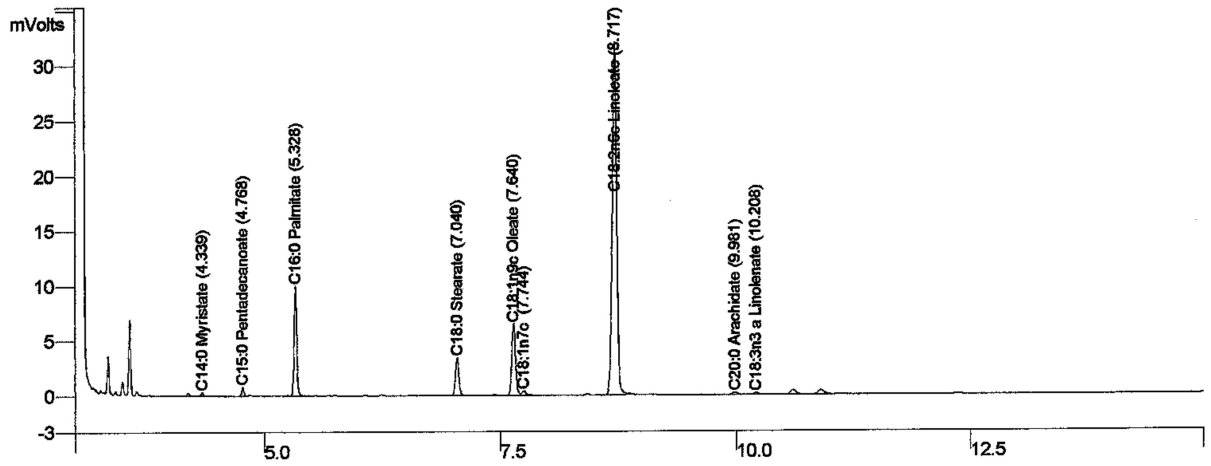

Fig2. Chromatogram Fatty acids content in *T.terrestris* seeds.

When it comes to the leaves, there are two main fatty acids, the linoleic acid (C18:2n 6c) and linolenic acid (C18: 3n 3c); ALA **(Table1)**

Roots fatty acid:We distinguish saturated fatty acids: palmitic (C16:0), stearic (C18:0), arachidic acids (C20:0), and polyunsaturated fatty acids: oleic (C18:1), linoleic (C18:2), acid linolenic (C18:3). The results of **(Table1)** indicate that the root is distinguished by the predominance of unsaturated fatty acids 65.26% of TFAs; polyunsaturated fatty acids constitute the major fraction (51%).

The analysis of the fatty acid content in the different organs of *Tribulus* showed that it is predominated by unsaturated fatty acids. However, qualitative differences and quantitative can be noted. **(Table1).**

The content of Fatty acid in the seeds differs from that of the leaves and the root. Indeed, in the leaves, we noted a significant contribution of essential fatty acids from the family (n-3): alpha-linolenic acid (ALA), while the major fatty acids in the root are linoleic and palmitic acids.

The seeds are distinguished by significantly higher proportions of fatty acids compared to the other two organs and include a source of essential fatty acids of the family (n-6); in particular linoleic acid is known for its nutritional value characterizing the lipids of reserves in oil seeds.

The human body can synthesize all the fatty acids it needs, with the exception of two: Linoleic acid (LA), an omega-6 fatty acid, and alpha-linolenic acid (ALA), an omega-3 fatty acid. These must be provided by nutrition and are a part of the acids so-called “essential” fats. These two categories of fatty acids are necessary for growth and cell repair, but can also be used to synthesize other fatty acids (such as arachidonic acid (AA), which is synthesized from linoleic acid).

Being an essential fatty acid, omega-6 is one of the essential nutrients for the proper functioning of the body [16]. If the dose is respected, omega-6, just like other fatty acids belonging to their category, acts as a message transmitter. They essentially play a role in vasomotion at the level blood circulation, in the assembly of blood platelets causing clots and inflammation [17]. These properties allow them in particular to stimulate the body's immune system, protect the arteries in the heart and act as anti-inflammatories. Synthesis of eicosanoids by arachidonic acid (AA) also promotes wound healing and alleviates allergic reactions [18], [19].

Table 1: Fatty acids content in different parts of the *T.terrestris*

Fatty acid	**Saturation**	**Seeds**	**Leaves**	**Roots**
Myristic acid	C14:0	0,360 ± 0,01	5,36 ± 0,06	n.d
Pentadecanoic acid	C15: 0	0,77 ± 0,05	n.d	n.d
Palmitic acid	C16: 0	11,84 ± 0,03	29,5 ± 0,03	15,92 ± 0,03
Stearic acid	C18: 0	5,52 ± 0,02	6,72 ± 0,02	6,12 ± 0,02
Oléic acid	C18:1n 9c	11,31 ± 0,07	5,19 ± 0,07	15,26 ± 0,07
Vaccenic acid	C18: 1n 7c	0,74 ± 0,01	n.d	n.d
Linoleic acid	C18: 2n 6c C18: 3n3a	66,26 ± 0,09 0,34 ± 0,07	16,66 ± 0,03	48,37 ± 0,23 1,63 ± 0,07
Arachidic acid	C20: 0	0,47 ± 0,05	1,62 ± 0,05	0,92 ± 0,05
Saturated Fatty acid	X	18,96	43,2	22,96
Insaturated Fatty acid	X	77,91	38,29	65,26
AL/ALA AGI/AGS	X	194,34	1,01	29,67

Omega-6s are used in medicine to strengthen the body's natural defenses against pathogens. Thanks to their anti-inflammatory properties, they can be used to treat inflammation caused problems such as rheumatoid arthritis or even arthritis. Stress and depression-related disorders can also be treated with omega-6 if these are of an inflammatory origin [18]. Moreover, omega-6s heals skin problems. Their healing effect is particularly effective in treating wounds. They can also overcome acne, rosacea, eczema and psoriasis. On the other hand, their interaction with omeg3 helps prevent coronary artery disease and obesity. This balance can reduce premenstrual discomfort and relieve pain due to fibro-cysts breasts. Thanks to their virtues, omega-6 can be used in treatments of prevention and cure of many diseases: Diabetes, cancer, muscle diseases, hormonal disorder. So far no side effects have been observed for omega-6. It should be noted, however, that their beneficial action depends solely from their interaction with other essential fatty acids, more particularly Omega 3. If the ratio between these two nutrients is unbalanced, the proper functioning of the organism is disturbed causing diseases.

Researchers agree that saturated fat should not exceed 10% of our total caloric intake. But what type of fat can we replace them with to keep a good cardiovascular health? Opinions currently differ within the scientific community: some give the advantage to omega-6, [20], [21] others to omega-9 [22]. Although the experts do not see eye to eye on the subject, the authors of a summary drawn up in [22], conclude that omega-9s are one step ahead of omega-6s. Both types of fat have positive effects, on “bad” cholesterol (LDL) and on total cholesterol levels, however omega-9s have additional beneficial effects on “good”

cholesterol levels (HDL), on hypertension and insulin sensitivity [22]. Moreover, as the omega-6/omega-3 ratio is too high in the modern diet, this gives another benefit to omega-9.

IV. Conclusion

In this study, the ethno botanical study of *T. terrestris*, which was collected from Tunisia flora, was conducted. Different organs of the plant were investigated to quantify and identify fatty acids composition considering the effective use of traditional medicine. The results showed the presence of different bioactive ingredients of *T. terrestris* in varying amounts according to the part of the plant studied. These compounds are known to be responsible for the antioxidant and anti cancer potential of plants. In this regard, the plant is a powerful natural source of bioactive compounds and may be beneficial in the preventive of free radical pathologies. Omega 3 fatty acids α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid; linoleic acid (major component). The plant has an important profile as it contains omega 6-9-7 fatty acids, including oleic acid, one of the omega 9 fatty acids. The data obtained in this study will help to understand the characteristics and advantages of this traditional herb used in ayerveda therapy and will be useful in the future to formulate food supplements.

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