Advances in Environmental Biology, 10(1) January 2016, Pages: 16-22

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Total Phenolics Contents, Flavonoïds Contents, and Fatty Acids Compositions in *Thymelaea hirsuta* L. Aerial Parts, Grown in Western Algeria

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Received 28 December 2015; Accepted 28 January 2016; Available online 24 February 2016

ABSTRACT

The Algerian flora contains a wide variety of plant species with potential to be used in medicinal applications. This study was carried out in order to quantify and to compare the fatty acids compositions, the phenolics and the flavonoïds contents of *Thymelaea hirsuta* leaves, flowers and twigs, and to determine the relationship that may exist between beneficial fatty acids and phenolics. Phenolic compounds and flavonoïds were extracted with methanol:water (80:20; v/v), and quantified photometrically. Methylated fatty acids were determined qualitatively and quantitatively by GC/MS. The results showed that leaves contain the higher concentration of total phenolics (19.83±0.42mgGAE/g DW) than flowers (16.78±0.67mgGAE/g DW) and twigs (6.17±0.82mgGAE/g DW). The flavonoïds contents were estimated at 5.00±0.17 and 5.17±0.06mgQE/g DW in leaves and flowers, respectively, and just 1.30±0.10mgQE/g DW in twigs. In addition, GC/MS analysis revealed 25 different fatty acids. The most dominant components were Oleic acid (Leaves: 15.56±0.12; flowers: 12.53±0.09; twigs: 12.29±0.05%), Adrenic acid (Leaves: 16.35±0.17; flowers: 5.03±0.08; twigs: 7.87±0.05%) and Palmitic acid (Leaves: 33.02±0.06; flowers: 33.02±0.06; twigs: 30.94±0.04%). The sum of unsaturated fatty acids (MUFAs+PUFAs) are most accumulated in leaves (38.14±0.04%), compared to flowers (29.98±0.03%), and twigs (28.23±0.04%). contents of UFAs, among plant parts, are positively correlated with phenolics (r = 0.79) and flavonoïds contents (r = 0.61), as well as omega3 (phenolics: r = 96.42%, Flavonoids: r = 86.7%). Findings of the study suggested that leaves are a better source of phenolic compounds which can play an effective role against unsaturated fatty acids peroxidization. They could be applied in pharmaceutical and medicinal applications, as well as natural food preservatives.

KEYWORDS: Total phenolic compounds, Flavonoïds, Fatty acids, *Thymelaea hirsuta*, Aerial parts.

INTRODUCTION

Nowadays, in many countries around the world, aromatic and medicinal plants have received a rising attention [40]. In Algeria, numerous researchers were interested to study and explore the bioactive constituents of their secondary metabolism, for allowing their extended use in folk medicine [6,39].

Thymelaea hirsuta (*T. hirsuta*), commonly known as "Methnane" in Algeria, is an evergreen shrubs belonging to the flowering plant family "Thymelaeaceae", which is native to the Mediterranean region, north of central Europe and east of central Asia. This plant is used in folk medicine as antidiabetic [7], purgative to treat the common cold disease in veterinary medicine, hypertension and as an antiseptic [21]. In western Algeria, *T. hirsuta* aerial part is claimed to treat human skin infectious, and it has proven its effectiveness.

Few authors have been interested to the biological properties of *T. hirsuta*. Its aerial part evaluated has been reported to be effective inhibitors of α-glucosidase *in vivo* [8,1]. Hypoglycemic and antidiabetic of this plant extracts were also highlighted [14]. It exhibited significant antitumor growth inhibition of human colon cancer HT-29 cells [2], and antimelanogenesis effect on B16 murine melanoma cells, using leaves extract. Antibacterial and antifungal effects of *T. hirsuta* extracts were also reported [37,4].

In addition, previous studies demonstrated that the aerial part of *T. hirsuta* showed a potential antioxidant resource, mainly due to their huge of phenolics and flavonoids contents (secondary metabolites) which are considered as the most important phytochemicals [2,37,3]. These natural bioactive compounds play beneficial effects in preventing cellular damages from oxidative stress caused by free radicals. It's largely known that free radicals are the causal agents of several diseases [34,9]. Besides, phenolic compounds are also considered as conservation factors and allow increasing the nutritional quality of foods such as lamb meat fatty acids [13].

All these works were focused on secondary metabolites. However, in our knowledge, there is no data in the literature on *T. hirsuta's* primary metabolites composition. Certain fatty acids, not synthesized by human body, are bioactive compounds and play an important role in therapeutics, such as cardiovascular disease and cancer [38,19]. Multiple studies have identified and confirmed that Omega-3 fatty acids can be beneficial for skin health such as psoriasis [24,5].

In order to provide additional scientific information about bioactive compounds available in this medicinal plant and to justify its traditional use, this study was conducted to evaluate total phenolic compounds and flavonoïds contents and the fatty acids profiles (for the first time) from the three parts of the plant (leaves, flowers and twigs), separately, and to determine the relationship that may exist between beneficial fatty acids and phenolics.

MATERIALS AND METHODS

Plant collection:

The fresh aerial parts of *Thymelaea hirsuta* were collected from Jdiouia region, Algeria (Lat. 35°55'33" N, Long. 0°49'23" E), in April 2012, at an altitude of 82m above the sea level. Leaves, flowers and twigs were separated and left drying at room temperature. After drying, they were crushed and ground to obtain a finely divided powder.

Extracts preparation:

One gram of each explant was isolated for preparation of the aqueous methanolic extract, while the rest was used for lipids extraction. Phenolics compounds were extracted exhaustively with 80% methanol in distilled water, using an Ultra turax, as described by Kim et *al*. [23] with slight modifications. Dry materials were extracted, three times, with 10mL of aqueous methanol 80% (v/v), during 20mn at 2000 rpm. Filtrates were mixed and vacuum filtered through Wattman paper ≠ 1. The mixture was adjusted at 20mL, and stored at -20°C until further use.

Determination of total phenolics content:

Total phenolic compounds contents of extracts were estimated by the method described by Milliauskas et *al*. [30], using Folin-Ciocalteu reagent. Absorbances were measured spectrophotometrically at 765nm. Based on the calibration curve of Gallic acid, quantification was expressed as Gallic acid equivalents (mg GAE/g DW).

Determination of total flavonoïds content:

The contents of flavonoïds in aqueous methanolic extracts were determined using aluminum chloride colorimetric method [11]. Absorbances at 415nm were read after 10min against a blank prepared without aluminum chloride. Results of flavonoïds contents were calculated with respect to Quercetin standard curve, and expressed as Quercetin equivalents (mg QE)/g DW).

Fatty acids extraction and methylation:

Total lipids from *Thymelaea hirsuta* leaves, flowers and twigs were extracted according the method of Folch et *al*. [15]. Samples were homogenized with Folch reagent (chloroform/methanol: 2/1: v/v) for 2min. After filtration and stirring, two phases were separated into separatory funnels, using sodium chloride 73%. A vacuum evaporator was used to dry the lipid extracts by evaporation of chloroform, and the percentage result lipids was calculated per 100g sample dry weight (w/w).

MEFA was prepared following the procedure of Morrison and Smith [32]: Briefly, 20mg of dried lipids were initially mixed with 1mL of NaOH 0.5N (prepared with methanol) into a closed test tube, at 70° C for 15min. Methylation was carried out by adding 1mL of $BF_3$14% (in methanol) at 70° C for 10min. Finally, the purified methyl esters with dH_2O and petrol ether were concentrated under nitrogen stream and stored at -20° C, for GC–MS analysis.

GC–MS analysis:

Methyl esters fatty acids were analyzed using Perkin Elmer Autosystem XL gas chromatograph, equipped with a flame ionization detector (hydrogen air), an automatic sample changer, and a polar silica capillary column (30m x 0.25mm internal diameter, Supelco). Stationary phase is a mixture of 80% cyanopropylphénylsiloxane and 20% biscyanopropyl. Injector and detector temperatures were 220° C and 240° C, respectively. Based on FA methyl standards, Fatty acids detected were known, and quantified by an internal standard (C17:0). Fatty acids were identified as a percentage of total FAs.

Statistical analysis:

All measurements were performed in triplicate and expressed as mean ± standard deviations. Statistically, significant data were compared using ANOVA and Student-Neuman-Keuls test, and *p*-values of less than 0.05 were considered statistically significant. Pearson's correlation coefficients were performed to compare the correlations between parameters.

RESULTS AND DISCUSSION

Phenolics and flavonoïds contents:

Total phenolics contents and total flavonoïds contents (Tab. 1) were determined from the calibration curves of Gallic acid (Y = 0.005x, R^2= 0.992), and Quercetin (Y = 530.5x + 0.018, R^2 = 0.999), respectively. In the current study, the TPC (F_2 = 285.029; $p \leq 0.05$) and flavonoïds (F_2 = 992.368; $p \leq 0.05$) contents in *Thymelaea hirsuta* extracts vary significantly according to the studied plant part. Indeed, the highest total phenolics value was founded in leaves aqueous methanolic extract (19.83±0.42mg GAE/g DW), against flowers (16.78±0.67mg GAE/g DW) and twigs (6.17±0.82mg GAE/g DW). However, flavonoïds seem to show equal content in leaves (5.00±0.17mg QE/g DW) and flowers (5.17±0.06mg QE/g DW). These compounds are, relatively, less available in twigs (1.30±0.10mg QE/g DW). Results of this study are in disagreement with the findings of Amari et *al*. [3], who showed that the flowers aqueous extract are the richest in total phenolics and flavonoïds contents. This is mainly due to the abundance of mucilage in leaves [16], reducing, consequently, the filtration of searched compounds. These quantities are also different from those reported by Akrout et *al*. [2] and Trigui et *al*. [37]. These conflicting data have been attributed to the extraction process [22,18], and the solvent used [31,20,35]. Several authors confirm that differences in phenolics and flavonoïds contents were obtained according to differences attributed to solvents polarities [10].

Table 1: Contents of total phenolics and flavonoïds in *T. hirsuta* aerial parts.

Aerial Parts	Total phenolics (mgGAE/g DW)	Total flavonoïds (mgQE/g DW)
Leaves	19.83±0.42a	5.00±0.17a
Flowers	16.78±0.67b	5.17±0.06a
Twigs	6.17±0.82c	1.30±0.10b

Data are expressed as mean±SD. mgGAE/g DW: mg of Gallic acid equivalent per g of dry weight; mgQE/g DW: mg of Quercetin equivalent per g of dry weight. Different letters in the same column indicate significantly different values ($P< 0.05$).

Fatty acids composition:

The fatty acids profiles of *Thymelaea hirsuta* aerial parts are summarized in Table 2 and 3.

In our knowledge, the fatty acids compositions of *Thymelaea hirsuta* aerial parts were never reported.

Quantification and qualitative analysis of *Thymelaea hirsuta* lipids showed differences in fatty acids, related to the plant part studied.

Percentages of total lipids in leaves and flowers appear to be similar (2.65 vs 3%); a slight difference of 0.35% was observed. However, twigs, less rich, recorded 1.97%; value much lower than those noted above (Tab. 2).

Saturated fatty acids (SFAs = capric, lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric acids) detected in leaves, flowers and twigs are present with different percentages: 61.86 – 70.02 – 71.77%, respectively (F_2 = 4353.24; p = 0). Specifically, palmitic acid (C_{16}) is the most predominantly. Its availability is more observed on leaves (46.79%), compared to flowers (33.02%) and twigs (30.94%). However, arachidic acid is shown, especially, by twigs (30.75%) and flowers (14.82%). Comparatively, stearic acid, less represented, records 10.51 – 8.35 and 6.81% respectively in flowers, leaves and twigs. The other fatty acids are either absent or insignificant.

In parallel, mono-unsaturated fatty acids (MUFAs) represented by hexadecenoic, palmitoleic , oleic, trans-vaccenic and eicosenoic acids are mainly observed in flowers (19.87%). However, leaves and twigs noted 15.61 and 14.99% (F_2 = 20108.06; p = 0), respectively; insignificant difference. The most abundant MUFA was oleic acid (C_{18}:1) and the one which showed the highest value among them. It was found to be 15.56% in flowers,

followed by leaves (12.53%) and twigs (12.29%). It has been reported that only oleic acid (C_{18}:1, cis-9) has antibacterial activity against range of Gram-positive bacteria and can eliminate *Staphylococcus aureus* through cell wall disruption [12], from skin which constitutes the most common sites for its development [29]. Smaller proportions of trans-vaccenic acid were also recorded (Leaves: 1.72%; Flowers: 1.95%; Twigs: 0.92%). Thus, hexadecenoic acid, absent in leaves and Palmitoleic acid are less than 1%. Regarding to mono-unsaturated long chain fatty acids, Eicosenoic acid amounted in the range from 0.32 to 0.65% is existing in all aerial parts. However, nervonic acid, present in small amounts, was exclusively detected in flowers (0.15%).

Table 2: Fatty acids compositions of *T. hirsuta* aerial parts.

Fattyacid	Squelettons	Leaves	Flowers	Twigs
TL%		2.65±0.09	3.00±0.11	1.97±0.06
Capric acid	C_{10}:0	0.39±0.01	0.18±0.00	0.12±0.00
Lauric acid	C_{12}:0	0.25±0.00	0.32±0.01	0.20±0.00
Myristic acid	C_{14}:0	4.89±0.02	2.36±0.01	1.94±0.00
Palmitic acid	C_{16}:0	46.79±0.04	33.02±0.06	30.94±0.04
Hexadecenoic acid	C_{16}:1 n-9	0.00±0.00	0.46±0.00	0.74±0.00
Palmitoleic acid	C_{16}:1 n-7	0.74±0.02	0.93±0.03	0.72±0.03
Stearic acid	C_{18}:0	8.35±0.06	10.51±0.04	6.81±0.06
Oleic acid	C_{18}:1 n-9c	12.53±0.09	15.56±0.12	12.29±0.05
transVaccenic acid	C_{18}:1 n-7	1.72±0.02	1.95±0.02	0.92±0.02
transLinoleic acid	C_{18}:2 n-6t	0.14±0.00	0.14±0.00	0.17±0.00
cis Linoleic acid	C_{18}:2 n-6c	0.39±0.00	0.30±0.00	0.13±0.00
γ-Linolenic acid	C_{18}:3 n-6	0.12±0.01	0.16±0.00	0.11±0.00
α-Llinolenic acid	C_{18}:3 n-3	0.23±0.00	0.76±0.01	0.22±0.01
α-Parinaric acid	C_{18}:4 n-3	0.00±0.00	0.17±0.00	0.09±0.00
Arachidic acid	C_{20}:0	0.20±0.01	14.82±0.13	30.75±0.11
Eicosenoic acid	C_{20}:1 n-9	0.53±0.01	0.65±0.02	0.32±0.00
Eicosadienoic acid	C_{20}:2	0.29±0.01	0.76±0.01	0.09±0.01
Dihomo-gamma-linolenic acid	C_{20}:3 n-6	0.00±0.00	0.15±0.00	0.00±0.00
Arachidonic acid	C_{20}:4 n-6	0.00±0.00	0.00±0.00	3.04±0.02
Eicosatrienoic acid	C_{20}:3 n-3	4.45±0.03	1.82±0.02	1.44±0.02
Eicosatetraenoic acid	C_{20}:4 n-3	0.41±0.01	0.27±0.01	0.10±0.03
Behenic acid	C_{22}:0	0.44±0.00	8.21±0.12	0.12±0.00
Adrenic acid	C_{22}:4 n-6	16.35±0.17	5.03±0.08	7.87±0.05
Lignoceric acid	C_{24}:0	0.00±0.00	0.00±0.00	0.26±0.02
Nervonic acid	C_{24}:1	0.00±0.00	0.15±0.01	0.00±0.00

Values are mean±SD (n=3).

Table 3: Saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, ω6 and ω3 of *T. hirsuta* aerial parts.

FAsub-class (%)	Leaves	Flowers	Twigs
SFAs	61.86±0.16c	70.02±0.12b	71.77±0.08a
MUFAs	15.61±0.03b	19.87±0.02a	14.99±0.03c
PUFAs	22.53±0.02a	10.11±0.02c	13.24±0.03b
UFAs	38.14±0.04a	29.98±0.03b	28.23±0.04c
ω6	17.15±0.03a	5.78±0.01c	11.31±0.02b
ω3	5.09±0.01a	3.58±0.01b	1.84±0.00c

SFAs: Saturated Fatty acids, MUFAs: Monounsaturated, PUFAs: Polyunsaturated fatty acids, UFAs: Unsaturated fatty acids.
Values (mean±SD, n= 3) in the same line followed by a different letter are significantly different ($p< 0.05$).

The most important point is the proportion of polyunsaturated fatty acids (PUFAs) estimated, it amounted to 22.53% in leaves. This value is two times higher (F_2 = 150452.7; p = 0) than that observed on flowers (10.11%) and twigs (13.24%). They are prime targets of ROS; more fatty acid is unsaturated and also it is likely to be peroxidized [17]. The ω-3 (F_2 = 89478.63; p = 0) and ω-6 (F_2 = 202493.5; p = 0) are prevalent in leaves (5.09% - 17.15%) compared to the flowers (3.58% - 5.78%) and twigs (1.84% - 11.31%). Indeed, these components belong to the series of fatty acids which play an important role in the development and maintenance of various organs, especially brain, and are involved in the prevention of various diseases such as cardiovascular diseases, psychiatric, neurological, dermatological and rheumatologic disorders [28,27,26]. Regarding to skin, Omega-3 polyunsaturated fatty acids (n-3 PUFA) are bioactive compounds showing a potential to protect the skin from ultraviolet radiation injury [33], and their deficiency causes skin problems including atopic eczema, acne and psoriasis [36].

Individually, linoleic (trans and cis), linolenic (α and γ) and α-parinaric acids are less available throughout the aerial parts of *Thymelaea hirsuta*. In addition, PUFAs long chain represented by eicosadienoic and Eicosatetraenoic acid exist also in small quantities. While,dihomo-gamma-linolenic and Arachidonic acids are exclusively obtained in flowers and twigs respectively. However, Eicosatrienoic acid seems to be more

accumulated in leaves. In contrast, adrenic acid was the major compound of PUFAs present, ranging from 5.03%, 7.87% to 16.35%, in the flowers, twigs and leaves, respectively.

It is important to signal that a proportional relationship was obtained between variations of these data and changes in total phenolics, according to the plant parts (Correlation coefficient r=0.79). Indeed, high phenolics content has been recorded on the plant part (leaves) having accumulated the highest percentage of unsaturated fatty acids (MUFAs+PUFAs), and the low values of phenolics were determined on flowers followed by twigs which showed a reduction in UFAs percentages (F_2 = 47438.93; p = 0) (Tab. 3). The positive correlation was also observed with total flavonoïds (r=0.61). In addition, a strong positive correlation were obtained between total phenolic compounds and Omega3 (r=96.42%), followed by flavonoids (r=86.70%).

These remarks seem to indicate that phenolics are necessary to protect UFAs against peroxidization. Our finding is in agreement with several reports. For example, Kireçci et *al*. [25] had suggested that antioxydants of *Laurus nobilis*, *Malva sylvestris* and *Arum italicum* methanolic extracts have a protective effect on polyunsaturated fatty acids.

Conclusions:

The current study was the first to identify the methylated FAs, available in *Thymelaea hirsuta* aerial parts. It confirms the presence of good positive correlation between phenolics and unsaturated fatty acids, including Omega3. The study findings suggest that leaves are the better source of phenolics which can be efficient against unsaturated fatty acids peroxidization. This work will be helpful to explore the active compounds in the pharmaceutical research field, especially the treatment of skin infection and as food preservatives. However, further studies need to be conducted on the evaluation of the antioxidant *in vitro* experiments against UFAs, and to investigate their clinical effects in the human body.

ACKNOWLEDGEMENTS

The authors thank Pr. Bouderoua K. and Dr. El-Affifi M., researchers at Abdelhamid Ibn Badis University (Mostaganem, Algeria), Laboratory of Food Technology and Nutrition, for providing helpful comments on the manuscript.

Conflict of interests:

The authors have not declared any conflict of interests.

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