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Evaluation of fatty acids profile and mineral content of *Retama monosperma* (L.) Boiss. of Morocco

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Abstract
The content of lipids and the fatty acid (FA) profile were determined for branches/leaves (BLs) and seeds (Sds) of *Retama monosperma*. The total lipids were extracted with hexane and further derivatives to FA methyl esters (FAME). The analyses of FAME samples were performed by gas chromatography coupled to a flame ionisation detector. The total lipids content in BLs and Sds were 0.3 % and 5% respectively. The results indicate that palmitic acid (C16: 0) is the most abundant saturated FA in both Sds and BLs followed by stearic acid (C18: 0), while the oleic acid (C18:1n9) is the dominant mono-unsaturated FA in the Sds. The two samples contained linoleic (C18:2n6) and linolenic acid (C18:3n3) as major polyunsaturated acids. The greatest proportion of linoleic acid was found in Sds, while linolenic acid is highest in BLs. The traditional use of the *Retama monosperma* as an antidiabetic is probably related to its FA. The results of mineral analysis showed that all Sds and BLs contained considerable amount of macro and micro elements.

Key words: *Retama monosperma*, Leguminosae, fatty acids, omega-3, omega-6, minerals

1. Introduction

The genus *Retama* belongs to the family Fabaceae (500 genera and 1,000 species). It includes three species (*R. monosperma, R. raetam* and *R. sphaerocarpa*) with a large distribution in the East Mediterranean regions, North Africa and on the Canary Islands [1-5]. In Moroccan traditional medicine, *Retama monosperma* (L.) Boiss. and *Retama raetam* (Forsk.) Webb. locally named as "*Rtem*" are used by traditional healers as an emetic, purgative, vermifuge, healing, vulnerary, sedative [3], anthelmintic, antiseptic [6] and antidiabetic [7, 8]. Also, *Retama raetam* fruits are used in the Saudi traditional medicine to treat a large variety of ailments including hypertension, hyperlipidemia and diabetes mellitus [9].

Retama monosperma (L.) Boiss. is a glabrescent large shrub that colonizes dune sands (The plant flowers from January to May), it is known as well as *R. raetam*, for its ornamental flowers and its important ecological roles in dune stabilization and soil fixation [10]. In Morocco, *R. monosperma* is common in the valleys, sandy regions and in the internal areas of the Great Atlas, where the climate is semi-arid [6].

Several research works on *Retama* genus have reported the chemical composition of alkaloid [11-13], essential oils [14-15] and flavonoids [16-17]. Many other articles describe their various pharmacological activities including analgesic [17], antibacterial [16, 18], antifungal, antiviral [19], cytotoxic [16 ;20-22], hypoglycemic, diuretic [23-24], anti-hypertensive [25] and antioxidant [14]. Moreover, it has been reported that the aerial parts of *Retama raetam* can be used as an inexpensive biosorbent and as suitable alternatives for the removal of copper ions from wastewater [26]. Recently, researchers have become interested in woody legumes due to their ecological importance [27-29] and biochemical components (protein, fat, fatty acids, flavonoids…) [30]. Thus, phytotoxic activity of *Retama raetam* (Forsk.) Webb. has also been demonstrated, suggesting a potential use for the development of ecological herbicides [31]. Also, it is reported that aqueous extract of this species had repellent effect [32].

Additionally, *Retama raetam* is an important dietary source for livestock species such as camels, goats, and sheep [10, 33]. According to Barakat et al.[34], *R. raetam* appears to represent a valuable candidate as forage resource in Sinai (Egypt) and it should be considered valuable nonconventional forage in the Mediterranean arid ecosystem.

Also, it has been showed that woody species like *R. monosperma* and *R. raetam* may create ‘‘islands of fertility’’ by improving availability of water and nutrients [35-36] or by protecting against direct irradiance and overheating [37-38]. In addition, legume species can increase soil fertility due to N enriched litter deposition or direct release of N from roots [28].
As demonstrated above, and in contrast to *R. raetam*, relatively few studies have been done on *R. monosperma* (L.) Boiss. originally from Morocco. The previous researches on this species have focused especially on its alkaloids [13]. Thus, a recent study [22] has shown that quinolizidine alkaloids contained in the dichloromethane fraction of *R. monosperma* extract, may act as potential *in vitro* cytotoxic agents against human cervical cancer cells through the induction of apoptosis.
In Morocco, *R. monosperma* is widely distributed. Knowing that *R. monosperma* is a good natural source of alkaloids and has medicinal and potential industrial values, its preservation and valorisation require a better understanding of the fundamental laws of ecology and biology. To our knowledge there are no data on its fatty acid profile and its mineral content. Continuing our effort on the valorisation of this locally available plant, we report herein the mineral contents and the total fatty acid methyl esters composition of Sds and BLs of *R. monosperma* taken under natural conditions in coastal dunes of Haouzia (El Jadida, Western Morocco).

2. Materials and Methods

2.1. Plant material

The aerial parts of *Retama monosperma* (branches/leaves and seeds) were collected from three adult shrubs on June 2012 from Haouzia site (El Jadida city, Atlantic coast, Morocco). The plant was identified by Dr. M. Fennane from Scientific Institute of Rabat, Morocco. Voucher specimens (Ref.77816 RAB) have been deposited at the herbarium of Institute.

2.2. Extraction of the seed oils

After air drying, seeds and branches/leaves were ground separately. 15g of each part were refluxed in hexane for 6 h using a Soxhlet apparatus; the solvent was evaporated under the reduced pressure by a rotary evaporator at 30 °. Residue obtained has been refluxed with 0.5 N sodium hydroxide solution in methanol (5 ml) for 10 min.

2.3. Preparation of fatty methyl esters (FAMEs)

Fatty acids in the lipid extracts were converted into methyl esters by means of 5 ml of 14% BF_3-MeOH solution and boiled for 2 min. Then 5 ml of heptane was added through condenser and boiled one more minute. The solution was cooled and 5 ml of saturated NaCl solution was added. Methyl esters were extracted with heptane (2 \ 5 ml), then the organic layer was separated using Pasteur pipettes for both samples, dried over anhydrous Na_2SO_4 and filtered for the each oil. The fatty acid methyl esters were recovered after solvent evaporation in vacuum for the both samples [39]. All determinations were performed in triplicate and the mean values were reported.

2.4. Gas chromatography analysis

The FA composition of the FAMEs was determined by capillary GC on a SP-2560, 100 m × 0.25 mm × 0.20 µm capillary column installed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 3396 Series II integrator and 7673 controller, a flame ionization detector, and split injection (Agilent Technologies Inc., Santa Clara, CA). The initial oven temperature was 140°C, held for 5 min, subsequently increased to 240°C at a rate of 4°C min−1, and then held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5 mL_min−1, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260°C. The split ratio was 30:1[40]. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analysed under the same conditions.

2.5. Determination of the mineral contents

About 1.5 g dried and ground sample (BLs and/or Sds) was put into a burning cup and then was incinerated in an oven at 550°C, until a constant weight was obtained (approx 4 h). Next, the ash was solubilized with 25 mL of HNO_3 50%, heated in a water bath for 30 min, filtered and the mineral contents determined with an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). [41].

3. Results and Discussion

3.1. Fatty acid profiles

The lipid contents in Sds and BLs were 0.3% and 5% respectively. According to Tulukcu [42], lipid contents were significantly influenced by genotypic factors, years, various physiological, geographical, ecological and cultural factors.

The FA compositions of BLs and Sds of *R. monosperma* are presented in Table 1. It is found that the predominant fatty acids are linoleic acid (18:2n6), oleic acid (18:1n9), linolenic acid (18:3n3), palmitic acid (C16:0) and arachidic (C20:0).

Palmitic acid (C16:0), arachidic (C20:0), behenic acid (C22:0) and stearic acid (C18:0) are major saturated FAs of investigated samples. The other minor saturated FAs are lauric (C12:0), myristic (C14:0), pentadecylic (C15:0), margaric (C17:0), tricosanoic (C23:0), lignoceric (C24:0) and pentacosanoic (C25:0); they are found at low levels in BLs while lauric acid and pentacosanoic acid were absent in Sds. Palmitic acid is the major saturated FA in both BLs and Sds. This FA is reported as very constant lipid constituent in the most of the leguminous genera seed oil, [43-44].

The present data show that oleic acid (18:1n9), linoleic acid (18:2n6) and α-linolenic acid (18:3n3) are major unsaturated FAs in studied samples. The highest oleic acid (C18:1n9) content, mono-unsaturated FA, is found in Sds (23,16%) and also found at the lowest level in BLs (6,38%). Several studies from Leguminoseae reported that oleic acid (18:1n9), ranged 6.9-22,4% [44], 8,3-26,8% [45], 8,8-34% [46] in the *Lathyrus* taxa. These findings clarified that oleic acid is found more variable in the legume seeds. Furthermore, palmitoleic acid (C16:1n9) is detected, as another monounsaturated FA, at low level in the BLs(1.13%) and at trace amount in Sds (0.52 %) in this work.

The major polyunsaturated FAs in the Sds and BLs of *R. monosperma* are linoleic (C18:2n6) and α-linolenic acid (C18:3n3). Linoleic acid (omega-6) was detected at a high level in Sds (45.83%), while linolenic acid (omega-3) is found in high level in BLs (22.16%). In general, forage food contains higher concentration of linolenic acid (18:3), whereas linoleic (C18:2n6), is contained mainly in cereals and seeds [47].

Table 1. FA profile of the Sds and BLs of *R. monosperma* of Morocco

RT (min)	FAs	Position of the insaturations and indices	BLs (%)	Sds (%)
6.43	Lauric	(C12 :0)	2.25	-
8.26	Myristic	(C14 :0)	1	0.30
9.28	Pentadecylic	(C15 :0)	0.50	0.16
10.37	Palmitic	(C16 :0)	**16.59**	**12.77**
10.88	Palmitoleic	16 :1n9	1.13	0.52
11.40	Margaric	(C17 :0)	0.91	0.27
12.47	Stearic	(C18 :0)	5.21	5.56
12.66	Oleic (omega-9)	18 :1n9	**6.38**	**23.16**
13.16	Linoleic (omega-6)	18 :2n6	**20.85**	**45.83**
13.52	Linolelaidic (omega-6)	18 :2n6t	**-**	1.77
13.81	Linolenic (omega-3)	18 :3n3	**22.16**	6.49
14.54	Arachidic	(C20 :0)	**11.75**	1.24
16.47	Behenic	(C22 :0)	**6.13**	0.71
17.51	Tricosanoïc	(C23 :0)	1.45	0.59
18.78	Lignoceric	(C24 :0)	3.48	0.64
20.35	Pentacosanoïc	(C25 :0)	0.34	-
∑poly-unsaturated			**43.01**	**54.09**
∑monunsaturated			**7.51**	**23.68**
∑saturated			**49.61**	**22.24**

Results of present data reveal that *R. monosperma* is richer in linoleic acid (C18:2n6), especially Sds (45.83%) than other some legume genus (*Vicia*, 4.3- 9, 4%; *Astragalus*, 23.9 - 37.4%) [30, 48]. Moreover, findings of Şahin et al. [44] indicated that linoleic acid compositions of *Lathyrus* taxa were between 40.7% and 59.9%. Linolelaidic acid (C18:2n6t) was not found in BLs of *R. monosperma*. But this FA is at a low level in Sds (1.77%). These results are very similar to those obtained by Emre et al. [46] for seeds of studied *Lathyrus* taxa.

In recent years, programs of healthy food are enriched with unsaturated FAs, especially omega-3 and omega-6. These two FAs are termed essential FAs because they can not be synthesized by man and are needed for optimum development and health [45, 47].

Omega-3 FAs have positive effect on heart health and potentially other diseases such as cancer, diabetes as well as neurological disorders. Consumption of recommended amounts of omega-3 FAs can contribute to improvement of general health and welfare of human population, especially young people.
The most important and present omega-6 FA is linoleic acid which is present in seeds of sunflower, gourd, soy bean, walnut, sesame and flax. Longer deficiency of linoleic acid in diet is fatal. However, it is important to point out that ratio of omega-6 and omega-3 FAs is extremely important, since these two substances act jointly in preservation of health. Inadequate ratio of these two essential FAs contributes to development of diseases, whereas adequate balance of these two acids contributes to maintaining and even improvement of health [47].
Different studies showed that legume seeds are rich in many nutrient components including protein, starch, dietary fibre, FAs and micronutrients such as vitamins, trace minerals [48-53]. The scientific literature has shown the ability of legume to decrease the glycemic index and cholesterolemy which is due not only to the protective role of dietary fibre but perhaps also to the favourable content of fatty acids [54] and also research recommendations suggest that intake of legumes should be increased for better health and treatment of chronic disease, such as cardiovascular disease, diabetes and cancer [55].
Present study showed that BLs and Sds of Moroccan *R. monosperma* have highest unsaturated FA compositions, 50.52% and 77.77% respectively. Oleic acid (C18:1n 9), linoleic acid (omega-6) and α-linolenic acid (omega-3) are major unsaturated FAs in Sds and BLs. Thus, the traditional use of the *Retama* genus as an antidiabetic is possibly related to its FAs.
In addition, results obtained recently by Belayachi et al. [56] suggest that bioactive compounds in hexanic extract of *R. monosperma* (leaves) act either alone or in combination to promote antileukemic activity. The major bioactive compounds identified in this extract were α-linolenic acid (13.97%), stigmasterol (10.34%), linoleic acid (9.98), β-sitosterol (7.92%) and campesterol (11.09%). According to Sretenović et al.[47], antioxidative and anticancerogenic properties are attributed to conjugated linoleic acid, and studies and results obtained on laboratory animals are very encouraging in prevention of breast, skin and colon tumours. In addition linoleic acid is considered to be beneficial to reduce the cardiovascular risk [57].
Furthermore, during our crops and surveys, we noticed that slim branches, leaves and seeds of plants (after natural falling) are grazed by camels and small ruminants. In this study, samples were collected in June when plant was at a mature stage. Sampling was during the dry season, because it's the period when this plant may be more important for grazing.
The Sds of *R. monosperma* are deficient in omega-3 FAs (6. 49%), and have excessive amounts of omega-6 FAs (47.6%), whereas in BLs the ratio omega-6 / omega-3 is low (0.94/1). According to Sretenović et *al*.[47], "we are what our animals eat". In fact, products obtained from animals on pasture contain significant quantities of two "good" fats, monounsaturated oils and stearic acid. They are also the richest known sources of conjugated linolenic acid and contain high amounts of vitamin E and beta carotin. Conjugated linolenic acid can exclusively be found in animal products. Good source of conjugated linolenic acid are beef and mutton, especially those heads of cattle and sheep on pasture since they contain significantly higher quantity of conjugated linolenic acid compared to animals fed concentrated food. Also, meat obtained from animals reared on pastures compared to those fed concentrated food has lower content of total fat and calories which makes it ideal for nutrition of modern consumers who spend most of the time sitting down.
This is reason why meat deriving from traditionally known regions where cattle is on pasture, are so valued and in demand [58].
In arid and semi-arid lands, animal feed shortages are a severe problem, having a negative impact on animal production. Under these adverse environmental conditions, low soil organic matter contents and low soil water availability result in deficiency and low annual forage productivity due primarily to the long summer dry season [59]. Therefore, the utilization of local feed resources, for animals is necessary and some xerophytic plants with adequate forage potential offer the opportunity to reduce feed shortages to livestock [60].

3.2. Mineral contents

In the earlier part of this century, scientists could qualitatively detect small amounts of several mineral elements in living organisms. The trace elements found in living organisms may be essential, that is, indispensable for growth and health, or they may be nonessential, fortuitous reminders of our geochemical origins or indicators of environmental exposure.

Mixtures of medicinal plants, grown wildly in various regions of the world, are prescribed by the traditional healers for diseases ranging from common colds to malaria, arthritis, ulcers, etc... Human, as well as animal, studies originally showed that optimal intake of elements, such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine, could reduce individual risk factors, including those related to cardiovascular disease [61]. Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases. Minerals are of critical importance in the diet, even though they comprise only 4-6% of the human body. Major minerals are those required in amounts greater than 100 mg per day and they represent 1% or less of body weight. These include calcium, phosphorus, magnesium, sulfur, potassium, chloride, and sodium. Trace minerals are essential in much smaller amounts, less than 100 mg per day, and make up less than 0.01% of body weight. Essential trace elements are zinc, iron, silicon, manganese, copper, fluoride, iodine, and chromium [61].

The mineral compositions of Sds and BLs of *R. monosperma* are shown in Table 2. Mineral contents vary widely depending on the part of plant. The BLs are richer in mineral elements than Sds. According to results, Fe, Mg, Zn, Ca, K, Na, and P contents were very high in both samples.

In this study, both parts of *R. monosperma* showed high K contents. The level of K in BLs (630452 mg/Kg) was found to be higher than those of Sds (5662. 83 mg/Kg). In fact, potassium has highest concentration in the leafy materials than other nutrients as it is an activator of some enzymes. One main feature of K is the high rate at which it is taken up by plant tissues. Usually the absorption of K depends on the soil type.

Also, potassium is the principal intracellular cation and mainly involved in membrane potential and electrical excitation of nerve and muscle cells [63]. The daily adult goat requirements are 1800–2500 mg/kg DM in their diets [64]. It seems that K content in foliage from *R. monosperma* growing in semiarid regions is greater than the required levels [65]. This fact may become a problem because high K concentrations can interfere with Na retention, absorption and Mg utilization [66].

Table 2: Mineral contents of *Retama monosperma*

Mineral (mg/kg)	BLs	Sds
Al	36.11	85.06
Ba	6.48	3.55
Cd	0.21	0.13
Cu	57.82	9.44
Fe	215.43	51.33
Mg	3439	1484
Pb	9.41	8.13
Zn	140.91	44.33
Mn	24.83	3
Ca	7459	4693.52
K	630452	5662.83
Na	2665.25	1228.16
P	1958	456.66

The highest levels of Ca, Mg, Na and P are found in BLs to be 7459 mg/kg, 3439 mg/kg, 2665.25 mg/kg and 1958 mg/kg respectively. Na and K are of great importance for many regulation systems in the body. Na is excreted in sweat by the body. Diarrhoea and vomiting causes the loss of Na and K. Tea, fruits, vegetables and coffee are good sources of K and Na. The minimum daily intake of Na and K are 2.4 g and 3.5 g [67].

The maximum amount of zinc and iron in BLs were determined to be 140.91 mg/kg and 215.43 mg/kg, respectively. Sds are the richest in Al (85.06 mg/kg).

Calcium contents of *R. monosperma* are found in high percentages in the samples analyzed, ranging from 4693.52-7459 mg/kg (Table 2). A dietary level of 4300 mg /kg calcium is required for feeding animals [68]. The maximum level is 20000 mg/kg of diet dry matter [66]. It seems that foliage from browse plants that grow in semiarid [35] regions have enough Ca. Calcium is the major component of bone and assists in teeth development [69]. Phosphorus ranges from 456.66 to 1958 mg /kg. Its content in BLs is four times greater than the Sds.

The concentration of Mg ranges from 1484 mg /kg (Sds) to 3439 mg /kg (BLs). Generally, the level of Mg in plants depends to a large extent on soil type. A magnesium concentration of 2000 mg/kg in plants is commonly regarded as the minimum ‘‘safe’’ dietary concentration for adequate animal health [68, 70]. Our data indicate that *R. monosperma* Sds are deficient in Mg.

The content of zinc ranged between 44.33 mg/kg in seeds and 140.91 mg/kg in BLs (Table 2). The physiological activities of the plant influence Zn absorption and the interactions with many elements like Fe, Mn and Cu also affect Zn uptake [62]. The concentration of zinc in *Artemisia herba alba* leaves, which is widely used in Moroccan medicine, was ranged between 377.89 and 798.21 mg/kg [62].The maximum tolerable zinc level has been set at 500 p.p.m. for cattle and 300 p.p.m. for sheep [71]. It seems that goats require about 40–50 mg /kg DM of Zn in their diets [72].

Aluminium contents are low in both samples and ranged from 36.11 mg/kg (BLs) to 85.06 mg/kg (Sds). Aluminum toxicity is a major factor limiting crop performance on acid soils that predominate under tropical climate. Aluminum interferes with the uptake, transport, and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn, and Zn [62].

Also, manganese concentrations are low in both samples and ranged between 3 mg/kg in Sds and 24.83 mg/kg in BLs. Imelouane et al. [62] reported that the range of Mn in *Artemisia herba alba* leaves was 501.59-1255.87 mg/kg. Magnesium is the most abundant intracellular divalent cation. It is an essential cofactor for a multitude of enzymatic reactions that are important for the generation of energy from ATP and for physiologic processes, including neuromuscular function and maintenance of cardiovascular tone. [73].

Furthermore, our results indicate that the BLs and Sds of *R. monosperma* contain little iron (Table 2). Imelouane et al. [62] reported that the concentration of Fe in *Artemisia herba alba* leaves was ranged between 3617 and 22053 mg/kg. The suggested Fe requirement for animals range between 30 and 100 p.p.m. and the maximum tolerable level for cattle is suggested as 1000 mg /kg. [71, 74-75]. Plants require more Fe than any other macro nutrients, because Fe deficiency in leaves lead to the iron chlorosis [76].

Cd and Pb contents are found in similarly small percentages in the analyzed samples, ranging respectively from 0.13 mg/kg to 0.21 mg/kg for Cd and 8.13 mg/kg to 9.41 mg/kg for Pb. The concentration of Cd and Pb may depend on the sampling location. In fact, *R. monosperma* was collected in deep forest area, and so these two toxicants are present in low quantities. The determination of heavy metals in environmental, biological, and food samples has drawn a significant attention due to the toxic and nutritional effects of these elements. [77]. Pb and Cd can be accumulated in biological systems becoming potential contaminants along the alimentary chain. These elements produce harmful effects on the human health, affecting several organ systems, such as the nervous, gastrointestinal, reproductive, and skeletal, and biochemical activities. Cd and Pb are elements of immediate concern due to their potential toxicity for living organisms, depending on the permissible daily dose for different toxic elements [78-79].

Conclusion

Although they are present in low quantities in branches/leaves and seeds of *Retama monosperma*, lipids are an important fraction in terms of quality, given their high unsaturated fatty acids, particularly oleic, linoleic and linolenic acid whose therapeutic virtues are considerable. The fact that the linoleic acid is considered to be beneficial in cancer and diabetic prevention provides some scientific basis for the traditional use of the plant as an antidiabetic, and also for its pharmaceutical indication as antileukemic. Based on this, further pharmacological investigations to screen other potential bioactivities of fatty acids of *R. monosperma* may be recommended.

This study showed the presence of some major and trace elements in *R. monosperma*. The highest mineral contents were Al, Fe, Mg, Zn, Ca, K, Na and P. However, the composition of plant is mainly dependent on the composition of the soil which is influenced primarily by the nature of the rocks from which the soil is derived.

This work contributes to the knowledge of the potential nutritional properties of *R. monosperma*. We hope that described here results revealing the chemical composition of fatty acids of *R. Monosperma* are important contribution to further studies on significant lipid components of this plant which would be useful tool for taxonomy and physiology of *Retama* genus. However, further research is needed to i) studied the effects of climatic changes on the fatty acids profile of *R. monosperma* and ii) assess nutritional value of fatty acids and minerals of *R. monosperma* in order to assess the plant’s nutrient status under extreme dry conditions.

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