Journal of Functional Foods 53 (2019) 237–247

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Detailed chemical composition and functional properties of *Ammodaucus leucotrichus* Cross. & Dur. and *Moringa oleifera* Lamarck

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ARTICLE INFO

Keywords:
Ammodaucus leucotrichus
Moringa oleifera
Nutritional value
Phenolic compounds
Cytotoxicity
Anti-inflammatory
Antibacterial activity

ABSTRACT

Edible *Ammodaucus leucotrichus* Cross. & Dur. and *Moringa oleifera* Lamarck are largely used in the Algerian Sahara. In this context, soluble sugars, fatty acids, organic acids, and tocopherols were characterized. Decoctions and hydroethanolic extracts were studied regarding their phenolic compounds by HPLC-DAD-ESI/MSn, and *in-vitro* cytotoxic, anti-inflammatory, and antibacterial activities were evaluated. Results indicate high contents of carbohydrates (glucose and sucrose) and significant levels of protein and mineral were recorded for both plants. Malic acid was the major organic acid alongside with α-tocopherol, while PUFA was predominant in *M. oleifera* and MUFA in *A. leucotrichus*. Flavonoid derivatives were the most abundant group, being luteolin-*O*-(malonyl-glucoside) the main compound in *A. leucotrichus* and quercetin-3-*O*-(6″-malonyl-glucoside) and 3-*O*-caffeoyl-quininc acid the major molecules in *M. oleifera*. Extracts exhibited significant cytotoxicity on Hela and MCF-7 cell lines. The anti-inflammatory activity was also higher in the hydroethanolic extracts, which also revealed the highest antibacterial effects, especially for Gram-positive bacteria.

1. Introduction

The abundance of bioactive molecules in plants is an important asset in the screening of new agents for therapeutic and agro-food purposes (Shahidi & Ambigaipalan, 2015). This may also elicit a long range of different physiological effects, due to their richness and varied nutritional constituents, deriving essentially from the primary metabolism, such as sugars, lipids, proteins, and ash. These metabolites are helpful for many biological and functional needs (Pires, Dias, Barros, & Ferreira, 2017), either enhancing the nutritional and organoleptic properties of food products (Shahidi & Ambigaipalan, 2015), or working alongside with the secondary bioactive metabolites, such as phenolic compounds. These molecules are applied due to their several functional properties, including anti-proliferative, anti-inflammatory (Sobral et al., 2016), as also antimicrobial (Vadhana, Singh, Bharadwaj, & Singh, 2015).

The Algerian Sahara has a rich and prestigious heritage of plant-based medicines (Benarba, 2016) and many species are used in traditional phytotherapy, which contributed for the development of new therapeutic pathways (Benarba, 2016; Ziani et al., 2015). *Ammodaucus leucotrichus* Coss. & Dur. (Apiaceae) is an endemic plant in North Africa and is widely used in traditional medicine, especially in the southern Algerian Sahara and Tassili regions (Louail et al., 2016). Leaves and seeds of this plant are consumed in the form of infusions and/or decoctions for several therapeutic cases, such as liver and digestive system ailments, gastroenteritis, diabetes, as also for blood pressure and chest pain (Halla, Boucherit, Boucherit-otmani, Zohra, & Rahmani, 2018; Louail et al., 2016). The fruits are often used as a spice during culinary preparation, and leaves are used as a flavoring herbal in teas (Halla et al., 2018; Louail et al., 2016). Nevertheless, most phytochemical and biological studies on *A. leucotrichus* are related to the volatile compounds from the essential oils (Gherraf et al., 2017; Halla et al., 2018), and few scientific studies have been conducted towards the phenolic compounds and their functional properties. According to a recent study conducted by Halla, Heleno, Costa, Fernandes, Calhelha, Boucherit, Rodrigues, Ferreira, and Barreiro (2018) using colorimetric reactions, flavonoids, condensed and hydrolysable tannins, coumarins, alkaloids, and terpenoids were found in the Algerian *A. leucotrichus* aqueous extract obtained by decoction and in the hydromethanolic extract. El Haci, Mazari, Gherib, and Atik Bekkara (2018) reported moderate

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https://doi.org/10.1016/j.jff.2018.12.023
Received 28 October 2018; Received in revised form 15 December 2018; Accepted 17 December 2018

levels of total phenols and flavonoids in the polar organic extracts (ethanolic, methanolic and acetone extracts) of *A. leucotrichus* from Bechar-Algeria. However, to the author's best knowledge no reports regarding the nutritional composition, individual phenolic compounds profile, cytotoxicity, anti-inflammatory, and antibacterial activity of this plant has been published until now.

Moringa oleifera Lamarck (Moringaceae) is a South Asian tree, commonly known as 'drumstick tree' or 'horseradish tree' (Anwar, Latif, Ashraf, & Gilani, 2007). Recently, it has attracted a lot of attention due to its nutritional value, as also health benefits (David et al., 2017) such as, inflammation treatments, diabetes, gastro-intestinal and infectious diseases along with cardiovascular, hematological, and hepatorenal disorders (David et al., 2017; Gopalakrishnan, Doriya, & Santhosh, 2016). The reason why it has been introduced in many parts of the world, including Algeria, where it is cultivated in some Saharan areas (Bachar and tamanrasset), is mainly due to its food security and nutritional proposes (Boukandoul, Casal, Cruz, Pinho, & Zaidi, 2017). Edible leaves and seeds of *M. oleifera* (eaten fresh, powdered or cooked) contain a varied profile of many nutrients, such as essential sulfur amino acids, protein and minerals (Maryann, Lord, Uchechukwu, & Chibuike, 2018). It is also a renewable source of polyunsaturated fatty acids (PUFA) (Stadtlander & Becker, 2017), tocopherols (γ and α isoforms) (Sánchez-Machado, Lopez-Cervantes, & Rıos Vazquez, 2006), β-carotene, and other vitamins, as well as phenolic compounds (Gopalakrishnan et al., 2016). Phytochemicals have been isolated from different parts of *M. oleifera*, such as zeatin, quercetin, sitosterol, caffeoylquinic acid, kaempferol, tannins, terpenoids, saponins, anthraquinones, and alkaloids (Nouman, Anwar, Gull, Newton, & Rosa, 2016; Upadhyay, Yadav, Mishra, Sharma, & Purohit, 2015). Anti-cancerous agents, like glucosinolates, isothiocyanates, glycoside compounds, and glycerol-1-9-octadecanoate have been reported to exercise an effective action on cancer cell lines, during the different stages of cancer evolution (Edwinanto, Septiadi, Nurfazriah, Anastasya, & Pranata, 2018; Tiloke, Phulukdaree, & Chuturgoon, 2013). Leaves of *M. oleifera* have a low caloric value and can be used in a healthy diet. The fruits (pods) are fibrous and are valuable to treat digestive problems and thwart colon cancer (Moyo, Masika, Hugo, & Muchenje, 2011).

Due to the multiple traditional uses of these species by the Saharan population in Algeria, but also its usage around the world, the aim of the present study was to determine the nutritional value and chemical composition regarding minerals, free sugars, organic acids, fatty acids, and tocopherols. Moreover, the biological potential as anti-proliferative, anti-inflammatory and antibacterial agents of the decoction and hydroethanolic extracts of both plant species was also studied. Furthermore, the targeted functional properties were related to their phenolic profile determined by HPLC-DAD-ESI/MSn.

2. Materiel and methods

2.1. Plant material

Aerial parts of spontaneous *Ammodaucus leucotrichus* Cross. & Dur. (locally named as "Kemoun msawef") and leaves of the cultivated *Moringa oleifera* Lamarck were harvested in September 2017, from the desert areas of south Algeria, such as Tamanrasset (Coordinates: 22°47′13″N, 5°31′38″E) and Béchar (Coordinates: 31°37′00″N, 2°13′00″W), respectively. Plant identification and characterization was conducted using Quezel and Santa (1963) botanical criteria and authenticated by the Botanical professors at the National Superior School of Agronomy Algeria. The collected biomass was separated, cleaned and shade air-dried in a well-ventilated room, at room temperature. Dried plants were grounded to a fine powder (~20 mesh) and stored at 4 °C for further analyses.

2.2. Chemical characterization of the plant material

2.2.1. Nutritional value

Crude proteins, fat, carbohydrates and total ash content of the all samples were analyzed according to AOAC procedures (AOAC, 2016), and expressed in g/100 g of dry weight. The crude protein content (N × 6.25) was estimates through the macro-Kjeldahl method, whereas the crude fat content was determined after a Soxhlet extraction with petroleum ether. Incineration at 550 ± 15 °C was used to measure the total ash content. While total carbohydrates were calculated by difference and energetic value was calculated as following: Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

2.2.2. Elemental mineral composition

For mineral elements determination, each sample (0.5 g) was weighed in a Teflon recipients and an acid digestion (69% HNO_3 and 30% H_2O_2) at 110 °C for 30 min, in a High Performance Microwave Digestion system (ETHOS one-Milestone), was performed. The digested samples were filtered and poured into a 100 mL vial. The Teflon recipients were rinsed repeatedly by ultrapure water to make a total volume of 100 mL, and then they were decanted into clean vials for the mineral determination. The absorbance was read in an atomic absorption spectrophotometer (Agilent Technologies, 240 FS AA) with nitrous oxide-acetylene flame, using different wavelength for each mineral element (Zn-213.9 nm, Ca-422.7 nm, Fe-248.3 nm, Mg-285.2 nm, Na-589 nm, and K-766.5 nm). The absorbance responses were compared with > 99.9% purity analytical standard solutions for AAS, prepared with high purity, $Fe(NO_3)_3$, $Cu(NO_3)_2$, $Mn(NO_3)_2$, Zn $(NO_3)_2$, $NaNO_3$, KNO_3, $CaCO_3$ and $Mg(NO_3)_2$, purchased from Sigma Aldrish TraceCERT® AAS Standards.

2.2.3. Free sugars

The identification and quantitative of the free sugars present in the dry sample were determined by HPLC couple to a refraction index detector (Knauer, Smartline system 1000, Berlin, Germany), as previously described (Barros et al., 2013). Peaks identification was carried out by comparisons of their relative retention time (Rt) with authentic standards. While, quantification was performed using the internal standard (IS, melezitose, Sigma-Aldrich, St. Louis, MO, USA). Results were processed in a Clarity Software (Data Apex, Prague, Czech Republic) and expressed in g per 100 g of dry weight.

2.2.4. Organic acids

Organic acids content were determined using ultra-fast liquid chromatography (UFLC) (Shimadzu 20A UFLC series, Shimadzu Corporation, Kyoto, Japan) coupled to a diode array detector (DAD) operating under the conditions described by Barros et al. (2013). The detected compounds were identified and quantified (215 and 245 nm, preference wavelengths) by comparing their peak area with calibration curves obtained from commercial standards. Results were processed using the LabSolutions Multi LC-PDA software and expressed in mg per 100 g of dry plant.

2.2.5. Fatty acids

The fatty acids content of the plant material was determined after trans-esterification as previously described by the authors Barros et al. (2013). The fatty acids profile was determined using a gas chromatographer (DANI model GC 1000, Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detection (GC- FID, 260 °C). The identification was carried out by comparing the relative Rt of FAME (Fatty Acid Methyl Esters) peaks of the samples with commercial standards. Results were processed using Clarity Software (Data Apex, Prague, Czech Republic) and expressed in relative percentage.

2.2.6. Tocopherols

Tocopherols (four structural isoforms) were analyzed with using an

HPLC (Knauer, Smartline system 1000) coupled with a fluorescence detector (excitation/emission detection at 290/330 nm) as previously described by Barros et al. (2013). Identification was performed by chromatographic comparisons with authentic standards and quantification of the compounds was carried out using the IS (tocol) method though calibration curves obtained from commercial standards. Results were processed using Clarity Software (Data Apex, Prague, Czech Republic) and expressed in mg per 100 g of dry weight.

2.3. Bioactive properties

2.3.1. Extracts preparation

The hydroethanolic extracts were prepared by adding an ethanol/water mixture (8:2, v/v, 30 mL) to 1 g of dry powdered plant, the mixture was macerated twice for 1 h under stirring agitation. The extracts were filtered and evaporated under vacuum (rotary evaporator Büchi R-210, Flawil, Switzerland) at 35 °C and the aqueous dark green residue was frozen (−20 °C), and then lyophilized (−49 °C and 0.041 bar, FreeZone 4.5, Labconco, Kansas City, MO, USA) before subsequent analyzes.

The decoction is the usual form of administration of these plants as described by traditional healers. Therefore, decoctions were performed in a laboratory scale, in which each plant sample (1 g) was mixed with distilled water (200 mL) and then are left to boil, afterword's they are cooked for an additional 5 min in a closed recipient, filtered under reduced pressure, frozen (−20 °C), and lyophilized.

2.3.2. Phenolic compounds analysis by HPLC-DAD-ESI/MSn

The phenolic compounds were determined following a methodology previously described by the authors Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). The extracts were prepared at a ratio of 10:1 (w/v) of dry extract/ultrapure water for the decoction extract and in ethanol/water (80:20 v/v, HPLC-grade) for the hydroethanolic extract. Afterwards, the samples were filtrated (Whatman 0.45 μM syringe filter), prior to the HPLC-DAD-ESI/MSn analysis (Dionex Ultimate 3000, Thermo Finnigan, San Jose, CA, USA). The compounds were detected using a diode array detector (DAD, recording at 280, 330 and 370 nm), and a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA), operating in negative mode. Identification was performed by comparing retention times (RT) and mass spectrum fragments with literature reported data or by comparison with commercially available standards (Extrasynthesis, Genay, France). Quantification was made on the basis of the UV–vis signal of each available phenolic standards, where calibration curves were obtained by injecting known concentrations (2.5–100 μg/mL). Results were processed using the Xcalibur®data system and expressed in mg per g of extract.

2.3.3. Cytotoxicity activity

The cytotoxicity was conducted as described by Barros et al. (2013), using a concentration of 8 mg/mL of the aqueous decoction and hydroethanolic extract, both dissolved in ultrapure water. The sulforhodamine B colorimetric assay was applied in order to determine the inhibitory growth activity of four human tumor cell lines: NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and MCF-7 (breast carcinoma).

The hepatotoxicity was determined using a primary culture of non-tumor liver cells (PLP2), which were prepared from a freshly harvested porcine liver according to a procedure established by the authors Barros et al. (2013).

Ellipticine was used as positive control. Colorimetric readings were recorded using a Microplate Reader ELX800 (Bio-Tek Instruments, Inc., Winooski, VT, USA), and results were expressed as GI_{50} values, which represents the samples concentrations required to inhibit 50% of cell growth.

2.3.4. Anti-inflammatory activity

The concentration of nitrite NO produced by lipopolysaccharide (LPS)-stimulated murine macrophages RAW 264.7 cell lines in culture medium, treated with different concentrations of the plant extracts, was determined by a method previously described by Sobral et al. (2016). For this purpose, the crude aqueous extract (decoction) and the hydroethanolic extract, were dissolved at a concentration of 8 mg/mL in water and the production of nitric oxide (NO) was measured using the Griess reagent system kit and Dexamethasone was used as a positive control. Results were expressed as EC_{50} values corresponding to the sample concentration achieving 50% of the inhibition of NO-production.

2.3.5. Antibacterial activity

Crude extracts were screened for antibacterial activity against a panel of Gram-positive and Gram-negative Multidrug resistant (MDR) bacteria donated from the Local Health Unit of Bragança and Hospital Centre of Trás-os-Montes and Alto-Douro-Vila Real, Northeast of Portugal, therefore there was no direct contact with the patients. Four Gram-positive bacteria: *Enterococcus faecalis,* isolated from urine, *Listeria monocytogenes*, isolated from cerebrospinal fluid, methicillin-sensitive *Staphylococcus aureus* (MSSA), isolated from wound exudate, and methicillin-resistant *Staphylococcus aureus* (MRSA), isolated from expectoration, and five Gram-negative bacteria: *Escherichia coli*, isolated from urine, *Proteus mirabilis*, isolated from wound exudate, *Klebsiella pneumoniae*, isolated from urine, *Pseudomonas aeruginosa,* isolated from expectoration and *Morganella morganii*, isolated from urine, were used to determine this bioactivity. The minimum inhibitory concentration (MIC) values were evaluated using a colorimetric assay of *p*-iodonitrotetrazolium chloride (INT) following a procedure ascribed by Pires et al. (2018). The MBC (minimal bactericidal concentrations) were determined by subculturing the culture from each negative well and from the positive control, and further incubated at 37 °C for 24 h.

Three negative controls were prepared, consisting of Mueller-Hinton broth (MHB), the extract, and a third with the medium and the antibiotic. A positive control was prepared with MHB and for each inoculum. Antibiotics, such as ampicillin and imipenem, have been used as positive controls for Gram-negative bacteria, while ampicillin and vancomycin were used for Gram-positive bacteria.

2.4. Statistical analysis

For all the extracts and tests, analyses were carried out in triplicate and the obtained values were expressed as the mean ± standard deviation (SD). Statistics treatments was performed by SPSS v. 22.0 program (IBM Corp., Armonk, NY, USA) using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. A student́s *t*-test was used to determine the significant difference among two different samples, with $\alpha = 0.05$.

3. Results and discussion

3.1. Chemical characterization

The macronutrients composition and energetic value results obtained for the studied plants are presented in Table 1. Carbohydrates were found to be predominate compounds in both plant species, ranging from 65.0 ± 1.0 g/100 g dw in *A. leucotrichus* to 56.6 ± 0.5 g/100 g dw in *M. oleifera*, where reducing sugars constitute a small amount, representing 8.23 and 6.74% of the whole carbohydrates found in the studied species, respectively. Soluble sugars are usually represented by three naturally occurring molecules and widely distributed, such as glucose, fructose, and sucrose (Barros, Morales, Sanchez-Mata, Oliveira, & Ferreira, 2015). Chromatographic analysis using HPLC-RI allowed their detection and quantification in the studied plant, with the highest total soluble sugars content in *A. leucotrichus*

Table 1
Nutritional and energetic value, soluble sugars, and organic acids composition in *A. leucotrichus* aerial parts and *M. oleifera* leaves.

	A. leucotrichus	*M. oleifera*	*p*-Students *t*-test
Crude fat (g/100 g dw)	11.1 ± 0.3	6.5 ± 0.2	< 0.001
Crude protein (g/100 g dw)	13.1 ± 0.9	22.8 ± 0.3	< 0.001
Total ash (g/100 g dw)	10.8 ± 0.2	14.1 ± 0.5	0.001
Macroelements			
Na (mg/100 g dw)	160 ± 6	319 ± 6	< 0.001
Mg (mg/100 g dw)	236.6 ± 0.4	382 ± 1	< 0.001
K (mg/100 g dw)	2283.1 ± 0.4	1626 ± 1	< 0.001
Ca (mg/100 g dw)	1555 ± 2	2785 ± 1	< 0.001
Microelements			
Fe (mg/100 g dw)	22 ± 2	39 ± 3	< 0.001
Zn (mg/100 g dw)	1.72 ± 0.04	3.37 ± 0.09	< 0.001
Cu (mg/100 g dw)	0.39 ± 0.03	0.81 ± 0.01	< 0.001
Mn (mg/100 g dw)	7.6 ± 0.4	5.21 ± 0.03	< 0.001
Total carbohydrates (g/100 g dw)	65 ± 1	56.6 ± 0.5	< 0.001
Fructose (g/100 g dw)	1.0 ± 0.1	0.36 ± 0.02	< 0.001
Glucose (g/100 g dw)	2.6 ± 0.1	0.420 ± 0.004	< 0.001
Sucrose (g/100 g dw)	1.8 ± 0.2	3.04 ± 0.07	< 0.001
Total soluble sugars (g/100 g dw)	5.4 ± 0.4	3.82 ± 0.09	< 0.001
Energetic value (Kcal/100 g dw)	413 ± 1	376 ± 1	< 0.001
Oxalic acid (g/100 g dw)	0.728 ± 0.008	1.35 ± 0.03	< 0.001
Malic acid (g/100 g dw)	2.5 ± 0.1	3.60 ± 0.008	< 0.001
Ascorbic acid (mg/100 g dw)	5.6 ± 0.1*	6.7 ± 0.2*	0.711
Total organic acids (g/100 g dw)	3.3 ± 0.1	4.97 ± 0.04	< 0.001

Values in mean values ± standard deviation.
* Results expressed in mg/100 g dw.

(5.4 ± 0.4 g/100 g dw), being the main sugar, the reducing monosaccharide glucose (2.6 ± 0.1 g/100 g dw). While *M. oleifera* leaves contained the lowest concentrations of soluble sugars. Similar values of carbohydrates (ranging from 54.6 to 57.6%) were reported by Amabye (2016) in a sample of *M. oleifera* obtained commercially from Mekelle, Ethiopia. Nevertheless, Oduro, Ellis, and Owusu (2008) reported a lower amount of carbohydrates (43.88 g/100 g) in the dry leaves of this species from Ghana. Samples of *M. oleifera* leaves from Ouagadougou (Yaméogo et al., 2011) also showed a lower level of carbohydrates (38.6 g/100 g of dry matter). Upadhyay et al. (2015) reported L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, and D-xylose to be predominant sugars in the purified whole-gum exudates of *M. oleifera*. The differences observed could be attributed to some exotic conditions, such as environmental and edaphoclimatic factors including temperature, topography and soil variations, but also to some biotic conditions (genetics, metabolism, between other factors) that may influence biochemical and physiological processes involved in the plant sugars' synthesis. To the author's best knowledge there is no report concerning carbohydrates and soluble sugars' content in *A. leucotrichus*.

Total ash content (Table 1) was also significant in both plant species, but *M. oleifera* (14.1 ± 0.5 g/100 g dw) revealed the highest content. The obtained results for *M. oleifera* are corroborate with the reported data from literature, in which a commercial sample of *M. oleifeira* from Ethiopia (Amabye, 2016) presented a similar ash content (10.71 to 11.18 g/100 g), while Oduro et al. (2008) studied the leaves of *M. oleifera* collected from Ghana and presented a lower amount (7.13 g/100 g). The amount of ash significantly influences the content of minerals, and indeed the minerals detected by AAS confirms this mineralogical richness. It was noticed a clear predominance of the macroelements and microelements in *M. oleifera*, in comparison to *A. leucotrichus*, with the exception of Cu. Previous works concluded that leaves of the Moringa tree appeared to provide a broad range of important minerals essential for the human diet, especially P, K, Ca, Mg, Mn, Fe, Al, Cu, Zn, and Na (Witt, 2013). Moyo et al. (2011) found Ca (3.65 g/100 g), P (0.3 g/100 g), Mg (0.5 g/100 g), K (1.5 g/100 g), Na (0.164 g/100 g), S (0.63 g/100 g), Zn (13.03 mg/kg), Cu (8.25 mg/kg), Mn (86.8 mg/kg), Fe (490 mg/kg), and Se (363 mg/kg) in South African *M. oleifera* leaves, which were similar to the amounts present in this study. Also similar to the current findings, Amabye (2016) also revealed high values of macroelements in *M. oleifera* (from Ethiopia), Ca (2016.5–2620.5 mg/100 g), K (1817–1845 mg/100 g), and Mg (322.5–340.6 mg/100 g). Kane et al. (2017) studied *M. oleifera* dried leaves from the botanical garden of Dakar University (Senegal), and reported that P (1675.52 mg/100 g), Ca (583.3 mg/100 g), and Mg (306.19 mg/100 g) as the major mineral elements, which were also the predominant macroelements in the kernels and leaves of *M. oleifera* from India (Stadtlander & Becker, 2017). The differences found regarding the mineral composition of the same species, may be attributed to the geographic diversity in the origin of these samples (Pires et al., 2018). Also, to the author's best knowledge there is no literature information regarding ash and mineral content in *A. leucotrichus*.

Concerning the protein fraction (Table 1), a significant high content was recorded in both plant species, thus *M. oleifera* revealed the highest levels (22.8 ± 0.3 g/100 g dw). Previous research in this species also described high protein levels, considering this plant as a rich source of protein for dietary supplement (Oyeyinka & Oyeyinka, 2016). Moreover, the same findings (22.42 g/100 g of dry weight) were also recorded by Sánchez-Machado, Núñez-gastélum, and Reyes-moreno (2009) in *M. oleifera* leaves from Mexico. These contents were also in accordance with those reported by Asante, Nasare, Tom-dery, and Ochire-boadu (2014), studing *M. oleifera* leaves from the semi-deciduous forest zone of Ghana. However, Guinea savanna *M. oleifera* leaves revealed slightly higher values (25.34 and 26.98 g/100 g dw) (Asante et al., 2014). Nevertheless, Amabye (2016) presented a lower content (10.74 to 11.48 g/100 g dry leaf powder) in the commercial sample of *M. oleifera* from Mekelle. Other studies have reported variable levels of protein contents in *M. oleifera* leaves ranging between 27.2 and 32.3 g/100 g dw (Moyo et al., 2011; Oduro et al., 2008; Stadtlander & Becker, 2017; Yaméogo et al., 2011). These findings highlights Moringa leaves as good sources of protein, with particular nutritional significance, as it may meet human's protein and energy requirements.

Different levels of crude fat were observed in the studied plants, with *A. leucotrichus* showing a higher content (11.1 ± 0.3 g/100 g dw), comparatively to *M. oleifera*, presenting remarkably lower contents (6.5 ± 0.2 g/100 g dw). These lower values of lipid contents were described by many previous scientific reports studying *M. oleifera* leaves. A content of 2.23 g/100 g of fat was found in *M. oleifera* leaves from Ghana (Oduro et al., 2008), while the commercial Ethiopian *M. oleifera* ranged from 10.21 to 10.31 g/100 g dw (Amabye, 2016). The differences found in the fat content could be due to the analyzed plant part, which has specific influence on the fat distribution within the plant (generally concentrated in seeds), that could be affected by the plant genetics', the photoperiod, climatic conditions (temperature), and also to the extraction procedures applied.

A. leucotrichus revealed a higher energetic value than Moringa leaves, this being due to its higher carbohydrates and lipid content. Oduro et al. (2008) reported a similar energetic value in *M. oleifera* leaves from Ghana (~310 kcal/100 g).

Three organic acids, such as oxalic, malic, and ascorbic acids, were identified and quantified in both studied species, being malic acid the predominant organic acid, followed by oxalic and ascorbic acids. *M. oleifera* showed the highest amounts of these compounds, with the exception of ascorbic acid, in which both species revealed a similar content. The noted variation between the species may be attributed to certain factors, including genetic and maturity levels of the species, which may affect the organic acids metabolism. However, according to Dias et al. (2016) several external processes, such as harvest and storage conditions, treatment, processing and preparation steps, may also contribute to the differences found in the organic acids composition. No data were found in literature regarding the organic acids profile for

Table 2
Fatty acids (relative percent) and tocopherols composition in *A. leucotrichus* aerial parts and *M. oleifera* leaves.

	A. leucotrichus	*M. oleifera*	*p*-Students *t*-test
C8:0	0.166 ± 0.001	0.327 ± 0.003	< 0.001
C10:0	0.13 ± 0.01	0.29 ± 0.01	< 0.001
C11:0	1.67 ± 0.07	0.415 ± 0.003	< 0.001
C12:0	0.26 ± 0.02	0.46 ± 0.02	< 0.001
C13:0	2.038 ± 0.002	0.84 ± 0.01	< 0.001
C14:0	0.87 ± 0.05	1.80 ± 0.02	< 0.001
C14:1	0.34 ± 0.02	0.57 ± 0.01	< 0.001
C15:0	0.37 ± 0.02	0.14 ± 0.01	< 0.001
C16:0	21.2 ± 0.7	17.160 ± 0.001	< 0.001
C16:1	1.06 ± 0.01	1.41 ± 0.01	< 0.001
C17:0	0.72 ± 0.01	0.47 ± 0.02	< 0.001
C18:0	0.101 ± 0.002	2.67 ± 0.05	< 0.001
C18:1n9	53.8 ± 0.4	1.70 ± 0.03	< 0.001
C18:2n6	1.99 ± 0.02	13.10 ± 0.06	< 0.001
C18:3n3	10.6 ± 0.4	51.5 ± 0.2	< 0.001
C20:0	1.012 ± 0.003	0.92 ± 0.01	< 0.001
C20:1	1.179 ± 0.002	0.065 ± 0.001	< 0.001
C20:2	0.089 ± 0.004	0.073 ± 0.001	< 0.001
C21:0	0.11 ± 0.01	0.085 ± 0.001	< 0.001
C22:0	1.23 ± 0.01	1.87 ± 0.03	< 0.001
C20:5n3	0.357 ± 0.004	0.297 ± 0.001	< 0.001
C24:0	0.741 ± 0.003	3.87 ± 0.04	< 0.001
SFA	30.6 ± 0.8	31.3 ± 0.1	0.102
MUFA	56.4 ± 0.4	3.75 ± 0.02	< 0.001
PUFA	13.0 ± 0.4	64.9 ± 0.1	< 0.001
PUFA/SFA	0.43 ± 0.02	2.07 ± 0.01	< 0.001
α-Tocopherol	0.69 ± 0.04	4.0 ± 0.1	< 0.001
β-Tocopherol	nd	0.029 ± 0.002	–
γ-Tocopherol	nd	0.41 ± 0.02	–
δ-Tocopherol	nd	0.068 ± 0.002	–
Total tocopherol (mg/100 g dw)	0.69 ± 0.04	4.5 ± 0.1	< 0.001

Values expressed in mean values ± standard deviation. nd - not detected; dw – dry weight; Caprylic acid (C8:0); Capric acid (C10:0); Undecanoic acid (C11:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Penta-decanoic acid (C15:0); Palmitic acid (C16:0); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); a-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosanoic acid (C20:1); cis-11,14-Eicosadienoic acid (C20:2c); cis-11,14,17-Eicosatrienoic acid; Behenic acid (C22:0); Lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

both species.

The GC-FID analysis of fatty acids revealed the presence of twenty-two molecules in both plants species, thus revealing significant different contents (Table 2). However, linoleic acid (C18:2n6), oleic acid (C18:1n9), α-linolenic acid (C18:3n3), and palmitic acid (C16:0) were the predominant fatty acids in both species. While PUFA (polyunsaturated fatty acids) predominated in *M. oleifera* leaves (64.9 ± 0.1%), due to the contribution of α-linolenic acid (ALA, 51.5 ± 0.2%) and linoleic acid (LA, 13.10% ± 0.06%); MUFA (monounsaturated fatty acids) were the main group of compounds present in *A. leucotrichus* (56.4 ± 0.4%), with the highest contribution of oleic acid (53.8 ± 0.4%). This fact lead to low ration of PUFA/SFA in *A. leucotrichus*, thus *M. oleifera* presented a very high ratio of PUFA/SFA, which is a very interesting criteria in the quality of crops and their biological functions (Dias et al., 2016).

Sánchez-Machado et al. (2009) also reported high percentages of α-linolenic acid (56.87%) in *M. oleifera* leaves from Sonora Mexico. Moyo et al. (2011) identified seventeen fatty acids in *M. oleifera* leaves from South-Africa, with α-linolenic acid (44.57%) as the most abundant fatty acid. The obtained amounts of ALA and LA in *M. oleifera* were higher than the published amount of Stadtlander and Becker (2017), who analyzed the lipid fraction of *M. oleifera* kernels from Nicaragua, India, and Ethiopia.

Moringa contains a higher percentage of PUFA, which is desirable for its inclusion in a healthy diet, as it may prevent the occurrence of certain diseases (Sánchez-Machado et al., 2009). Recently, essential fatty acids, such as ALA and LA, have been considered as a functional food and/or nutraceutical, which promote the endogenous biosynthesis of long chain n-3 PUFA, being important for the eicosanoids biosynthesis, which are viewed as important bio-regulators of many cellular processes (Arbex et al., 2015).

The tocopherols content in both studied species is shown in Table 2. *M. oleifera* revealed the presence of all four isomers, while *A. leucotrichus* only presented α-tocopherol. This vitamer was the most abundant in *M. oleifera*, followed by γ-tocopherol, β-tocopherol, and δ-tocopherol. Sánchez-Machado et al. (2006) found α-tocopherol as the main isomer in *M. oleifera* leaves (744.5 μg/g) from Sonora, Mexico. The Algerian *M. oleifera* oil sample from kernels showed a significant higher value of total tocopherol (287–327 mg/kg), as well as the individual amounts of α-tocopherol (195.8–263.2 mg/kg), β-tocopherol (4.7–10.5 mg/kg), γ-tocopherol (51.2–77.8 mg/kg), and δ- tocopherol (6.1–8.6 mg/kg) (Boukandoul et al., 2017). This higher content could be due to the fact that the previous authors use different plant parts, which certainly contain a higher content of this lipophilic vitamin.

Globally, the differences found between the obtained values and those reported in literature may be explained by several factors responsible for these variations, such as the impact of soil and climatic conditions, the maturity of analyzed samples, or changes in the extraction method applied.

3.2. Phenolic profiling by HPLC-DAD-ESI/MS

The chromatographic characteristics of the identified phenolic compounds are presented in Tables 3 and 4, for *A. leucotrichus* and *M. oleifera*, respectively. *A. leucotrichus* revealed the presence of 7 phenolic compounds, 2 phenolic acid derivatives and 5 flavones (apigenin and luteolin derivatives), while *M. oleifera* presented 12 compounds, of which 3 were phenolic acid derivatives and 9 were flavonoids, mainly flavonols (quercetin, kaempferol and isorhamnetin glycoside derivatives) and flavones (apigenin derivatives).

Regarding, *A. leucotrichus* extracts (Table 3), peak 3^{Al} revealed a pseudomolecular ion at *m/z* 601 and based on its MS/MS fragmentation pattern, it was identified as di-*O*-caffeoyl-malonylquinic acid, as previously identified in a plant species of the same family *Centella asiatica* (Maulidiani, Abas, Khatib, Shaari, & Lajis, 2014). Similarly, peak 6^{Al} ($[M\text{-}H]^-$ *m/z* 687) revealed 86 u (malonyl moiety) higher then peak 3^{Al}, being tentatively identified as di-*O*-caffeoyl-dimalonylquinic acid. The remaining compounds (peaks 1^{Al}, 2^{Al}, 4^{Al}, 5^{Al}, and 7^{Al}) corresponded to flavone derivatives, in which peak 2^{Al} (luteolin-7-*O*-glucoside) was positively identified in comparison with the commercial standard. Two apigenin derivatives were found in the extracts, peak 1^{Al} ($[M\text{-}H]^- m/z$ 593) corresponded to a *C*-glycoside due to its characteristic losses of 90 and 30 u moieties, being tentatively identified as apigenin-6,8-*C*-diglucoside, as previously described by Makita, Chimuka, Steenkamp, Cukrowska, and Madala (2016) and Karthivashan, Arulselvan, Alimon, Ismail, and Fakurazi (2015). Peak 7^{Al} ($[M\text{-}H]^-$ *m/z* 473) presented one MS^2 fragment at 269, corresponding to apigenin aglycone with the loss of 42 u + 162 u (acetyl and hexosyl moieties), therefore being tentatively identified as apigenin-*O*-(acetyl-hexoside). Finally, the two remaining compounds presented a pseudomolecular ion at *m/z* 533 with two MS^2 fragments at 489 and 285, revealing the loss of a malonyl (−86 u) and hexosyl (−162 u) moiety, and therefore tentatively identified as luteolin-*O*-(malonyl-hexoside) isomer 1 and 2.

Luteolin derivatives, such as luteolin-7-*O*-glucoside and luteolin-*O*-(malonyl-hexoside) were the major compounds present (Table 3), and the hydroethanolic extract revealed the highest content. To the author's best knowledge there is no reports regarding the individual identification of phenolic compounds in this species. Nevertheless, El Haci et al.

Table 3
Retention time (Rt), wavelengths of maximum absorption in visible region (λmax), mass spectral data, tentative identification and quantification of phenolic compounds in the extracts of *A. leucotrichus* aerial parts.

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	References	Quantification (mg/g extract)		*p*-Students *t*-test
							Hydroethanolic	Decoction	
1^{Al}	10.0	330	593	473(100), 383(10), 353(27)	Apigenin-6,8-*C*-diglucoside	Karthivashan et al. (2015) and Makita et al. (2016)	0.409 ± 0.001	0.27 ± 0.01	< 0.001
2^{Al}	19.2	347	447	285(100)	Luteolin-7-*O*-glucoside	Standard compound	11.7 ± 0.5	3.8 ± 0.4	< 0.001
3^{Al}	21.0	300, 329	601	557(80), 515(100), 395(47), 353(10)	Di-*O*-caffeoyl-malonylquinic acid	Maulidiani (2014)	1.5 ± 0.1	0.41 ± 0.004	< 0.001
4^{Al}	22.2	341	533	489(66), 285(100)	Luteolin-*O*-(malonyl-hexoside) isomer 1	DAD, MS	1.9 ± 0.1	1.08 ± 0.01	< 0.001
5^{Al}	23.7	346	533	489(71), 285(100)	Luteolin-*O*-(malonyl-hexoside) isomer 2	DAD, MS	8.87 ± 0.03	2.4 ± 0.1	< 0.001
6^{Al}	24.3	300, 329	687	643(22), 601(100), 557(20), 515(10), 395(5), 353(5)	Di-*O*-caffeoyl-dimalonylquinic acid	Maulidiani (2014)	2.4 ± 0.3	nd	–
7^{Al}	28.2	330	473	269(100)	Apigenin-*O*-(acetyl-hexoside)	DAD, MS	1.40 ± 0.01	0.941 ± 0.001	< 0.001
					Total phenolic acids		3.9 ± 0.3	0.408 ± 0.004	< 0.001
					Total flavonoids		24.2 ± 0.4	8.5 ± 0.5	< 0.001
					Total phenolic compounds		28.1 ± 0.7	8.9 ± 0.5	< 0.001

Values expressed in mean values ± standard deviation. nd–not detected. Three phenolic compounds were used for quantification: compound 1^{Al}- apigenin 6-*C*-glucoside (y = 107025x − 61531; R^2 = 0.9989; LOD = 0.19 µg/mL; LOQ = 0.63 µg/mL); compounds 2^{Al}, 4^{Al}, 5^{Al}, and 7^{Al}- apigenin 7-*O*-glucoside (y = 10683x − 45794; R^2 = 0.999; LOD = 0.10 µg/mL; LOQ = 0.53 µg/mL); compounds 3^{Al} and 6^{Al}- chlorogenic acid (y = 168823x − 161172; R^2 = 0.9999; LOD = 0.20 µg/mL; LOQ = 0.68 µg/mL).

(2018) studied the total phenolic and flavonoid contents of *A. leucotrichus* fruits from Algeria; Sebaa, Marouf, Kambouche, and Derdour (2018) also studied the fruits of this species, but performed a preliminary screening of group of compounds through a silica TLC assay, revealing the presence of anthracenes, tannins, alkaloids, phenolic acids, cardiotonic glucoses, saponins, flavonoids, and coumarins. Although, in the present study only phenolic acids and flavonoids were detected, these differences could be due to the different plant parts studied, as also to the extraction solvents applied. Finally, Halla et al. (2018) described the presence of the following group of compounds, flavonoids, tannins, coumarins, alkaloids, and terpenoids, in *A. leucotrichus* hydromethanolic extracts from Algeria, but the authors did not mentioned the part studied. These authors determined phenolic compounds through colorimetric reactions. Nevertheless, none of the mentioned authors presented a full characterization using chromatographic methodologies.

Meanwhile, *M. oleifera* is a well-studied plant species, in which all the detected phenolic compounds (Table 4) have been previously identified (Amaglo, Bennett, Lo Curto, Rosa, Lo Turco, Giuffrida, Lo Curto, Crea, & Timpo, 2010; Bennett et al., 2003; Coppin et al., 2013; Karthivashan et al., 2015; Karthivashan, Fard, Arulselvan, Abas, & Fakurazi, 2013; Makita et al., 2016, 2017; Nouman et al., 2016). Thus, to the author's best knowledge, *M. oleifera* with Algerian origin has not been previously reported in literature. Moreover, none of the mentioned authors revealed the exact phenolic profile, but some of the herein identified compounds have been previously described. Therefore, considering the pseudomolecular ion, fragmentation pattern and UV–vis spectra found, the studied herein compounds were tentatively identified. Peak 1^{Mo} and 3^{Mo} ($[M-H]^-$ *m/z* 353) and peak 2^{Mo} ($[M-H]^-$ *m/z* 337) were assigned as 3-*O*- and 4-*O*-caffeoylquinic acid, and 3-*p*-coumaroylquinic acid, respectively. These assumptions were taken into account, due to the hierarchical fragmentation pattern described by Clifford, Johnston, Knight, & Kuhnert, 2003, but also by using literature data described by other authors. Makita et al. (2017) studied *M. ovalifolia* leaves from Namibia using an UPLC-ISCID-MS/MS, and profiled various isomers of chlorogenic acids in the aqueous methanol (80%) extract, which allowed the positive identification of compounds 1^{Mo}, 2^{Mo} and 3^{Mo}. Nouman et al. (2016) revealed the presence of compounds 1^{Mo} and 2^{Mo} in the hydromethanolic extracts (70%) of *M. oleifera* seeds from cultivars collected from China and Pakistan. While, Bennett et al. (2003) only revealed the presence of compound 1^{Mo} in the methanolic extract (70% v/v) of *M. oleifera* young leaves from many locations in Nicaragua and Malawi. This compound (peak 1^{Mo}) was also present in the methanolic extract (70%) of the Ghanaian *M. oleifera* stems, leaves, and flowers (Amaglo et al., 2010).

The remaining compounds refer to flavonoid derivatives, such as quercetin (λ_{max} around 350 nm and an MS^2 fragment at *m/z* 301), kaempferol (λ_{max} around 348 nm, MS^2 fragment at *m/z* 285), isorhamnetin (λ_{max} at 354 nm, MS^2 fragment at *m/z* 315), and a flavone, apigenin (λ_{max} around 336 nm, MS^2 fragment at *m/z* 269). Compounds 5^{Mo} (quercetin-3-*O*-rutinoside), 6^{Mo} (apigenin-6-*C*-hexoside), 7^{Mo} (quercetin-3-*O*-glucoside), and 10^{Mo} (quercetin-3-*O*-rhamnoside), were positively identified in comparison with the commercial standards. While compounds 8^{Mo} and 9^{Mo} ($[M-H]^-$ *m/z* 549), 11^{Mo} ($[M-H]^-$ *m/z* 533), and 12^{Mo} ($[M-H]^-$ *m/z* 563) were identified as malonyl-glucoside derivatives of quercetin, kaempferol and isorhamnetin, respectively. Finally, compound 4^{Mo} ($[M-H]^-$ *m/z* 593) was identified as apigenin-6,8-*C*-diglucoside, taking into the findings described above for *A. leucotrichus* extracts.

Quercetin-3-*O*-(6″-malonyl-glucoside) was the most abundant compound present in *M. oleifera*, followed by the phenolic acid, 3-*O*-caffeoylquinic acid.

Makita et al. (2016) studied an 80% aqueous methanol extract of *M. oleifera* from three different locations in Namibia. These authors profiled the phenolic composition using an UHPLC-ESI-qTOF-MS system, revealing the presence of 17 compounds of which five were found in the

Table 4
Retention time (Rt), wavelengths of maximum absorption in visible region (λmax), mass spectral data, tentative identification and quantification of phenolic compounds in the extracts of *M. oleifera* leaves.

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	References	Quantification (mg/g extract)		*p*-Students *t*-test
							Hydroethanolic extracts	Decoction extract	
1^{Mo}	4.9	322	353	191(100), 179(47), 173(5), 161(3), 135(10)	3-*O*-Caffeoylquinic acid	Amaglo et al. (2010), Bennett et al. (2003), Makita et al. (2017) and Nouman et al. (2016)	6.5 ± 0.2	7.3 ± 0.1	0.001
2^{Mo}	6.0	313	337	191(8), 173(5), 163(100), 155(5), 137(3), 119(5)	3-*p*-Coumaroylquinic acid	Makita et al. (2017), and Nouman et al. (2016)	2.1 ± 0.1	1.8 ± 0.1	0.005
3^{Mo}	6.3	326	353	191(20), 179(55), 173(100), 161(5), 135(8)	4-*O*-Caffeoylquinic acid	Makita et al. (2017) and Nouman et al. (2016)	4.83 ± 0.03	3.0 ± 0.4	< 0.001
4^{Mo}	10.0	338	593	473(100), 383(15), 353(25)	Apigenin-6,8-*C*-diglucoside	Karthivashan et al. (2015) and Makita et al. (2016)	2.65 ± 0.03	1.41 ± 0.08	< 0.001
5^{Mo}	17.9	352	609	301(100)	Quercetin-3-*O*-rutinoside	Amaglo et al. (2010), Bennett et al. (2003), Coppin et al. (2013) and Makita et al. (2016)	1.52 ± 0.01	1.257 ± 0.002	< 0.001
6^{Mo}	18.5	336	431	341(27), 311(100)	Apigenin-6-*C*-hexoside	Karthivashan et al. (2015, 2013), Makita et al. (2017) and Nouman et al. (2016)	0.8 ± 0.1	0.40 ± 0.01	0.001
7^{Mo}	19.1	351	463	301(100)	Quercetin-3-*O*-glucoside	Amaglo et al. (2010), Bennett et al. (2003), Coppin et al. (2013), Karthivashan et al. (2015, 2013), Makita et al. (2016) and Nouman et al. (2016)	2.75 ± 0.02	1.50 ± 0.01	< 0.001
8^{Mo}	20.1	350	549	505(10), 463(27), 301(100)	Quercetin-3-*O*-(6″-malonyl-glucoside)	Amaglo et al. (2010), Bennett et al. (2003), Coppin et al. (2013) and Makita et al. (2016)	13.5 ± 0.5	6.4 ± 0.2	< 0.001
9^{Mo}	21.1	350	549	505(14), 463(33), 301(100)	Quercetin-3-*O*-(X″-malonyl-glucoside)	Amaglo et al. (2010), Bennett et al. (2003) and Coppin et al. (2013)	2.5 ± 0.1	1.6 ± 0.1	< 0.001
10^{M}	22.6	352	447	301(100)	Quercetin-3-*O*-rhamnoside	Nouman et al. (2016)	1.50 ± 0.02	1.262 ± 0.001	< 0.001
11^{Mo}	24.6	343	533	285(100)	Kaempferol-3-*O*-(6″-malonyl-glucoside)	Amaglo et al. (2010), Bennett et al. (2003), Coppin et al. (2013) and Makita et al. (2016)	3.0 ± 0.1	2.03 ± 0.01	< 0.001
12^{Mo}	25.9	350	563	285(100)	Isorhamnetin-3-*O*-(6″-malonyl-glucoside)	Amaglo et al. (2010)	1.84 ± 0.04	1.52 ± 0.06	< 0.001
					Total phenolic acids		13.5 ± 0.3	12.1 ± 0.6	0.006
					Total flavonoids		30.0 ± 0.7	17.4 ± 0.6	< 0.001
					Total phenolic compounds		43.5 ± 0.4	29 ± 1	< 0.001

Values expressed in mean values ± standard deviation. Four phenolic compounds were used for quantification: compounds 1^{Mo} and 3^{Mo}- chlorogenic acid (y = 168823x − 161172; R^2 = 0.9999; LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL); compound 2^{Mo}- *p*-coumaric acid (y = 301950x + 6966.7; R^2 = 0.9998; LOD = 0.68 μg/mL; LOQ = 1.61 μg/mL); compounds 4^{Mo} and 5^{Mo}- apigenin 6-*C*-glucoside (y = 107025x − 61531; R^2 = 0.9989; LOD = 0.19 μg/mL; LOQ = 0.63 μg/mL); and compounds 5^{Mo}, 7^{Mo}, 8^{Mo}, 9^{Mo}, 10^{Mo}, 11^{Mo} and 12^{Mo} - quercetin 3-*O*-glucoside (y = 34843x – 160173; R^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL).

Table 5
Cytotoxic and anti-inflammatory properties of *A. leucotrichus* and *M. oleifera* decoctions and hydroethanolic extracts.

		A. leucotrichus		*M. oleifera*		
Cytotoxicity	Cell lines	Hydroethanolic	Decoction	Hydroethanolic	Decoction	Ellipticine
Growth inhibition values (GI_{50}, μg/mL)	NCI-H460	179 ± 3c	> 400	226.5 ± 0.8b	235.9 ± 0.4a	1.03 ± 0.09
	HeLa	79.8 ± 0.2*	> 400	> 400	142.9 ± 0.9*	1.91 ± 0.06
	HepG2	126.8 ± 0.8b	> 400	99.2 ± 0.4c	199.2 ± 0.7a	1.1 ± 0.2
	MCF-7	54 ± 1c	> 400	192 ± 3a	186.0 ± 0.8b	0.91 ± 0.04
	PLP2	239.5 ± 0.3c	> 400	327 ± 1a	313 ± 4b	3.2 ± 0.7
Anti-inflammatory						
Nitric oxide NO-production (EC_{50}, μg/mL)	RAW264.7	251 ± 14c	> 400	294 ± 10b	321 ± 8a	16 ± 1

GI_{50} values (mean ± SD) correspond to the sample concentration achieving 50% of growth inhibition in human tumor cell lines or in liver primary culture PLP2. NCI-H460: Non-small cell lung carcinoma, HeLa: Cervical carcinoma, HepG2: hepatocellular carcinoma, MCF-7: Breast carcinoma. EC_{50} values (mean ± SD) correspond to the sample concentration achieving 50% of the inhibition of NO-production. RAW264,7: Murine macrophages. In each row different letters mean significant differences between the more than two species ($p < 0.05$). When only two samples were present a *p*-Students *t*-test was used to determine the significant difference with $\alpha = 0.05$.

* Means a significant difference between the samples ($p < 0.05$).

present study (compounds 4^{Mo}, 5^{Mo}, 7^{Mo}, 8^{Mo}, and 11^{Mo}). While, Nouman et al. (2016) profiled three of the identified compounds (6^{Mo}, 7^{Mo} and 10^{Mo}) in the hydromethanolic extract (70%) of *M. oleifera* seeds obtained from different cultivars collected from China and Pakistan. Bennett et al. (2003) studied *M. oleifera* ethanolic extract (70%) from Nicaragua, Malawi, Tanzania and Senegal using an LC-UV–Vis-ESI-MS system. These authors presented five compounds (4^{Mo}, 5^{Mo}, 7^{Mo}, 8^{Mo} and 9^{Mo}) in common with the herein studied extracts. Additionally, Karthivashan et al. (2013) revealed the presence of two of the identified compounds (6^{Mo} and 7^{Mo}) in the hydroethanolic extracts of *M. oleifera* leaves from Malaysia, while compounds 4^{Mo} and 6^{Mo} were described in another work, using the aqueous extracts of the same species from Malaysia (Karthivashan et al., 2015). Moreover, Amaglo et al. (2010) and Coppin et al. (2013) identified compounds 5^{Mo}, 7^{Mo}, 8^{Mo}, 9^{Mo} and 11^{Mo} in *M. oleifera* leaves from Ghana and *M. oleifera* leaves collected from African sub-Sahara, respectively. Nevertheless, Amaglo et al. (2010) also detected compounds 4^{Mo} and 12^{Mo}, respectively.

According to the phenolic compounds' quantification results (Tables 3 and 4), both plant species revealed higher values for the hydroethanolic extract in comparison to the decoction extract. *A. leucotrichus* revealed a high concentration in flavonoids (24.2 ± 0.4 mg/g extract), due to the presence of luteolin derivatives, such as luteolin-7-*O*-glucoside (11.7 ± 0.5 mg/g extract) and luteolin-*O*-(malonyl-hexoside) isomer 2 (8.87 ± 0.03 mg/g extract). Moreover, *M. oleifera* hydroethanolic extracts presented a higher amount of total phenolic compounds (43.5 ± 0.4 mg/g extract), mostly due to the presence of total flavonoids (30.0 ± 0.7 mg/g extract), and especially to the presence of flavonol glycosides, such as quercetin-3-*O*-(6″-malonyl-glucoside) (13.5 ± 0.5 mg/g extract), kaempferol-3-*O*-(6″-malonyl-glucoside) (3.0 ± 0.1 mg/g extract) and quercetin-3-*O*-glucoside (2.75 ± 0.02 mg/g extract).

The same quantitative relation was previously reported for the hydroethanolic extract (70%) of *M. oleifera* young leaves from Managua (Nicaragua) recording, 10.8, 2.6, and 4.1 mg/g dw of the same compounds, respectively (Bennett et al., 2003). Moreover, these authors also reported that the *M. oleifera* young leaves extract (ethanolic 70%) from Dakar (Senegal) and Zion Church (Malawi), also contained high amounts of quercetin-3-*O*-glucoside (6.3 mg/g and 2.6 mg/g dw respectively) and quercetin-3-*O*-(6″-malonyl-glucoside) (3.5 mg/g and 3.9 mg/g dw respectively). Amaglo et al. (2010) studied the ethanolic extract (70%) of *M. oleifera* leaves and although reporting the presence of quercetin-3-*O*-(6″-malonyl-glucoside) (1.5 mg/g fw) and quercetin-3-*O*-glucoside (0.65 mg/g fw), these were not the main compounds present. Out of the three main flavonoids present in our sample, only quercetin-3-*O*-glucoside was present in several cultivars of *M. oleifera* leaves from Pakistan, thus the quantities were not comparable, and apigenin-8-*C*-glucoside was reported as the main flavonoid (Nouman et al., 2016).

An appreciable amount of total phenolic acids was also detected in the current study, being 3-*O*-caffeoylquinic acid (3-CQA), followed by 4-*O*-caffeoylquinic acid (4-CQA), and 3-*p*-coumaroylquinic acid (3-CoQA) the most abundant compounds, respectively. 3-CQA was also found as the main phenolic acid in *M. oleifera* old and young leaves (6.2 and 8.9 mg/g dw, respectively) as reported by Bennett et al. (2003), which also showed similar values for this compound in other *M. oleifera* from different regions. Amaglo et al. (2010) also revelaed the presence of 3-CQA (0.8 mg/g fw) and 4-CQA (1.26 mg/g fw) in *M. oleifera* flowering season leaves. While, Nouman et al. (2016) also identified 3-CQA as the main phenolic acid and smaller amounts of 3-CoQA in different cultivars of *M. oleifera* leaves from Pakistan.

The differences found in the phenolic composition profile and quantification could be due to the geographical origin of the plant and the different parts of the sample used, as also to the different extraction procedures. The applied methodologies for the individual phenolic compounds quantification could also lead to possible differences found in the amounts present, because the methodology could differ in their sensitivity and selectivity, and the standard compound used to quantify each one of the phenolic compounds present.

3.3. Bioactive properties evaluation

Herbal-induced cytostatic and cytotoxic effects can be therapeutically effective when directed to attack tumorigenesis proliferations (Kmail, Lyoussi, Zaid, & Saad, 2015). *A. leucotrichus* and *M. oleifera* extracts were screened for their cytotoxic activities using four different cancer cell lines (Table 5). This assay reports the toxicity effects of the plant extracts against cell proliferation and metabolism (Son & Anh, 2013). The GI_{50} values for the hydroethanolic extract were in general lower (higher activity) than the ones exhibited by decoction extracts (Table 5), showing a significant dose dependent cytotoxic effect. The cell lines MCF-7, HeLa, and NCI-H460 were the most susceptible for the hydroethanolic extract of *A. leucotrichus*, while HepG2 was more susceptible to *M. oleifera* hydroethanolic extract. *A. leucotrichus* decoction did not show any cytotoxic effects, while *M. oleifera* decoction extract presented moderated anti-proliferative activity. Nevertheless, regarding the cytotoxic effects in non-tumor cells, all the extracts, with the exception of *A. leucotrichus* decoction, presented certain inhibition effect, thus the GI_{50} value were higher than the ones needed to inhibit the human tumor cell lines.

Therefore, these results indicate that both plant extracts (except *A. leucotrichus* decoction) induced cytotoxic effects in all human tumor cell lines, and could be related to the diverse phenolic constituents of each plant extract. The mechanism of action of phenolic compounds has been related to the cell cycle block or delay in the cell proliferation,

Table 6
Antibacterial activity (MIC and MBC, mg/mL) of *M. oleifera* and *A. leucotrichus* decoctions and hydroethanolic extracts.

	A. leucotrichus				*M. oleifera*				Antibiotics					
	Hydroethanolic		Decoction		Hydroethanolic		Decoction		Ampicillin		Imipenem		Vancomycin	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria														
Escherichia coli	5	> 20	5	> 20	10	> 20	20	> 20	< 0.15	< 0.15	< 0.0078	< 0.0078	nt	nt
Klebsiella pneumoniae	10	> 20	20	> 20	20	> 20	20	> 20	10	20	< 0.0078	< 0.0078	nt	nt
Morganella morganii	5	> 20	20	> 20	5	> 20	10	> 20	20	> 20	< 0.0078	< 0.0078	nt	nt
Proteus mirabilis	20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	< 0.15	< 0.15	< 0.0078	< 0.0078	nt	nt
Pseudomonas aeruginosa	2.5	> 20	20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	0.5	1	nt	nt
Gram-positive bacteria														
Enterococcus faecalis	> 20	> 20	> 20	> 20	10	> 20	10	> 20	< 0.15	< 0.15	nt	nt	< 0.0078	< 0.0078
Listeria monocytogenes	5	> 20	20	> 20	2.5	> 20	2.5	> 20	< 0.15	< 0.15	nt	nt	nt	nt
MRSA	5	> 20	5	> 20	10	> 20	20	> 20	< 0.15	< 0.15	nt	nt	< 0.0078	< 0.0078
MSSA	1.25	> 20	5	> 20	0.625	> 20	5	> 20	< 0.15	< 0.15	nt	nt	0.25	0.5

MRSA – Methicillin resistant *Staphylococcus aureus*; MSSA – methicillin susceptible *S. aureus*; MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration, nt – not tested.

cytotoxicity based on DNA damage leading to an apoptosis, inhibition of angiogenesis, and interactions with topoisomerase (Carocho & Ferreira, 2013). Edwinanto et al. (2018) indicated that the flavonoids (quercetin, kaempferol and myricetin) found in Moringa leaves can induce apoptosis through intrinsic pathways by inhibition of mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase 1/2 (ERK 1/2), c-Jun N-terminal protein kinase 1 (JNK), and protein kinase C (PKC). Other authors (Tiloke et al., 2013) found Moringa leaves to be a potential source of antitumor agents, especially due to the presence of niaziminin, a thiocarbamate, that exhibits inhibition of tumor-promoter induced Epstein–Barr virus activation. In a study using Swiss mice, *M. oleifera* leaf paste increased glutathione-S-transferase (GST), as well as the crude ethanolic extract of seeds, which exhibited anti-tumor potential against Epstein-Barr virus-early antigen (EBV-EA) (Goyal, Agrawal, Goyal, & Mehta, 2007). Additionally, aqueous extract of *M. oleifera* induced apoptosis in KB carcinoma cells (Sreelatha & Padma, 2011), and can act as an anticancer agent in lung cancer by inducing cellular apoptosis and subsequent cell death (Tiloke et al., 2013). The phytochemical constituents of *M. oleifera* extracts, such as glucosinolates, isothiocyanates, niazimicin, niaziminin, and quercetin, were shown to possess anti-proliferative effects in a diverse of cell lines (Purwal, Pathak, & Jain, 2010; Tiloke et al., 2013). In addition, niazimicin and 4-(4′-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate were identified as natural anticancer agents and were positively compared with the recommended chemotherapeutic drug. These phytochemicals enhanced the activity of cellular prostatic acid phosphatase and possessed less toxicity, thus showing potential as a potent and safe natural agent in prostate cancer therapy and drug design (Inbathamizh & Padmini, 2011).

Macrophages (Kupffer cells) play an essential role in the anti-inflammatory process as well as in xenobiotic metabolism (Mahajna et al., 2015). The binding complex of LPS to the cluster of differentiation (CD) 14 protein triggers a signal cascade involving a nuclear factor to enhance the expression of inflammation-related genes (Guo, Sakulnarmrat, & Konczak, 2014). In general, the results presented in Table 5, showed that the studied plant extracts, effectively reduced the expression of ant-inflammatory enzymes iNOS in LPS-activated murine macrophages, and this reduction was accompanied by the decrease of the nitric oxide (NO) levels (lower EC_{50} values). To verify whether the reduction of the decrease of NO production, was measured employing the Griess reagent system kit. Among both extracts, the hydroethanolic showed the highest inhibitory effects in NO production by LPS-stimulated RAW264.7 cells (EC_{50} = 251 μg/mL for *A. leucotrichus* and 294 μg/mL for *M. oleifera*), while *M. oleifera* leaves decoction was less active (321 μg/mL), and *A. leucotrichus* decoction did not revealed anti-inflammatory activity. Several authors (Catarino, Talhi, Rabahi, Silva, & Cardoso, 2016; Sobral et al., 2016) reported that plant phenolic compounds act as anti-inflammatory molecules by different levels of inhibitory action in the LPS-induced NO production. The polyphenols that have a higher number of hydroxyl groups (Gardi et al., 2015), such as the ones identified in this study, are probably the activators of NO-production inhibition. Moreover, a hydromethanol extract (70:30, v/v) of *M. oleifera* leaves collected from sub-Saharan Africa was found to exhibit an anti-inflammatory activity (Coppin et al., 2013). According to these authors, the main molecules detected in the extract were flavonoid glycosides, especially quercetin and kaempferol glycoside derivatives, being related to the anti-inflammatory activity revealed by the extract. Likewise, Upadhyay et al. (2015) also reported anti-inflammatory activity using a crude ethanolic extract of *M. oleifera* dried seeds, finding an excellent inflammation inhibition. These authors used a carrageenan induced inflammation in the hind paw edema of mice, and the extract revealed 85% inhibition at a dose of 3 mg/kg of body weight. Moreover, the same effeteness was noted for the infusions extract of flowers, leaves, roots, and bark, and the seeds, showing an anti-inflammatory and diuretic activity at a concentration of 1000 mg/kg of body weight. To the author's best knowledge, the cytotoxicity and anti-inflammatory activity of *A. leucotrichus* has not been previously studied.

Certain established drugs have become less effective against many infections agents (Gupta et al., 2017), and antibiotic resistance becomes an alarming issue, which turns the discovery of new active antibacterial agents a ground breaking research (Stojković et al., 2013). The decoction and hydroethanolic preparations of *A. leucotrichus* and *M. oleifera* were evaluated for their antibacterial potential against multi-resistant pathogenic strains (Table 6), such as Gram-positive bacteria (*E. faecalis, L. monocytogenes, S. aureus*) and Gram-negative bacteria (*E. coli, K. pneumoniae, M. morganii, P. mirabilis* and *P. aeruginosa*). The results shown in Table 6, indicate that the extracts were moderately active against both Gram-negative and Gram-positive bacteria, being the later more sensible to both plant extracts. In general, the hydroethanolic extracts were more effective than the decoction extracts. *M. oleifera* hydroethanolic extract showed a good antibacterial activity, especially against *S. aureus* (MIC = 0.625 mg/mL) and *L. monocytogenes* (MIC = 2.5 mg/mL). As mentioned above, *M. oleifera* decoction showed to be relatively less active against the studied bacteria. Likewise, *A. leucotrichus* hydroethanolic extract revealed lower MIC values than the decoctions. However, both extracts obtained from the plant had no effect on *P. mirabilis* and very low effect on *K. pneumoniae,* even at the highest concentration tested (20 mg/mL). The differences observed between the Gram-negative and Gram-positive bacteria response could be due to cell membrane permeability or either to the genetic factors,

the outer membrane of the Gram-negative bacteria acts as a barrier to many environmental substances including antibiotics (Gyawali & Ibrahim, 2014). MBC values were not obtained for any of the plant extracts tested (values > 20 μg/mL).

Several extracts of *A. leucotrichus* fruits using different solvents (methanol, ethanol, butanol, dimethyl ether and dichloromethane) from Béchar-Algeria were evaluated for their antimicrobial activity against eight bacterial strains and three human pathogenic fungi, revealing an interesting antimicrobial profile (Sebaa et al., 2018).

Also, the antimicrobial activity of a petroleum ether extract obtained from *M. oleifera* leaves from India, was tested against pathogenic bacteria and showed a maximum inhibition of *P. aeruginosa,* followed by *P. vulgaris*, and *B. subtilis* (Anupama, Pranay, Varma, & Kumar, 2013). Moreover, the same authors observed a maximum inhibition for *E. coli* with the chloroform extract, which had comparable results to Gentamycin. The antimicrobial activity of the aqueous extract from *M. oleifera* seeds, was also investigated against several bacterial stains, where the 4(α-L-rhamnosyloxy) benzyl isothiocyanate was identified to act on several bacteria and fungi, inhibiting the growth of *Mycobacterium phlei*, *Bacillus subtilis*, and *S. aureus* (Goyal et al., 2007).

According to the phenolic compounds composition reported above, flavonoids glycoside derivatives, especially apigenin and quercetin derivatives have been found to be important factors during the bacterial pathogenic process (Gyawali & Ibrahim, 2014). These compounds may act as antibiotics, due to their ability to complex with the extracellular and soluble proteins, and also with the bacterial cell walls, often leading to their inactivation and loss of function (Cushnie & Lamb, 2005; Wink, 2015). According to a previous work (Ziani et al., 2018), the location and number of hydroxyl groups on the phenol group, could also be related to the microorganisms growth inhibition, with evidence that with the increase of hydroxylation, the antimicrobial potential is also higher.

4. Conclusion

This study aimed to chemically characterize the nutritional value and chemical constituents of two edible plants *A. leucotrichus* and *M. oleifera*, and further explore two types of extracts (hydroethanolic and decoctions) regarding their phenolic composition and bioactive potential in terms of cytotoxicity, anti-inflammatory, and antibacterial activities. High levels of proteins, soluble sugars (glucose, fructose and sucrose), PUFA (α-linoleic, oleic and linoleic acids) and ashes were present in both plants, indicating that these species could be included in the daily diet as sources of nutrients. The chromatographic analyses showed that both plants are sources of bioactive compounds, especially with the identification of luteolin-*O*-(malonyl-glucoside) as the main compound in *A. leucotrichus*, and quercetin-3-*O*-(6″-malonyl-glucoside) and 3-*O*-caffeoylquininc acid in *M. oleifera.* The presence of these phenolic compounds in both plant species gave insight into understanding the significant pharmacological properties that were demonstrated, especially by the hydroethanolic extracts, which seemed to be more active against the tested tumor cell lines and murine macrophages RAW 264.7 NO production, and inhibited the growth of clinical MDR bacteria. Thus, these species could be proposed as new food ingredients with nutraceutical and functional properties, validating the medicinal principle behind the ethnobotanical practices associated to these plant species.

However, it is worthwhile to further investigate *in*-vivo biological effects, in order to identify the most promising extracts and to isolate the active compounds.

Ethic statement

This statement declares that the manuscript entitled “Detailed chemical composition and functional properties of *Ammodaucus leucotrichus* Cross. & Dur. and *Moringa oleifera* Lamarck”, does not involve the use of human subjects and animal experiments.

Conflict of interest

The authors declared that there is no conflict of interest.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013), L. Barros and R.M. Calhelha contract. The authors are also grateful to the Interreg España-Portugal for financial support through the project 0377_Iberphenol_6_E.

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