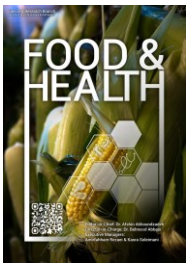

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Physico-chemical and nutritional value characterization of Mangrove leaf from Iran
Running title: Physico-chemical and nutritional value of Mangrove leaf

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ABSTRACT

In this study, the well-recognized tropical leaf of mangrove obtained from Hara forests of Qeshm Island located on the southern coast of Iran was investigated for its physicochemical properties and bioactive agents. The crude fiber, ash, fat, moisture, carbohydrate, and protein of Mangrove leaf were respectively obtained 16.36±0.8, 6.33±0.2, 2.06±0.5, 6.7±0.03, 44.32±0.65 and 24.23±0.3 (g/100 g). Total phenol content (TPC), total tannin content (TTC), total flavonoid content (TFC), and antioxidant activity of leaf extract were respectively gained 98.11±0.21 mg GA/g leaf, 3.4±1.15 mg GA/g leaf, 118.75±1.50 mg QE/g leaf, and 91.63±1.61 %. The predominant sterol of Mangrove leaf oil was found β-sitosterol (71.598±0.015 %). The most important fatty acids composition detected in Mangrove leaf oil were oleic acid (30.32±0.45 %), linolenic acid (23.13±0.21%), linoleic acid (18.23±0.24%), palmitic acid (12.83±0.15 %), and stearic acid (5.09±0.11%), palmitoleic acid (0.57±0.10%), respectively. These results showed that Mangrove leaf could be used as a therapeutical plant.

1. Introduction

The incidence of diabetes and other diseases calls for finding foods with complex carbohydrates and rich in phytochemical compounds such as polyphenols, healthy fatty acids, and dietary fibers (1,2). Approximately, all natural antioxidants are extracted from plant substances including vegetables, spices, different parts of herbs, fruits, and seeds which after detection of their bioactive components proposed for applying in food industry or medicin (3,4).

Mangrove is known to be dominant for the tropical and subtropical coastal areas and well recognized by its high productivity and growth potential even in poor nutrient soil and harsh conditions like high salinity, water log, acidity, etc (4). A very unique aspect of Mangrove is the adaptation and the ability to get along with the environmental changes especially through surface elevation and lateral migration over time to reach higher elevations and overcome the problematic effects of sea level rise on one side and other climate related changes such as temperature, storminess, salinity and so on via showing high tolerance and resistance to damage caused by such environmental factors (4,5). Functionally, mangrove constitutes the input for natural sedimentary rocks thus offers more healthy and desired soil. It is worth to mention that the constituents of mangrove that contribute to the formation of organic matter in the surrounding environment are mainly the fatty acids (6). In addition Mangrove is of ecological importance and is a link between terrestrial and marine ecosystems in terms of maintenance and stability of various organisms through producing the organic matter necessary for survival of local environment and that can be transported (7,5). Almost every part of any tree from leaves to fruits is of important value for medicine and food (8,9). The *R. mucronata* mangrove leaf has a thick layer of wax which can protect leaf against extreme radiation from sunlight and disease and also keep water in the leaf' tissue (10). There are some chemical compounds in mangrove leaves which showed the antioxidant (11,12), anti-inflammatory (13,14) and antidiabetic (15,16) functions. Jha et al. (2023) optimized and characterized physicochemical of extracted polysaccharide purified from Sonneratia caseolaris Mangrove leaves which the extracted polysaccharide proposed for applying in functional food, pharmacological and cosmetic industries (17).

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In this study, Mangrove leaf which sampled from Harra Island (Avicennian marina, as known locally "harra" tree) located in Qeshm of Iran was investigated. None of the previous analytical studies has reported its composition or possible applications. the famous Persian Mangrove leaf has been subjected to this study for some physical/chemical properties and nutritional value to anticipate the possible future applications of this leaf..

2. Experimental

2.1. Materials

All the chemicals applied in the current study including, n-hexane (99.99%), orthophosphoric acid (85%), methanol (99.9%), and acetonitrile (99.99%) were of HPLC grade and purchased from the Merck (Darmstadt, Germany). The reference materials of fatty acid methyl esters (FAMEs) (Supelco® 37 Component FAMEs methylene chloride), sterols (including stigmasterol, β-sitosterol, campesterol, stigmastenol and etc.) and polar phenol compounds (including vanillin, p-coumaric acid, caffeic acid, tyrosol, vanillic acid, quercetin, gallic acid, luteolin, cinnamic acid, syringic acid, catechin, and ferulic acid) were purchased from the Sigma-Aldrich (Los Angeles, USA). Other materials like glacial acetic acid, and potassium iodide were also purchased from the Merck Company.

2.2. Preparation of Samples

The samples **(**Mangrove leaves**)** were collected from Harra Island (*Avicennian marina,* as known locally "harra" tree) located in Qeshm of Iran. Samples were collected in the winter of 2023. Samples were transferred to the lab immediately, dried at ambient temperature, and finely grounded. Then the powders were kept in a dark container away from light until required for analysis.

2.3. Oil extraction

The oil extraction was done using the ISO 659: 2009 method. Briefly, an empty flask was heated at 103 ºC for 1h, cooled in a desiccator, and weighted carefully. Then, 150 g of dried Mangrove powder was transferred into a 500 ml flask containing 350 ml n-hexane and shaken for 30 h at 150 rpm in the darkness at room temperature. The flask content was filtered through a 0.45 µm propylene filter paper and then 100 mL hexane was used for any possible residue on the filter paper. The obtained filtrate was poured into the empty weighted flask (as mentioned above) and the solvent was removed using a rotary evaporator (Heidolph, Heizbad WB, Germany) for 45-60 min at 35 oC). Finally, the flask was re - desiccated and weighed to figure out the weight of the extracted oil based on the difference in weight.

2.4. Determination of physico-chemical properties

The following standard official methods presented by Association of Official Analytical Chemists (AOAC) were applied for determination of the Mangrove leaf powder: crude fat by Soxhlet apparatus (method No. 960.39), ash content by ignition (method No. 923.03), moisture by vacuum oven (method No. 925.10), crude protein or total nitrogen by the Kjeldhal instrument using 6.25 as a conversion factor for calculating of protein content (method No. 979.09) and crude fiber (method No. 978.10). In addition, total carbohydrate content was calculated by subtracting the sum percentage of moisture, protein, lipid and ash from 100 (18).

2.5. Determination of fatty acid composition

According to the ISO 12966-2:2017 method the occurring fatty acids were isolated and prepared for the chromatographic analysis of the fatty acid profile. Briefly, 0.1 g of sample (extracted oil from mangrove leaf) was weighted and 200 µL of methanolic potassium hydroxide (2 N) was added to it and vortexed, then 2 mL of hexane was added and vortexed, after one-minute upper layer injected to GC.

A Young Lin 6100 (Korea) Gas chromatography (GC) instrument equipped with Cp-Sil88 analytical column (100 m × 0.25 mm × 0.25 µm) and flame ionization (FID) detector was put into use for the identification and analysis of the distinct individual obtained peaks in accordance with ISO 12966-1:2014 standard analytical data. The injector and detector temperatures were set at 240 oC and 260 oC, respectively and the column was isothermally programmed at 175 oC. The injection volume was 1 µL.

2.6. Determination of sterols

The sterol composition of the extracted Mangrove oil was studied according to the ISO12228:2014 method, using thin layer chromatography. The peaks of cholesterol, stigmasterol, Δ-7-campesterol, campesterol, sitostanol, β-sitosterol, Δ-5-23-stigmastadienol, Δ-5-avenasterol, Δ-7-avenasterol, Δ-7-stigmastenol, and Δ-5-24-stigmastadienol in the Mangrove leaf oil were identified by comparing the retention times of the peaks of individual sterols with those of standard sterols obtained from GC-FID (Yung Lin 6500, Korea). For this purpose, a fused-silica capillary column with the dimensions of 30 m and 0.32 mm i.d. (SE-52 or SE-54) and a uniform thickness of 0.30 µm was used. An isothermal program was applied at 265 ºC. The detector and injector temperatures were set at 300 and 280 °C, respectively. The split ratio was adjusted at 1:20. The hydrogen was applied as a carrier gas at a flow rate of 1 mL/min. Total sterol was expressed as mg/100 g of the leaf oil with respect to the internal standard (5-α-Cholestane).

2.7. Extraction of phenolic compounds

The phenolic components of Mangrove leaf oil extract were extracted and isolated using a methanol: water solution of (80:

20) proportion following the explained procedure by Ghreishi Rad et al., (2023) for phenols extraction.

2.8. Determination of Total Phenol Content (TPC)

TPC was determined according to the colorimetric assay (19). Briefly, 200 µL of extract, 800 µL of deionized water, and Folin-Ciocalteu reagent (100 µL) were mixed and incubated for 5 min at room temperature. Then, sodium carbonate (Na 2CO3, 300 µL) (20% (w/v)) was added to the mixture and for 2 h incubated again in a darkness at 25 °C. Then, absorbance of the solution at 765 nm was obtained by UV/Vis Spectrometer (Lambda 25–Perkin Elmer, USA). The calibration curve of gallic acid (GA) standard was obtained in the range of 0–200 µg/mL. TPC was obtained as mg GA equivalent per g dry matter.

2.9. Determination of Total Tannin Content (TTC)

For determination of TTC of the extract of Mangrove leaf, UV/Vis spectrometer (Lambda 25-Perkin Elmer, USA) was applied at 725 nm. TTC was reporeted based on the GAE/g dry matter. Briefly, 100 µL of extract was added to distilled water (750 µL), then, Folin-Ciocalteu reagent (500 µL) and sodium carbonate (1000 µL, 35 % (w/v)) were mixed then shaked at room temperature. The mixture was diluted to 10 mL with distilled water and incubated for 30 min (20).

2.10. Determination of Total Flavonoid Content (TFC)

TFC of leaf extract of Mangrove was measured with the colorimetric assay and expressed as mg quercetin equivalent (QE)/g dry matter (calibration curve of quercetin was obtained in the range of 0–100 µg/mL). Briefly, 150 µL of sodium nitrite ($NaNO_2$, 5 % (w/v)) was added to 200 µL of Mangrove leaf extract and then incubated for 6 min at room temperature. Then, AlCl $3.6H_2O$ (150 µL, 10 % (w/v)) was added and incubated again for 6 min. Then, NaOH solution (800 µL, 10 % (w/v)) was added to the mixture and incubated at 25 °C for 15 min. The control sample (blank), instead of Mangrove leaf extract, was distilled water. The absorbance of blank and sample was recorded at 510 nm (19).

2.11. Determination of antioxidant Activity Percentage (AA%)

The antioxidant activity percentage of the sour cherry kernel oil extract was determined according to the described method by Khadem et al. (2019). 1 mL of DPPH solution (0.1 mM) was added to 3 mL of sour cherry kernel oil extract and, then, put it in a dark place for 30 min at room temperature. The absorbance of the sample was determined at 517 nm by UV/Vis spectrophotometer. Comparison of DPPH radical scavenging activity to the control was obtained using DPPH scavenging activity (%) formula:

$$AA\% = \frac{A_0 - A_1}{A_o}$$

where A_0 and A_1 are control absorbance and sample absorbance, respectively.

2.12. Statistical analysis

All obtained results were statistically analyzed using Excell software and displayed as mean ± standard deviation (SD). The significance of the difference was explicated at p-values ≤0.001. All determination was made in triplicate.

3. Results and Discussion

3.1. Physico- chemical properties of mangrove leaf

The Ash, fat, moisture, and protein compositions of Mangrove leaf powder are given in Table 1. It is important to mention that the crude fiber refers to one type of fiber; the insoluble dietary fiber portion which is left behind and can't be extracted upon the solvent extraction process. Dietary fibers in general can help boost the immune system, attenuate blood glucose and lipid risks, and present anticancer and antitumor activity (21). According to Table 1, It can be found that the obtained fiber content (16.36±0.8%) comparable and somehow higher than that of popular cereals such as wheat, millet, rice and so on, implying that it is of high energy value as food (21,9). In addition, Table 1 displayed a low level of ash (6.33± 0.2%) which implied that the Mangrove leaf is edible and can be taken as a supplement and therapeutic product (22). Finally, regarding the carbohydrate (44.32±0.65%) and protein contents (24.23±0.3%), Mangrove leaves has appeared to be rich in protein and carbohydrate thus increasing the nutritional significance of its uptake as a food supplement and most important looking a good product as food and no-food item for economical and investment purposes (10). Also, the moisture content of mangrove leaves was obtained 6.70 ±0.03 % which is an activity index for stability and susceptibility to microbial contamination (10).

Table.1. Physicochemical characterizations of the Mangrove leaf.

Physical characterization (g/100g)	Mean±SD
crude fiber	16.36±0.8
Ash	6.33± 0.2
Fat	2.06±0.5
Moisture	6.70 ±0.03
Protein	24.23±0.3
Carbohydrate	44.32±0.65

It was reported that R. mucronata mangrove green leaves had ash (9.32 ±0.61 %), protein content (6.79 ± 0.17 %), total fat (2.62 ± 0.61 %), moisture content (19.95 ± 0.70 %), carbohydrate (61.32 ± 1.77 %) which the amounts of its ash, moisture and carbohydrate were higher than those reported in our study. The mangrove leaves of harra Qeshm Island of Iran contained higher protein and fiber which the high protein of leaves influences on growth fish. The high carbohydrate content showed that these leaves can be considered as a rich source of energy and benefit for immune system if they are

used as a food supplement (23). Also, ash, fat, moisture, fiber, and protein in R. mucronate leaves were reported 1.81 ± 0.13 %, 0.29 ± 0.20 %, 34.91 ± 0.41 % , 0.78 ± 0.65 % and 11.32 ± 0.35 % by Kaur et al., (2019), respectively, which these results were completely different in comparison with our results.

3.2. Antioxidant properties

Polyphenols are a diverse and large class of compounds containing two or more phenolic groups and occurring widely in plant foods. These are considered life span essentials and play a big role in maintaining a long-term healthy body functioning and minimizing or preventing the incidence of diseases through their anti-carcinogenic, anti-mutagenic, antiviral, anti-estrogenic, anti-inflammatory, anti-oxidation and platelet aggregation inhibiting effects (24). The mangrove leaves extract contained a high level of TPC which was higher than cereals and other carbohydrate rich foods. According to the Table 2 given, the antioxidant activity of the Mangrove leaves extract was very high which supports the excessive advantageous properties of the Mangrove leaves in inhibiting and treating human diseases offering a better health life span. Ariyanto et a al., (2019) reported that tanin of R. mucronata mangrove green leaves obtained 2.42 ± 1.39 mg/g and we obtained 3.4 ± 1.15 mg/g dry powder of mangrove leaf. In another study, the tannin contents of R. mucronata mangrove leaves were ranged from 2.26 to 3.86 mg/g (25). Balakrishnana et al., (2016) reported that the tannin content of R. mucronata mangrove leaves obtained between 1.23 and 5,452 mg/g dry matter. There are many factors affecting the TTC such as reproductive development, age and the amounts of regulating compounds as hormones (10). Malik et al., used different solvents like methanol, water, and hexane for extraction of polyphenol content of mangrove leaves and antioxidant activity percentage, TPC and TFC extracts were obtained from 99.28 to 99.33 %, from 2.39 ± 4.97 mg GAE/g to 64.28 ± 3.05 mg GAE/g, from 25.67 ± 0.06 mg QE/g to 37.90 ± 4.76 mgQE/g for five mangrove leaves species such as B sexangula, B. cylindrical, R. apiculata, A. alba and L. racemosa, respectively (26).

Table.2. Phenolic composition and antioxidant activity of the extract of Mangrove leaves.

Phenolic composition	Amount
TPC (mg GA/g dry matter)	98.11 ± 2.55
TTC (mg GA/g dry matter)	3.4 ± 1.15
TFC (mg QE/g dry matter)	118.75 ± 1.50
Antioxidant activity (%)	91.63 ± 1.61

TPC: total phenolic compound, TTC: total tannin content, TFC: total flavonoid content

3.3. Phytosterols

In general, sterols are considered to be minor compounds in vegetable and olive oils and they are detected for distinguishing purity of vegetable oils (27). β- sitosterol is a common phytosterol known for its plenty positive health effects such as anti-inflammatory and antipyretic influences especially in the treatment of lung inflammations, asthma, and bronchospasm via its inhibitory action mode. Above all it prohibits cancer (breast, colon and prostate) and suppresses intestinal cholesterol absorption (18,27). The individual sterol contents of mangrove leaf oil were determined, and results were presented in Table 3. Results showed that the predominant sterols in the mangrove leaf oil were β-sitosterol (71.598±0.015 %) and stigmasterol (21.116±1.0 %), respectively while the other sterols showed zero or very low abundance especially the harmful cholesterol which is not recommended for healthy diets. Hogg and Gillan, (1983) reported sterol compositions of eleven mangrove leaves which results showed that major sterols containing ß-sitosterol and stigmasterol ranged from 47.8 to 80.7 and 4.4 to 28.6 %, respectively.

Table.3. Sterol composition of the Mangrove leaf oil.

Mean±SD	Sterols (%)
Cholesterol	0.0546±0.01
Brassicasterol	0.0753±0.01
Ergosterol	0.0044±0.001
24-Methylenecholesterol	0.0135±0.005
Campesterol	1.44±0.15
Stigmasterol	21.116±1.0
Delta-7-Campesterol	0.0247±0.01
Clerosterol	1.13±0.07
β-sitosterol	71.598±0.015
Sitosatnol	0.087±0.005
Δ-5-avenasterol	3.13±0.25
Δ-5,24-stigmastadienol	0.0989±0.02
Δ-7-stigmastenol	0.927±0.05
Δ-7-avenasterol	0.437±0.04
Erythrodiol	2.456±0.35
Uvaoul	0.086±0.03
Total Sterol (µg/g)	510 ±5.73

3.4. Fatty Acids Analysis

Fatty acids are important constituents of all plant cells, nutrients and metabolites contributing to cell membrane construction, energy production, metabolism, and storage (19,20). The fatty acids composition of mangrove leaves oil is summarized below in Table 4. Results showed that oleic acid

(30.32±0.45 %), linolenic acid (23.13±0.21%), linoleic acid (18.23±0.24 %), and palmitic acid (12.83±0.15 %) were the predominant detected fatty acids in the extracted oil of mangrove leaves, respectively. Results showed that polyunsaturated fatty acids (PUFAs) were higher than monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). This oil is a rich source of omega 3 and omega 6. Hogg and Gillan, (1983) reported fatty acid profiles for eleven mangrove leaf oils that C18:3 (ranged from 32.9 to 46.8 %) had higher levels than C16:0 (ranged from 22.2 to 28.5 %), C18:2 (ranged from 11.1 to 23.6 %) and C18:1 (ranged from 2.6 to 12.0 %), respectively. In general, fatty acids take a main part in many functions of the skin especially the trans-epidermal water loss from skin. Some polyunsaturated fatty acids known as Vitamin F including arachidonic acid linoleic acid and linolenic acid are necessary for the protection and growth of skin. In addition, lauric acid is a potentially inexpensive antimicrobial agent suitable for external application and infection control in hospitals. In conclusion the current study verifies the significance of Mangrove in therapy and medication (19,20).

Table.4. Fatty acid profile examination of Mangrove oil.

Fatty Acid	Mean±SD
C16:0	12.83±0.15
C16:1C	0.57±0.10
C18:0	5.09±0.11
C18:1C	30.32±0.45
C18:2C	18.23±0.24
C18:3C	23.13±0.21
C20:0	2.49±0.10
C20:1C	1.34±0.08
C22:0	1.09±0.05
C24:0	1.83±0.25
C24:1C	0.84±0.02
MUFA	33.07 ±1.78
PUFA	41.36 ±0.45
SFA	23.33 ±0.65

4. Conclusion

In conclusion, Mangrove leaf contains high quantities of carbohydrate and proteins and could be applied as a rich source of these two compounds. Also, results showed that antioxidant activity percentage, flavonoids and total phenol content of mangrove leaf is considerable which highlighted its specialness and uniqueness.

5. Data availability

The datasets are available from the corresponding author on reasonable request.

7. Acknowledgement

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