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ARTICLE

Characterization of Fatty Acids Profile in Some Moss Species in Syria

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Abstract: Most studies on bryophyte species in Syria were carried out for bryofloristic purposes; however, research on biochemical contents is still limited. The present study aimed to determine the fatty acids (FAs) content in four moss species (*E. praelongum*, *R. murale*, *B. erythrorrhizon* and *A. riparium*) widely spread in certain regions in Syria using GC-MS; fatty acids extraction by *n*-hexane was assisted by ultrasound to increase the extraction yield. The four moss species contained very long chain-polyunsaturtaed fatty acids (vl-PUFAs) larger than C18, like 20:5 (6–12%) and 20:4 (1–13%), and they also contained very long chain-saturated fatty acids (vl-SFAs), such as 24:0 (1–1.4%) and 26:0 (0–0.5%). FAs concentrations were highly different among the studied bryophytes. Palmitic acid 16:0 was identified as the main component in both *E. praelongum* and *R. murale*, while linoleic acid 18:2 and linolenic acid 18:3 constituted the main components in *B. erythrorrhizon* and *A. riparium*. FAs analysis showed a very high content of vl-PUFAs ranging between 12% and 24% for linoleic acid 18:2, from 19% to 21% for linolenic acid 18:3 and from 1% to 13% for arachidonic acid 20:4. Statistical analysis using Neighbor joining algorithm showed two clusters according to the original regions and confirmed the role of environmental factors on fatty acids content. The present study aimed to underline the importance of bryophytes as nutritionally rich species for further appropriate utilizations.

Keywords: Fatty acids, GS-MS, Mosses, Syria.

Introduction

Bryophytes are the second largest group in the plant kingdom with about 25000 species, including mosses, liverworts and hornworts. They can be found in all ecosystems, from dry desert to humid rainforest, from hot tropical area to the cold Arctic, with exception of the marine ecosystems[1,2]. Bryophytes are taxonomically placed between algae and pteridophytes. They are generally small plants that grow on different substrata, such as soils, rocks, trees, …etc.[3–5]. Bryophyte studies conducted so far showed that these plants were used ethnobryologically and, in different countries, as medicinal plants to treat wounds and burns due to their large content of biologically active compounds[6–10]. Besides, using bryophytes as a food source was limited to periods of famine. Around the pole, bryophytes are widely used to feed animals[11,12].

When compared to algae and higher plants, the phytochemistry of bryophytes is still poorly understood and has been neglected for a long time, possibly because they are morphologically small and it is also difficult to collect pure samples after elimination of all accompanying materials (e.g. dead-tree leaves, soil, twigs and even small animals)[13–16].

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In the last few years, bryophytes were reported to contain a large number of compounds showing biological activity[17–19]. Different fatty acids (FAs) and other organic compounds such as terpenoids, flavonoids, lipids, sterols…etc., were obtained as potentially significant chemical compounds of bryophytes[20–23].

FAs are widely distributed in nature and their importance as nutritional substances and metabolites was clearly proved. They have important physiological and biological functions[24–26]. Lipids and FAs in algae, mosses and some higher plants have been analyzed to obtain a better understanding of their chemotaxonomic relevance. Mosses lipids contain, besides common FAs, FAs with 4 and 5 double bonds, such as arachidonic acid (20:4, ω-6) and eicosapentaenoic acid (20:5, ω-3). None of these FAs are found in higher plants[26,27]. In addition, the FAs fingerprint is characterized by a very high content of polyunsaturated fatty acids (PUFAs)[28] and the distribution of FAs is used as a criterion of classification in bryophytes[29,30].

Environmental factors are keys to enhance PUFAs production. The content of ω-3 and ω-6 FAs is affected by many factors, such as temperature, light, pH and nutrition. Low-temperature treatments in some moss species were shown to increase the ω-3 PUFAs content and decrease that of ω-6 PUFAs, although growth deficits were also observed[31]. High contents of PUFAs are important for bryophytes to survive under low temperature and harsh environment, where some bryophytes can even survive at −14 ^{o}C[31].

Previous work suggested the unique presence of some FAs in mosses with high content of vl-PUFAs, especially C18 and C20. Differences in FAs contents between families may have chemotaxonomic significance[24,29]. The present study aimed to characterize FAs profiles in four bryophyte species (*E. praelongum, R. murale, B. erythrorrhizon* and *A. riparium*) grown abundantly in Latakia and Al-Zabadni in Syria.

Materiales and Methods

The four moss species investigated in this study (*E. praelongum, R. murale, B. erythrorrhizon* and *A. riparium*) were collected from Latakia and Al-Zabadani in Syria in February 2021. The species were classified according to the morphological characteristics (sporophyte: capsule, seta length; gametophyte: leaves (length and width at the base, middle and apex), shape of cells and their dimensions at the base, middle and apex of leaf, length and width of the main rib (costa), margins of leaf) by comparing them with the taxonomic key to mosses of the Middle East and Africa[32,33]. Moss samples were carefully selected and cleaned; they were dried in oven at 40 °C until constant mass. The dried samples obtained were ground in a mill, 1.00 g of each bryophyte sample was weighed into 250-mL bottles, 100 mL of *n*-hexane was added, then treatment with ultrasound (100 W) in an ultrasound bath (Digital Ultrasonic Cleaner) was conducted for 3 h at a constant temperature of 25 °C (temperature was regulated by regular addition of cold water to keep it constant). The bottles were shaken in a shaker (JSR, JSSI-100C) for 72 h at 140 rpm/min. Extracts were filtered and stored at 4 °C until analysis[34]. After removal of the solvent by rotary evaporator, the crude extract was dissolved in 1 mL *n*-hexane. Then, 5 mL KOH solution (1.0 M) in methanol was added to 100 µL of the extract solution. The final solution was vortexed, filtered and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS)[24].

Agilent Technologies 7890A GC system was used with capillary HP-5ms (5%-Phenyl)-methylpolysiloxane column (30 m × 250 µm × 0.25 µm). Helium was used as a carrier gas at an initial flow of 1.2 mL/min. The oven temperature was kept at 100 °C for 1 min, then increased in segments as follows: 1) to 160 °C at a rate of 40 °C/min, 2) then directly to 180 °C at a rate of 2.5 °C/min, 3) then directly to 185 °C at a rate of 2 °C/min and remaining constant at 185 °C for 8 min, 4) then to 195 °C at a rate of 5 °C/min, 5) then directly to 230 °C at a rate of 10 °C/min and remaining constant at 230 °C for 10 min, 6) then to 250 °C at a rate of 20 °C/min, 7) then directly to 300 °C at a rate of 40 °C/min and remaining constant at 300 °C for 1 min. The total run time was 39.75 min, and the injection volume was 1 µL at a split ratio of 20:1. The mass spectrometer was operated in the electron impact mode at an ionization energy of 70 eV from 35 Da to 650 Da; the ion source temperature was thereby 230 °C. The

retention time (RT, min) and the MS fragmentation patterns of the known compounds were compared with literature. All peaks were quantified by peak area, then peak areas of FAs were determined after baseline correction and normalized to the total peak area of all FAs as percentage.

Statistical analysis for Neighbour Joining clusters was carried out by means of PAST (4.03) software.

Results

All four species were classified according to the classification keys presented in the experimental part. The FAs yields were 1.19%, 1.82%, 2.41% and 2.63% for *Eurhynchium praelongum* (Hedw) Schimp, *Rhynchostegium murale* (Hedw) Schimp, *Brachythecium erythrorrhizon* Schimp and *Amblystegium riparium* (Hedw) Schimp, respectively. High amounts of vl-PUFAs > C18 are thereby contained, like arachidonic acid 20:4 and eicosapentaenoic acid 20:5, as well as smaller amounts of vl-SFAs, such as tetracosenoic acid 24:0 and hexacosanoic acid 26:0. The extracted FAs included 19 different FAs as indicated in Table 1. Details concerning GC-MS results and the different percentages of SFAs and PUFAs are presented in Tables 1 and 2.

Table 1. Results of fatty acids analysis by GC-MS.

Peak No.	Fatty acid	IUPAC name	Common name	Retention time, min	*E.pr*[1]	*R.mu*[2]	*B.er*[3]	*A.ri*[4]
1	14:0	Tetradecanoic acid	Myristic acid	8.244	0.73	0.5	0.42	0.79
2	16:1 (7-Z)	7-Hexadecenoic acid	Palmitoleic acid	12.091	1.49	1.06	0.66	0.97
3	16:0	Hexadecenoic acid	Palmitic acid	12.717	25.79	20.02	17.39	19.3
4	18:3 (6Z,9Z,11E) ω-6	(6Z,9Z,11E)-Octadecatrienoic acid	γ-Linolenic acid	17.835	0.37	0.5	0.26	0.68
5	18:2 (9-12-Z) ω-6	9-12- Octadecadienoic acid	Linoleic acid	18.554	12.44	17.16	20.45	24.15
6	18:3 (9-12-15- Z) ω-3	9-12- 15-Octadecadienoic acid	Linolenic acid	18.889	20.14	19.14	21.04	20.25
7	18:1	Octadecenoic acid	-	19.085	0.18	0.78	0.72	0.74
8	18:0	Octadecanoic acid	Stearic acid	20.128	4.67	2.81	6.74	3.95
9	20:4 (5-8-11-14-Z) ω-6	5-8-11-14-Eicosatetraenoic acid	Arachidonic acid	25.202	1.81	13.36	13.52	11.32
10	20:5 (5-8-11-14-17-cis all) ω-3	cis-5-8-11-14-17-Eicosapentaenoic acid	-	25.381	11.58	6.41	12.77	11.52
11	20:3 (8-11-14-Z) ω-6	8-11-14-Eicosatrienoic acid	-	25.644	11	1.67	1.41	0
12	20:4 (8-11-14-17-Z) ω-3	8-11-14-17-Eicosatetraenoic acid	-	25.8	5.21	0.14	0.39	0.86
13	20:2 (11-14-Z)	11-14-Eicosadienoic acid	-	26.048	0	0.28	0.27	1.41
14	20:1 (9-Z)	9-Eicosanoic acid	-	26.133	0.05	1.45	0	0.28
15	20:0	Eicosanoic acid	Arachidic acid	26.644	0.41	0.44	0.48	0.4
16	22:1	Docosenoic acid	Erucic acid	30.451	0.27	9.45	0	0.03
17	24:1 (15-Z)	15-Tetracosenoic acid	Nervonic acid	36.977	0.15	0.85	1.25	0.7
18	24:0	Tetracosenoic acid	Lignoceric acid	37.598	1.19	1.35	1.45	1.02
19	26:0	Hexacosanoic acid	-	39.417	0	0.52	0.03	0.06

E.pr[1] (*Eurhynchium praelongum*), *R.mu*[2] (*Rhynchostegium murale*), *B.er*[3] (*Brachythecium erythrorrhizon*) and *A.ri*[4] (*Amblystegium riparium*).

Palmitic acid 16:0 (25.79%) was found to be the main component in *E. praelongum*, followed by linolenic acid 18:3 (20.14%), linoleic acid 18:2 (12.44%), eicosapentaenoic acid 20:5 (11.58%) and eicosatrienoic acid 20:3 (11%). The main compounds in *R. murale* are palmitic acid 16:0 (20.02%), linolenic acid 18:3 (19.14%), linoleic acid 18:2 (17.16%), arachidonic acid 20:4 (13.36%) and erucic acid 22:1 (9.45%). However, *A. riparium* and *B. erythrorrhizon* showed different proportions of FAs. The main FAs in *B. erythrorrhizon* are linolenic acid 18:3 (21.04%), linoleic acid 18:2 (20.45%), palmitic acid 16:0 (17.39%), arachidonic acid 20:4 (13.52%) and eicosapentaenoic acid 20:5 (12.77%). *A. riparium* contained linoleic acid 18:2 (24.15%), linolenic acid 18:3 (20.25%), palmitic acid 16:0 (19.3%), eicosapentaenoic acid 20:5 (11.52%) and arachidonic acid 20:4 (11.32%).

Neighbour Joining analysis of the content of fatty acids showed two clusters. Each one contains two moss species, which are clearly separated according to the original region: *E. praelongum* and *R. murale*, which were collected in Latakia, belonged to the same cluster; however, *B. erythrorrhizon* and *A. riparium* from Al-Zabadani were classified in the other one (Figure 1).

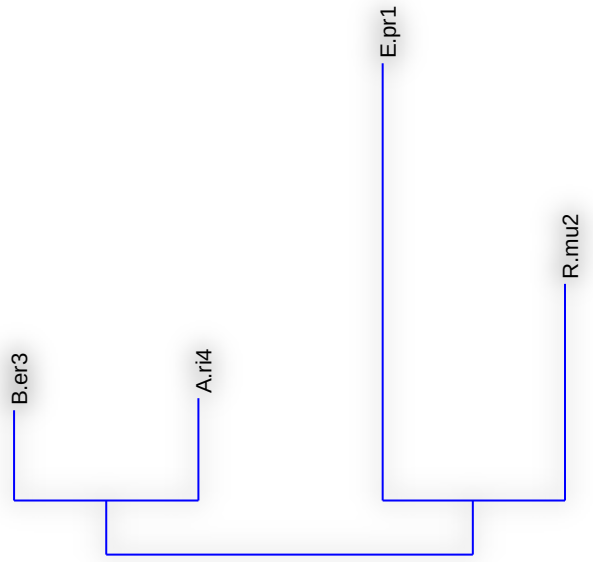

Figure 1. Dendogram obtained by Neighbour Joining cluster analysis of FAs percentages: *E.pr* (*Eurhynchium praelongum*), *R.mu* (*Rhynchostegium murale*), *B.er* (*Brachythecium erythrorrhizon*) and *A.ri* (*Amblystegium riparium*).

Discussion

Four bryophyte species were collected in two Syrian regions: *Eurhynchium praelongum* (Brachytheciaceae) and *Rhynchostegium murale* (Brachytheciaceae) from Latakia and *Brachythecium erythrorrhizon* (Brachytheciaceae) and *Amblystegium riparium* (Amblystegiaceae) from Al-Zabadani.

When the polarity of the target compounds is unknown, the search may start with the two-phase solvent system composed of hexane-ethyl acetate-methanol-water at different volume ratios, which has a moderate degree of polarity. A non-polar solvent such as hexane is applied to elute non-polar compounds (such as fatty acids). Many studies used hexane for the extraction of lipids and fatty acids from mosses as a nonpolar solvent[18,21,35–37]. The application of ultrasound in assisting the extraction was reported to increase the permeability of cell walls and produce cavitation[38]. Other advantages are shorter processing time, low temperature of application, lower energy consumption and solvent requirement. Many studies compared several extraction methods and recommended the ultrasound method[13,38–40].

SFAs varied from myristic acid 14:0 to hexacosanoic acid 26:0 with percentages

ranging from 0.03% to 6.74%; an exception is palmitic acid 16:0 with a percentage of 17–25%. PUFAs also varied from palmitoleic acid 16:1 to nervonic acid 24:1; similar results were obtained by other researchers[28,31]. Erucic acid 22:1 was exclusively found in (*R. murale*) (9.45%), which suggests *R. murale* to be a promising source of erucic acid (Table 2). SFAs, like 16:0, and UFAs, like 18:2, 18:3, 20:5 and 20:4, represented the highest values. Generally, it was noted that UFAs percentages (64–72%) were higher than those of SFAs (25–32%). Our results are comparable to those obtained in similar studies[15,16,25,29]. Some differences were noted between the investigated species, such as the percentages of SFA 16:0 in *E. praelongum* (25.79%) and *R. murale* (20.02%), which were higher than those obtained in *A. riparium* (19.3%) and *B. erythrorrhizon* (17.39%) (Table 2). PUFAs content, such as 18:3 and 18:2, in *A. riparium* and *B. erythrorrhizon* (collected in Al-Zabadani) was higher than that in *E. praelongum* and *R. murale* (collected in Latakia). The high percentages of 18:3 and 18:2 could be explained by the low temperature during the period of collection in Al-Zabadani region (February). Compared to Latakia region, Al-Zabadani is generally characterized by lower temperatures. Our results are coherent with other studies reporting values in the same range[31]. Aydin also indicated that the nutritional differences in habitats lead to a difference in the carbon source, which affects the activity of the enzymes which are responsible for the synthesis of fatty acids[29].

Table 2. Main fatty acids in moss species.

E. pr	***R. mu***	***B. er***	***A. ri***
16:0 (25.79 %)	16:0 (20.02 %)	18:3 (21.04 %)	18:2 (24.15 %)
18:3 (20.14 %)	18:3 (19.14 %)	18:2 (20.45 %)	18:3 (20.25 %)
18:2 (12.44 %)	18:2 (17.16 %)	16:0 (17.39 %)	16:0 (19.3 %)
20:5 (11.58 %)	20:4 (13.36 %)	20:4 (13.52 %)	20:5 (11.52 %)
20:3 (11 %)	22:1 (9.45 %)	20:5 (12.77 %)	20:4 (11.32 %)

E.pr (*Eurhynchium praelongum*), *R.mu* (*Rhynchostegium murale*), *B.er* (*Brachythecium erythrorrhizon*) and *A.ri* (*Amblystegium riparium*).

This research describes four moss species as rich sources of vl-PUFAs, that are usually found in algae and fish. The high content of vl-PUFAs in mosses highlights their potential for biotechnological applications, pharmaceutical industry, food and cosmetics industries[25,29]. It is well known that vl-PUFAs, especially omega-3 (ω-3) and omega-6 (ω-6), are essential for human health, especially for brain development as they are the main constituents of human brain phospholipids. They are also recommended to prevent cardiovascular diseases. FAs are not synthesized in human body, and must be obtained from food, which makes mosses highly recommended as new biological sources of these compounds[21,31].

Bryophytes are used for various purposes in many cultures. The chemical contents of bryophytes may vary significantly according to their types and they may show a wide diversity. Plant size, leaf morphology and anatomy affect fatty acids composition and properties[30]. Seasonal changes, water level, moisture exposure and nutritional elements found in the different habitats also affect fatty acids composition[28,29]. Differences in carbon sources in use and the environmental factors (light, temperature, moisture and soil composition) affect enzymes activity, which are responsible for the synthesis of these FAs[41,42]. Neighbor joining clusters confirmed the role of environmental conditions on fatty acids content as well as the authenticity of the chemical criteria in taxonomy.

Conclusions

In this study, FAs were extracted from four bryophyte samples and analyzed by GC-MS. Total FAs showed 19 different FAs, where the main components were: Palmitic acid 16:0, linolenic acid 18:3, linoleic acid 18:2, arachidonic acid 20:4 and eicosapentaenoic acid 20:5. It was found that *R. murale* could be a promising source of erucic acid, while *E. praelongum* represents an interesting reservoir of eicosatrienoic acid. Our results indicate that PUFAs are present in higher amounts than SFAs, except for palmitic acid (16:0). It is important to mention that the importance of mosses comes mainly from their composition in nutritional elements such as FAs, which are necessary for human health. Mosses are promising organisms in genetic modification as valuable sources of FAs and many other components.

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