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GC-MS analysis of *Moringa oliefera* root and stem branch extracts grown in Ramadi City, Iraq

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ABSTRACT

In the present study, phytochemical analyses were performed on the methanolic and ethanolic extracts of *Moringa oliefera* root and branches of the stem using Gas Chromatography-Mass spectrometry (GC-MS). This plant has therapeutic properties in its different parts. GC-MS analysis revealed the presence of many phytochemicals in root methanolic extracts involving 14 active compounds. It was found that a high percent area is 2-furancarboxylaldehyde (50.07%). Ethyl aldehyde had the lowest area (0.49%). In contrast, ethanolic extract of root contained 19 active compounds as GC-MS isolated and characterized. The technique confirmed that 6-octadecenoic acid was the highest compound (59.43%), while N-sulfinyl butylamine has rarely been detected in plant extracts (0.12%). Many other phytochemicals were also detected. Similarly, methanol extract for stem branches included three active compounds. A 5-hydroxymethylfufural was the most abundant (63.06%), while 2-acetyl-2H-tetrazole possessed the lowest area (9.70%). Ethanol extract contained six isolated active compounds. Few major compounds were found with a high percent peak area in branches by ethanol extract including 5-hydroxymethylfurfural (55.81%), whereas, the lowest one was hydrazinecarboxaldehyde (0.49%).

Key Words: Moringa, Branch, Root, Extract, Gas Chromatography.
Article type: Research Article.

INTRODUCTION

Medical plants have long been utilized to cure a variety of healthy conditions due to their active biological components. Medicinal plant extracts have been used as preventative agents against certain infections and xenobiotic toxicity in a large number of studies (Saleh *et al.* 2016; Bamola *et al.* 2018; Asraa *et al.* 2019; Salmerón-Manzano *et al.* 2020; Al-Hadidy & Mostafa 2022). *Moringa oliefera* belongs to the Moringaceae family and is spread in Africa, especially Ethiopia, Kenya, and Sudan. This species tropically is grown, originally in India, but known in Africa (Padayachee & Baijnath 2012; Abdulateef *et al* 2021). Each part of the Moringa has a lot of active chemical and biological components (Kasolo *et al.* 2010; Mbikay 2012; Ameh & Alafi 2018; Al-Obaidi *et al.* 2021; Iriani *et al.* 2023). Various extracts of its roots, bark, leaves, flowers, immature pods, and mature fruits have been reported to have cardiac and circulatory stimulant, antifertility, antitumor, antipyretic, antispasmodic, anti-inflammatory, antiulcer, hypotensive, hypolipidemic, hypoglycemic, hepatoprotective, antioxidant, antifungal, and antibacterial activities, implying promising therapeutic potential (Mbikay 2012). The leaves were shown to exhibit antioxidant properties (Saini *et al.* 2014). The seed extract of Moringa was

effectively used to reduce the toxicity of CPbNP in rats (Al-Obaidi *et al.* 2021). Moringa ethyl acetate was discovered to contain two bioactive glycosylated flavonoids (isoquercitrin and astragalin) as well as phenolic acid (3-O-caffeoylquinic acid; Oldoni *et al.* 2021). Furthermore, they have indicated three high-antioxidant-activity phenolic compounds, the chemical composition of hydro-ethyl crude extract rich in flavonoids glycosylated, which could be intimately related to antihyperglycemic action. It was reviewed that this plant ornamented a high nutritional, nutraceutical, and therapeutic compounds in different parts possessed vital significance viz. protein, flavonoids, saponins, phenolic acids, tannin, isothiocyanate, lipids, minerals and vitamins (Dzuvor *et al.* 2021). These components contribute pharmacology and health benefits as antimicrobial, anticancer, antidiabetic, antioxidant, antihypertensive, anti-inflammatory and cardioprotective properties (Islam *et al*. 2021). It was found that the supplementation of the basal diet with 3.2% moringa improved the antioxidant defence system and biochemical blood indices during early gestation in Beetal goats (Afzal *et al.* 2021). The goal of this research was to use GC mass spectroscopy to evaluate the chemicals identified in the methanolic and ethanolic root and stem branch extracts of *M. oleifera* in Iraq.

MATERIALS AND METHODS

Moringa oleifera was planted in Anbar Governorate, Iraq. The tree's roots and branches were removed, cleaned, dried, and ground into powder using a Laboratory Grinder. Roots and branches of the stem were extracted using the Soxhlet device in solvent methanol or ethanol. A total amount of 300 g of the sample was used. The extract was subsequently concentrated using a vacuum rotary evaporator after 72 h of extraction.

GC-MS analysis

In the Iraqi Ministry of Sciences and Technology, a Shimadzu GC-2010 plus-Japan chromatograph was employed; Column: ZB-5MS Capillary Column (30 m × 0.25 mm, ID 0.25 μm), Carrier Gas: UHP Helium, Injection Temperature: 280.00 ℃, Detector Temperature: 280.00 °C, Injection Mode: Split, Flow Control Mode: Pressure, Injector Pressure: 100.0 kPa, Total Flow: 47.3 μL min^{-1}, Column Flow: 1.43 μL min^{-1}, Linear Velocity: 44.1 cm/min, Injection Volume: 1 μL, Run Time: 35 min. A freshly prepared sample (10 mg mL^{-1}) was used.

RESULTS

GC/MS analysis of Root extract

Results from GC-MS and chromatogram plot illustrated that the number of active compounds differed in the two used alcoholic extracts of moringa roots. Methanolic extracted involved 14 active compounds separated via GC-MS. These compounds were characterized according to standard compounds found in electronic libraries coupled with the device. So, 2-furancarboxylaldehyde was the predominant compound with an area of 50.07%, followed by 5H-1-pyrimidine of 31.37%. Whereas, Ethylaldehyde possessed the lowest area of 0.49%. In contrast, ethanolic extract of root contained 19 active compounds as GC-MS isolated and characterized. The technique confirmed that 6-octadecenoic acid was the highest compound of 59.43%, followed by n-nonadecanoic acid of 16.39%, 5H-1-pyrimidine of 12.48%, of total area percentage as N-sulfinylbutylamine was the lowest one of 0.12%.

Stem branches GC-MS analysis

Table 2 demonstrated that methanol extract of Moringa branches was different from ethanol extract where methanol extract possessed three isolated active compounds using GC-MS technique. At the same time, ethanol extract included six active compounds. 5-hydroxymethylfurfural was the predominated compound in a methanolic extract with an area of 63.06%, followed by Trans-2-butene oxide of 27.24%. At the same time, 2-acetyl-2H-tetrazole possessed the lowest area% of 9.70. In contrast, the dominated compounds in ethanol extract also recorded 5-hydroxymethylfurfural with an area of 55.81%, followed by 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one of 25.19%, and 2-acetyl-2H-tetrazole of 11.81%, respectively. In contrast, the lowest compound was hydrazinecarboxaldehyde of 0.49%. The methanol extract was similar to ethanol one in two compounds. They extracted the same two compounds as GC-MS separated and isolated them. These compounds were 5-hydroxymethylfurfural, thereby yielding 118.87%, for both extract and 2-acetyl-2H-tetrazole with pooled area of 21.51% for both extracts.

Table 1. Active compounds, RT and their area%, detected in Moringa roots extracts.

Peak#	Methanol extract				Ethanol extract			
	RT	**Area (%)**	**compounds**	**Mol. weight**	**RT**	**Area (%)**	**Compounds**	**Mol. weight**
1	2.044	0.49	Ethylaldehyde	44	2.145	0.53	Monofluoroacetylene	44
2	3.484	2.19	2-furaldehyde	96	3.484	0.23	2-furaldehyde	96
3	5.573	1.59	Benzoicaldehyde	106	5.567	0.12	N-sulfinylbutylamine	119
4	7.629	1.15	3-furancarboxylic acid	126	8.624	12.48	5H-1-pyrindine	117
5	8.647	31.37	5H-1-pyrinidine	117	10.065	2.10	2-furancarboxaldehyde	126
6	8.715	0.76	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	144	12.125	0.98	Benzylisothiocyanate	149
7	10.206	50.07	2-furancaboxyl aldehyde	126	12.618	0.19	1,5-heptadien-3-yne	92
8	12.124	0.56	Benzylisothiocyanate	149	13.728	0.30	N-benzylurea	150
9	12.624	0.55	1,5-heptadien-3-yne	92	16.633	0.37	3,5-octadiyne	106
10	13.810	0.71	Fura[2,3-c] pyridine	133	17.134	0.39	1H-pyrrole-2-carbonitrile	92
11	14.124	2.29	Pentanoic acid	102	19.370	16.39	n-nonadecanoic acid	298
12	15.445	0.79	n-formylethylamine	73	19.564	2.13	Methyl2-methylhexacosanoate	424
13	19.299	1.64	Propanoic acid	128	21.246	59.43	6-octadecenoic acid	282
14	21.134	5.84	Cyclopentane	364	21.540	0.40	2,6-dimethyl-3-octanol	158
15					24.108	0.26	Thujaketone	140
16					24.595	0.35	1(isopropylsulfonyl)butene	164
17					25.719	0.65	(4E)-3-methyl-4-hexan-2-one	112
18					25.947	2.57	(4E)-2,3,3-trimethyl-4-nonene	168
19					28.196	0.15	(4E)-4-hepten-2-one	112

Table 2. Active compounds, RT, and their area (%) detected in moringa branches extracts.

Peak#	Methanol extract				Ethanol extract			
	RT	**Area%**	**compounds**	**Mol weight**	**RT**	**Area%**	**compounds**	**Mol weight**
1	7.560	9.70	2-acetyl-2H-tetrazole	112	7.596	11.81	2-acetyl-2H-tetrazole	112
2	8.639	27.24	Trans-2-butene oxide	72	8.656	25.19	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	144
3	9.981	63.06	5-hydroxymethylfufural	126	10.013	55.81	5-hydroxymethylfurfural	126
4					15.693	0.49	Hydrazinecarboxaldehyde	60
5					19.288	4.99	A-methylpropanoic acid	88
6					22.164	1.70	1,5-diaminotetrazole	100

DISCUSSION

Iraqi *Moringa oleifera* phytochemical analysis of methanol and ethanol extracts from roots and branches of stems displayed a presence of major classes of bioactive phytochemicals (Tables 1 and 2). It was found that most of these compounds exhibit diverse pharmacological activities (Gu *et al.* 2014). Similar studies were carried out in different countries. Roots that were taken from *M. oleifera* planted in Karachi gardens contained 102 compounds

in petroleum ether and dichloromethane extracts, which indicated interesting biological activity such as hydrocarbons, fatty acids, esters, alcohols, isothiocyanate, thiocyanate, pyrazine, aromatics, cyanides, steroids, halo compounds, urea and N-hydroxylamine derivatives, (Faizi *et al.* 2014). Sharma *et al.* (2011) reported that the Moringa roots alcoholic extract contains many compounds, including Moringine, spirachin, moringinine, 1,3-dibenzyl urea, p-cymene, alpha- phellandrene, Deoxy-niazimicine,4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate. Amaglo *et al.* (2010) investigated the kinds and concentrations of phytochemicals (non-nutrients) and nutrients in *M. oleifera* cultivated in Ghana. He found rhamn ose and acetyl-rhamnose-substituted glucosinolates, with varied profiles depending on the tissue. Palmitic and oleic acid were abundant in the roots, whereas palmitic acid was found in the stems and branches. In all tissues, potassium, magnesium, and calcium were the most abundant minerals. Anti-inflammation, antilithic, and antifertility properties have been discovered in the root extract (Muangnoi *et al.* 2012). Drumstick tree *(M. oleifera*) roots had active compounds that might be employed as natural anticancer medications; *in vitro*, they were effective against leukaemia cells. The findings also support the significance of roots as a source of high-value metabolites (Abdellatef *et al.* 2010). Manaheji *et al.* (2019) found in their study that the roots methanolic extracts of African generation of *M. oleifera* effectively reduced pain in rats. On the other hand, Paul & Didia (2012) studied the effect of methanolic extract of the root of Moringa planted in Nigeria and concluded that roots distorted the histo-architecture of both liver and kidneys of guinea pigs and these effects are time-dependent and dose-dependent. Vats & Gupta (2017) reported that the *M. oleifera* from Rajasthan, India contained 29 chemical compounds in bark, and the basic constituent identified was epiglobulol (41.68%). *M. oleifera* has also been recorded as a rich source of the compound 5-hydroxymethylfurfural in the methanol and ethanolic extracts of the stems' branches (Saini *et al.* 2016). In the present study, 5-hydroxymethylfurfural was the predominant compound of stems' branches. In addition, Foidl *et al.* (2001) reported the aqueous and hydroalcoholic extract of Moringa stem containing 4-hydroxyl mellein, vanillin, octacosonoic acid, beta- sitosterone and beta-sitosterol. The root and stem barks of *M. oleifera* Lam., growing in the northern Nigerian city analysis, revealed three phenol compounds including Quercetin glucoside, Quercetin rhamnoglucoside (rutin), and Chlorogenic acid. The extracts exhibited strongly *in vitro* antioxidant activity (Atawodi *et al.* 2010). In comparing the constituents of roots and branches of the stem in this study with the above studies, a clear difference was observed, besides the similarity with results of other studies. The texture of the soil, irrigation, climate, fertilizers, besides other factors, maybe the causes of these differences in the phytochemical contents of roots and branches of stems.

CONCLUSION

The results of a GC-MS study of Iraqi *M. oliefera* root and stem branches were beneficial. The quantity and quality of the compounds generated and preserved reflect the nutritional and/or therapeutic value of these phytochemicals. The compounds 2-furancarboxylaldehyde and dodecanoic acid were higher in the root. The major phytochemical in the stem branches was 5-hydroxymethylfufural.

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