Study of the Chemical Composition, Amino Acid, Flavonoids and Vitamin C of Moringaoliefera Leaves Extract Grown in AL-Ramadi- Iraq

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Abstract

This study was conducted to reveal the chemical components, amino acids, flavonoids and vitamin C of the aqueous leaves extract of Moringaoliefera plant grown in AL-Ramadi city in Iraq, because these herbal plants have medicinal and nutritional importance.material and method.The leaves were collected from MoringaOleifera tree grown in the home garden, aqueous extract was prepared and GC-MS analysis, amino acid diagnostics, flavonoids diagnosis and vitamin C analysis were performed. Results sixteen chemical constituents were identified in the leaf aqueous extract; The most important of these compounds Decanoic acid(1.14%),17- Octadecynoic acid (29.24%), (7.51%). 12 types of essential and non-essential amino acids were diagnosed.Extraction of phenolic compound showed presence of quercetine(245.7 ppm) and kaemferal(299.6 ppm). Vitamin C content in fresh samples of leaves of M. olifera were determined by the HPLC method was (250.2 ppm).

Keywords: *GC-MS analysis, HPLC, Flavonoides,Vitamines, Aqueous Leaves Extracts, Moringaoliefera.*

Introduction

Moringaoleifera is one of the best known, widely distributed and naturalized species of a monogeneric family Moringaceae[(1)].The tree ranges in height from 5 to 10 m[(2)].It can grow well in the humid, tropics or hot dry lands, can survive destitute soils, and is little affected by drought[(2)].M.oliefera tree tolerates a wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0 [(3)].

Medicinal plants, (Moringaoleifera) is one of the plants which have its customary use in most of the disease management. M. oleifera is native to the subHimalayan tracts of India, Pakistan, Bangladesh and Afghanistan,now it is cultivated all over the world[(4,5)].

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The World Health Organization (WHO) estimated that approximately, 75%-80% of the world's population used herbal plant as traditional medicines for their primary health care need [(6,7)].

Moringaoleifera is known with the following local names some of these: horseradish tree and ben tree in English, a AL-Rawag tree in Arabic, Nuggekayee in Kanada and kelor in Indonesia, simply known as moringa[(7)]. Moringaoleifera is a common vegetable in Nigeria and many other countries Indians used the leaves by dried, ground into afne powder, and used as a supplement by adding small amounts to soup, bread dough, and stews. Moringa leaf powder has been promoted as a health food in many countries due to its high nutritional profle. It has been suggested that leaf powder could be added to food aid provided with vital nutrients and that moringa trees should be planted in areas with low food security as a bufer against malnutrition [(6,7)]. This study therefore intends to evaluate the Gas chromatography–mass spectrometry (GC/MS) analysis

of the chemical constituents of the aqueous extract of Moringaoleifera leaves grown in AL-Ramadi-Iraq,to To determine which amino acids are present,and the anti-oxidant of quercetin,kaempferol and vitamin C of Moringaoliefera leaves extract.

Material and Method

The fresh leaves of Moringaoleifera were collected from the tree that was cultivated in the house garden and were identified by taxonomist in the Department of Biology, Anbar University, Ramadi,Iraq .

Preparation of samples

The leaves were destalked, washed and shade dried at ambient temperature with constant turning averts fungal growth.the leaves was later milled to obtain the vegetable leaf meals using an electric blender and stored in 40C temperature in refrigerator in well labeled airtight containers for analysis.

Preparation of Extract

40 gms of dried powdered leaves ofMoringaoleifera was extracted successively with 300 ml of distilled water in an orbital shaker for 24 hrs at room temperature. The extracts were filtered using filter paper to remove extractable substances, at every 3 hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C.

GC-MS analysis

GC-MS analysis was done at the University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi. GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan) system was employed for GC-MS analysis, which comprises the headspace sampler (AOC-20s) and autoinjector (AOC-20i). The system was equipped with mass selective detector with an ion source having temperature 220°C and interface temperature 260°C. Capillary column used for MS analysis was Rt × 5MS capillary column with 30 mm × 0.25 mm (length × diameter) and 0.25 µm of film thickness. The temperature of the injector was adjusted to 250°C, possessing a split injection mode. The initial temperature applied was 80°C (3 minutes), which was further programmed to increase to 280°C at a ramp rate of 10°C/minutes. Helium (>99.99%) was used as carrier gas with 40.5 cm/seconds of linear velocity. The total flow programed was 16.3 ml/minutes, with column flow of 1.21 ml/ minutes.

Amino acid extraction:

About 5 mg solid samples were weighed with an accuracy of 0.01 mg and approximately 100 mg liquid samples were weighed with an accuracy of 0.01 mg then 1 ml of 6 M hydrochloric acid solution as hydrolysis agent was added, the tube was covered and placed in the aluminum thermo block at 100°C±20°C for 24 hours for hydrolysis , Using a pipette, a volume of 100 µl of hydrolyzed is introduced in a vial placed in evaporation to remove moisture with nitrogen gas; The dried amino acid residues were dissolved in a volume of 100 µl of acetonitrile; They are derivative with a volume of 100 µl of OPA(orthophethalene aldehyde); The sealed vial is subjected to ultrasound for 1 minute;· The vial is placed in the thermo block at 100°C+2°C for 30 min. to complete the derivatization reaction; The vial is placed in the gas chromatograph sample stand; 10 injections of 100 µl per sample are performed [8].

Ultrasonic Extraction of Phenolic Compounds:

The phenolic compounds were extracted from homogenized plant sample (10.0 g) using ethanol/water (70/30) solvent. Extraction process was carried out using Ultrasonic Bath (USA) at the room temperature for 1 hour [9]. After filtration, 5 mL of liquid extract was used for extraction yield determination. Solvent was removed by rotary evaporator under vacuum (Slovenia), and was dried at 60°C to the constant mass. Dry extracts were stored in the glass bottles at 4°C to prevent oxidative damage until analysis.

Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a SYKAMN HPLC chromatographic system equipped with a UV detector), Chemstation , a Zorbax Eclipse Plus-C18-OSD .25cm, 4.6mm column. The column temperature was 30ºC the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-4 min, 80 % B ; 5-10 min, 60 % B; . and flow-rate of 1.1 mL/min. The injected volume of samples 100 µL .and

standards was 100 µL and it was done automatically using autosampler. The spectra were acquired in the 280 nm.

Vitamin C extraction

About 10g of sample fresh were separately weighed before extraction. After weighting of different samples, extraction was done with 25 mL of extracting solution, containing 5% meta-phosphoric acid , at 10°C in the dark. Extraction process was performed using a shaker for 4 hours with continuous shaking[10]. All extractions were carried out in triplicate and obtained solutions were then filtered andstored at 4°C before analysis. The injection of each extract into HPLC system was performed twice. HPLC model SYKAMN (Germany) using to analysis for vitamin C in sample. The liquid chromatographic method used for the determination of L-ascorbic acid (AA) consisted of an isocratic elution procedure with UV-Visible detection at 245 nm. Separations were carried out on a reverse phase column Luna C18 (25 cm * 4.6 mm The mobile phase employed was a mixture of 0.5% NaH2PO4 and acetonitrile (93:7). Flow rate of the mobile phase was 1.2 mL/min and an injection volume of 20 µL was used in quantitative analysis. The temperature of analytical column was keptconstant at 25°C.

Results and Discussion

Sixteen peaks were determined from the chromatogram of the aqueous leaf extract of Moringaoleifera. These peaks indicate the presence of sixteen compounds (1-16) in the extract. The molecular formula, percentage content and molecular mass of the compounds are shown in (Table 1). These compounds consist of mainly hydrocarbons, fatty acids, alcohols,and esters .

The composition of the extract comprises: Propanone, 2,2-dimethyl-1-(4-phenoxyphenyl)-, 1,2-Benzenediol, O-(4-butylbenzoyl)-O'-(isobutoxycarbonyl) , Decanoic acid, 1-Pentadecyn,11- Octadecenoic acid, cis-5-Dodecenoic acid, Dipalmitin, Z-7-Tetradecenal, Methyl 2-oxodecanoate,2-Monoolein, 2-Hexadecyloxirane, cis-3-Hexenyl pyruvate, 4-(2-Methyl-cyclohex-1-enyl)-but-3-en-2-one, Z,E-2,13-

Octadecadien-1-ol, 9-Icosyne.

Moringaoliefera leaf extract was rich with long chain hydrocarbon (Alkanes, Alkenes) ,alcohols,fatty acids and another compound, For example, the compound Decanoic acid is one of the components that has straight-chain saturated fatty acid. It has a role as an antibacterial agent, an anti-inflammatory agent, a human metabolite, a volatile oil component, a plant metabolite and an algal metabolite.Decanoic acid non-toxic ,used to make esters for perfumes and fruit flavors and as an intermediate for food-grade additives [8]. Also the other chemical compound is 17-Octadecynoic acid, it is is an acetylenic fatty acid that is octadecanoi acid (stearic acid) which has been doubly dehydrogenated at positions 17 and 18 to give the corresponding alkynoic acid.

It has a role as a P450 inhibitor, an EC 1.14.14.94 (leukotriene-B4 20-monooxygenase) inhibitor and an EC 1.14.15.3 (alkane 1-monooxygenase) inhibitor. It is a long-chain fatty acid, an acetylenic fatty acid, a terminal acetylenic compound and a monounsaturated fatty acid [11,12]. Z-7-Tetradecenal is the main compound at (40.90%). It is also the main chemical compound in the hexane extract of PremnamucronataRoxb leaves, which has great activity against E. coli and S. aureus [13].

Table (1): Important phytochemical detected by GC-MS of aqueous extract of Iraqi M .oliefera leaves

Peak	compound		Molecular formular	Molecular weight	Retention time	Percentage content
1	Propanone, 2,2-dimethyl-1-(4-phenoxyphenyl)-		C17H18O2	254	18.650	0.13
2	1,2-Benzenediol, O-(4-butylbenzoyl)-O'-(isobutoxycarbonyl)-		C22H26O5	370	19.633	0.18
3	Decanoic acid		C10H20O2	172	20.542	1.14
4	1-Pentadecyne		C15H28	208	21.500	0.98
5	11-Octadecenoic acid, methyl ester		C19H36O2	296	21.683	1.36
6	17- Octadecynoic acid		C18H32O2	280	22.367	29.24
7	4-Hydroxyheptanohydrazide		C7H16N2O2	160	22.550	0.92
8	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester		C20H40O2	317	23.558	7.51
9	Z-7-Tetradecenal		C14H26O	210	25.183	40.90
10	Methyl 2-oxodecanoate		C11H203	200	25.292	1.08
11	2-Monoolein		C21H40O4	356	26.992	7.21
12	Pentadecanal		C15H30O	226	27.250	1.32
13	cis-3-Hexenyl pyruvate		C9H14O3	170	27.671	0.37
14	4-(2-Methyl-cyclohex-1-enyl)-but-3-en-2-one		C11H16O	164	32.200	1.21
15	Z,E-2,13-Octadecadien-1-ol		C18H34O	266	32.917	4.89
16	1-Undecyne		C11H20	152	33.242	1.55

Pentadecanal(1.32%), It is a component of essential oils from plants like Solanumerianthum and Cassia siamea. It has a role as an antimicrobial agent, a volatile oil component and a plant metabolite. It is a long-chain fatty aldehyde and a 2,3-saturated fatty aldehyde [14]. 1-Undecyne(1.55%), It has a role as a metabolite [15]. 16 chemical compounds were also identified in the alcoholic extract of Moringaoleifera leaves cultivated in Nigeria [16].The results of amino acid profile analysis showed that there were 12 types of amino acids contained in M.oleifera leaves as shown in Table 2.

The essential amino acid content included threonine (31.4 mg/gm), leucine (77.2 mg/gm), valine (10.3 mg/gm), methionine (88.5 mg/gm), tryptophan (12.3 mg/gm) while non-essential amino acids include aspartic acid (101.2 mg/gm), glycine (58.7 mg/gm), arginine (62.3 mg/gm), alanine (33.5 mg/gm), serine (35.6 mg/gm), cysteine (58.7 mg/gm), Glutamic acid (142.2 mg/gm)

The results of the analysis showed that glutamic acid was an amino acid with the highest concentration, and the lowest was Valine, while a previous study of the amino acid extraction of *Moringaolifera* leaves cultivated in northern Nigeria showed that glutamic acid was the highest concentration and the amino acid methionine the lowest concentration[17].

Table 2: Amino acid detected by Amino Acid Analyzer

NO	Retention Time(min)	Response	Amount(mg/gm)	Compound name
1	7.91	358.9	12.3	Tryptophan
2	9.12	314.8	35.6	Serine
3	9.93	123.6	77.2	Lucien
4	10.50	1252.5	142.2	Glutamic acid
5	11.82	347.8	31.4	Threonine
6	13.34	212.5	39.6	Glycine
7	13.92	356.5	62.3	Arginine
8	15.13	654.1	33.5	Alanine
9	16.55	250.8	58.7	Cysteine
10	17.14	724.8	10.3	Valine
11	19.83	435.4	88.5	Methionine
12	22.15	333.5	101.2	Aspartic acid

The composition of amino acids can be a determinant of the characteristics and activities of proteins contained in a substance. Since *Moringa* contains high concentrations, it is an important food source for treating malnutrition resulting from protein deficiency, especially in children and infants [18].Udenigwe and Aluko (2011) said that amino acids which have high inhibitory activity against DPPH radicals(2,2-diphenyl-1picrylhydrazyl) are types of hydrophobic, aromatic and acidic amino acids.

This indicates that the higher the concentration of acidic, aromatic and hydrophobic amino acids in a sample, the potential as an antioxidant will be very good [19].The results of the study showed that the concentration of quercetin of *Moringaoleifera* leaves grown in Ramadi-Iraq is 245.7 ppm, and kaempferol 299.6 ppm, In a previous study to extract flavonoides from *Moringaolifera*leaves, conducted in 21 cities in Africa, showed that the percentage of quercetin ranged from the lowest concentration of 0.07 % in the city of Binga to the highest concentration of 1.26% in the city of Lusaka, the percentage of kaempferol ranged between 0.05% in Dodowa city and 0.67% in Chikupi city[20]. and in another study, quercetin and kaempferol were estimated in the leaves of *Moringaoleifera* cultivated in India. the concentration of quercetin was 1099 ppm and the concentration of kaempferol was 133ppm [21].

Quercetin is a polyphenolic flavonoid with potential chemopreventive activity. Quercetin, ubiquitous in plant food sources and a major bioflavonoid in the human diet, may produce antiproliferative effects resulting from the modulation of either EGFR or estrogen-receptor mediated signal transduction pathways [22]. Although the mechanism of action is not fully known, the following effects have been described with this agent in vitro: decreased expression of mutant p53 protein and p21-ras oncogene, induction of cell cycle arrest at the G1 phase and inhibition of heat shock protein synthesis. This compound also demonstrates synergy and reversal of the multidrug resistance phenotype, when combined with chemotherapeutic drugs, in vitro [23]. Quercetin also produces anti-inflammatory and anti-allergy effects mediated through the inhibition of the

lipoxygenase and cyclooxygenase pathways, thereby preventing the production of pro-inflammatory mediators [24].

Kaempferol is a tetrahydroxyflavone , acting as an antioxidant by reducing oxidative stress, it is currently under consideration as a possible cancer treatment. It has a role as an antibacterial agent, a plant metabolite, a human xenobiotic metabolite, a human urinary metabolite and a human blood serum metabolite [25].

Results showed, the leaves of M.olieferaolieferacontaine (250.2 ppm) of Vitamin C Table 4. Presence of vitamin C in M.oliefera leaves are sufficient to prevent free radical damage and oxidative stress,SoM.oliefera leaves provide a healthy and medical food for the body,as they contain effective antioxidant and anti –inflammatory compounds that increase the immunity [26,27].

Table 3 : Concentration of flavonoides of Moringaoliefera leaves extract in Iraq

Compound name	Retention time	Area	Hight	Amount ppm
Quercetine	3.820	988.414	166.398	245.7 ppm
Kaempferol	7.017	616.789	88.070	299.6 ppm

Table 4: Table 1. Content of ascorbic acid (AA) in leaves of M. oleifera

Retention time	Area	Hight	Amount ppm
6.573	81.433	19.307	250.2 ppm

In another study conducted in Bangladesh to find out the level of vitamin C content in different parts of Moringaoleifera plants, it was found that the amount of vitamin C in mature leaves ranged between 51.26mg/100g to 150.15 mg / 100 g, and the highest concentration of vitamin C was in Moringaolifera flower77.5mg/100g to 224.67 m9/100g[10].There are studies confirming that the content of MoringaOlivera tree of vitamin C is equivalent to 2-4 times what is found in plants known for their vitamin C content, such as lemon tree[28].There is another study confirming that the content of vitamin C in Moringa extract wass 1.371g per 100g of extract,Thus, MoringaOleifera is an important source of Vitamin C[29].

Conclusion

The GC-MS analysis of Moringaolivera leaves grown in Iraq-Ramadi showed the presence of compounds of economic and medical value, as it was observed that there are compounds that contribute as anti-microbial and anti-inflammatory such as Decanoic acid, 17-Octadecynoic acid, Z-7-Tetradecenal and Pentadecanal, where the highest percentage of the effectiveness of biochemical compounds represented 73%. just as the leaves of Moringa Oliveira are considered an important food source because it contains high concentrations of essential amino acids and the highest concentration of the amino acid Glutamic acid (142 mg / gm), and the results of the HPLC analysis showed the presence of quercetin compounds (245.7 ppm) and

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