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GC-Mass Spectrometry Analysis of Iraqi *Moringa Oleifera* Seeds Extract

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Abstract

Moringa oleifera is an important traditional medicinal plant usedfor the treatment of many health problems. It is widely cultivated across the world but it has not well known orwell-studied in Iraq. Therefore, the aim of this study is to identify the bioactive compounds present in acetone:methanol (1:1) seeds extractof Iraqi Moringa olieifera by using GC-Mass Spectrophotometry.

The results of this analysis revealed 41 compounds which include ,terpenes (cis-beta-Ocimene 0.97%, Cedrene 0.20%, Cedrenol 1.40%, Verrucarol 0.29 , Dehydroxy-isocalamendiol 0.09% , beta-Myrcene 0.14% , 3-Thujene 0.06%, Ocimene 0.72% , Sabinene 0.37% Aromadendrene 1.54%) , phenols (0.34%, Eugenol 0.49%, cis-Isoeugenol 1.21%, Methoxyeugenol 0.26%, Ferrocene 0.57%, Phenol, 2-methoxy-4-(1-propenyl)-, (Z) 0.34 , Oxabicyclo[3.3.0]octan-2-one -7-neopentylidene) 0.61%) , fatty acid methyl esters (n-Tridecanoic acid 0.15% , Undecanoic acid 0.09% , Pentadecanoic acid 34.43%, 4-Hexadienal 1.52% , Dodecanoic acid 11.02% , cis-Vaccenic acid 21.39%, Hexadecenoic acid 0.15% , Eicosanoic acid 0.03%), acid (myristicin,7.8%), Phenyl propanoid (Asarone, 4.99%), Antioxidant (-tert-Butyl-4-hydroxyanisole, 1.38%), Drug (Berbine, 2,3,9,10-tetramethoxy, 0.73%), Drug (Thiazolo, 0.63%), aldehyde (Trimethylsilyl vanillin, 0.61%), hormone (Octenoic acid, 0.46%), Amine (ethyl ester, 0.42%), Carboxylic acid (Dimethylandrost-5-en-3-one, 0.28%), Terpenoid (Lavandulol, 0.19%), Heterocyclic compound (Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl, 0.18%), Volatile oil (Butanoic acid, 0.16%), Fatty alcohol (1-Heptatriacontanol, 0.12%), Hydroxy carbon-alkene-(Carene, 0.11%).

It can be concluded from this study that the seeds are considered a rich source of bioactive compounds.

Keywords: *GC-MS analysis, Bioactivecompounds, Methanol:Acetone Extract, Iraqi Moringa oliefera.*

Introduction

Medical plants are commonly used as a source of medicinal products or against the toxicity of xenobiotics [1,2,3]. World Health Organization reports that 4 billion people (80 percent of the world's population) use herbal medicinal goods for most of the primary health care [4].

Moringa oleifera is among 13 species of Moringa trees that grow in different countries around the world [5].

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Seeds of *M. oliefera* tree are useful for medicinal, practical food preparations, water purification, and biodiesel production. [6]. *M. oliefera* seeds are a good source of proteins, lipids, vitamins, minerals, micronutrients, and bioactive phytochemicals [7,8,9]. The aqueous seeds extract of *M. oliefera* inhibit bacterial and fungal growth [10]. The methanol: acetone seed extract of Iraqi *M. oliefera* is found to reduce the toxicity of nanoparticles of lead in the rat (Unpublished results).

This study aims to estimate the compounds found in methanol: acetone seeds extract of Iraq *M. oleifera* by using GC mass spectroscopy.

Material and Meathods

Plant collection

The ripe dried pods of*M.oleifera* were collected from the trees planted in National Iraqi herbarium (in Baghdad), Iraq, andwas identified bya taxonomist in the Desert center, University of Anbar, Iraq.

Preparation of samples

The seeds were removed from dried pods, dehusked, kept at shade ambient temperature (45C) to remove any possible moisture. The seeds were later milled to obtain seed powder using a blender and stored it in the refrigerator in airtight containers for analysis.

Preparation of extract

The dried powdered seeds (300gms) were extracted using Soxhlet and thimble with 1000 ml of solvent (methanol: Acetone1:1) for 48 hours. The extract was then evaporated with a rotary evaporator. The dried extract was stored at 4°C in a sterile container.

GC-MS analysis

The chromatograph used was Shimadzu GC-2010 plus-Japan in the Iraqi ministry of sciences and technology; Column: ZB-5MS Capillary Column (30 m x 0.25 mm, I.D. 0.25 µm), Carrier Gas: UHP Helium, Injection Temperature: 280.00˚C, Detector Temperature: 280.00 °C, Injection Mode: Split, Flow Control Mode: Pressure, Injector Pressure: 100.0 kPa, Total Flow: 47.3 µl/min, Column Flow: 1.43 µl/min, Linear Velocity: 44.1 cm/min, Injection Volume: 1 µL, Run Time: 35 minutes. A freshly prepared sample(10mg/ml) was used.

Results and Discussion

GC-MS is an important device used for the analysis of bioactive chemical constituents according to their mass to charge ratio.

Forty one bioactive compounds were identified in seeds extract (methanol:aceton 1:1) of Iraqi *M. oleifera* by GC-MS analysis and detailed in Figure 1., the compounds included terpenes (cis-beta-Ocimene 0.97%, Cedrene 0.20%, Cedrenol 1.40%, Verrucarol 0.29 , Dehydroxy-isocalamendiol 0.09% , beta-Myrcene 0.14% , 3-Thujene 0.06%, Ocimene 0.72% , Sabinene 0.37% Aromadendrene 1.54%) , phenols (0.34%, Eugenol 0.49%, cis-Isoeugenol 1.21%, Methoxyeugenol 0.26%, Ferrocene 0.57%, Phenol, 2-methoxy-4-(1-propenyl)-, (Z) 0.34 , Oxabicyclo[3.3.0]octan-2-one -7-neopentylidene) 0.61%) , fatty acid methyl esters (n-Tridecanoic acid 0.15% , Undecanoic acid 0.09% , Pentadecanoic acid 34.43%, 4-Hexadienal 1.52% , Dodecanoic acid 11.02% , cis-Vaccenic acid 21.39%, Hexadecenoic acid 0.15% , Eicosanoic acid 0.03%), acid (myristicin,7.8%), Phenyl propanoid (Asarone, 4.99%), Antioxidant (-tert-Butyl-4-hydroxyanisole, 1.38%), Drug (Berbine, 2,3,9,10-tetramethoxy, 0.73%), Drug (Thiazolo, 0.63%), aldehyde (Trimethylsilyl vanillin, 0.61%), hormone (Octenoic acid, 0.46%), Amine (ethyl ester, 0.42%), Carboxylic acid (Dimethylandrost-5-en-3-one, 0.28%), Terpenoid (Lavandulol, 0.19%), Heterocyclic compound (Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl, 0.18%), Volatile oil (Butanoic acid, 0.16%), Fatty alcohol (1-Heptatriacontanol, 0.12%), Hydroxy carbon-alkene-(Carene, 0.11%).

The following examples revealed the biological activity of each of the above compounds. Myristicin was found to act as prophylactic effects against colitis in mice, the myristicin treatment before the induction of colitis significantly changed the colonic oxidative stress by elevating the antioxidant enzymatic activities, and reducing the lipid peroxidation [(11)].

Asarone may be developed to a therapeutic agent to manage cognitive weakening related to conditions such as Alzheimer [(12)]and increase of chemosensitivity by preventing tumor glycolysis in gastric cancer [(13)].

Methoxyeugenol has anticancer activity and antimicrobial activity [(14)]. Pentadecanoic acid, suppresses the stemness of mcf-7/sc human breast cancer stem-like cells through JAK2/STAT3 signaling [(15)]. Dodecanoic acid, may elicit apoptosis in certain cancer cells [(16)] and exerted antiproliferative activity in diverse types of tumor cells [(17)]. When fed vaccenic acid over 16 weeks, rats exhibited lowered total cholesterol, lowered LDL cholesterol, and lower triglycerides levels[(18)].

The crude seeds extract of *M.oliefera* was also found to have protective effects against, the toxicity of lead nanoparticles in rats (Unpublished results), oxidative DNA damage [(19)], and against bacterial growth [(20)]

Conclusions

The result of a GC-mass analysis of Iraqi *M. oliefera* was valuable. The number and quality of the compounds manufactured and stored in the seeds of *M. oleifera* reveal the nutritional and/or medicinal importance of these phytochemicals. The compounds, pentadecanoic acid, Cis-Vaccenic acid, dodecanoic acid, and myristicin constitute about 74.64% of the total contents of extraction.

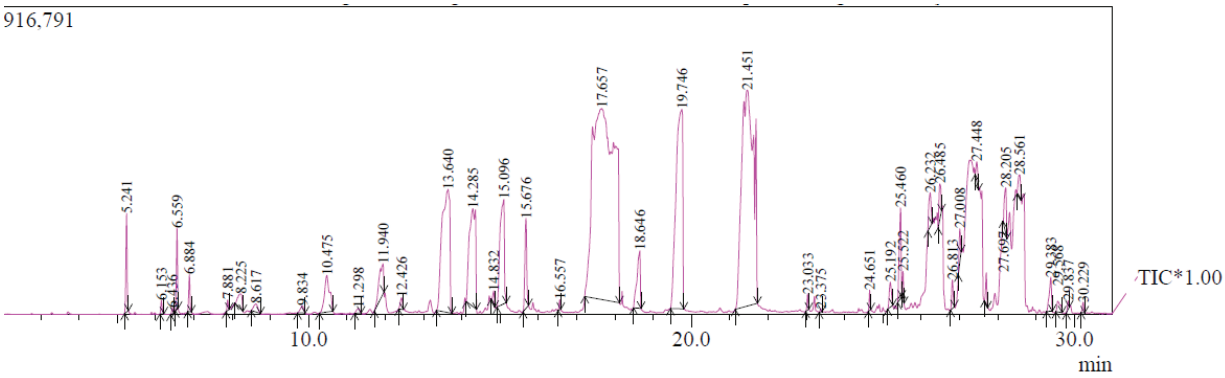
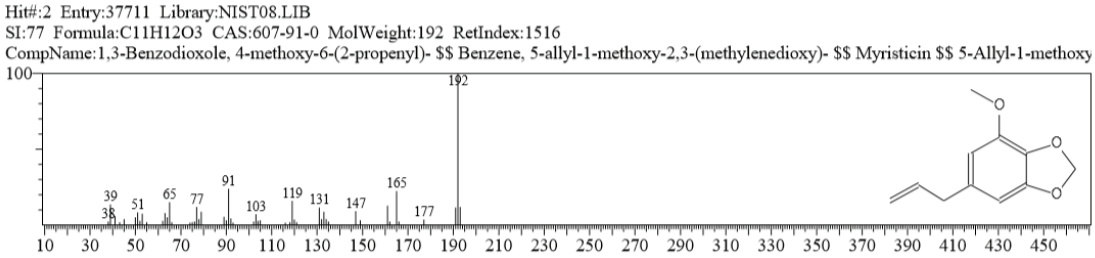
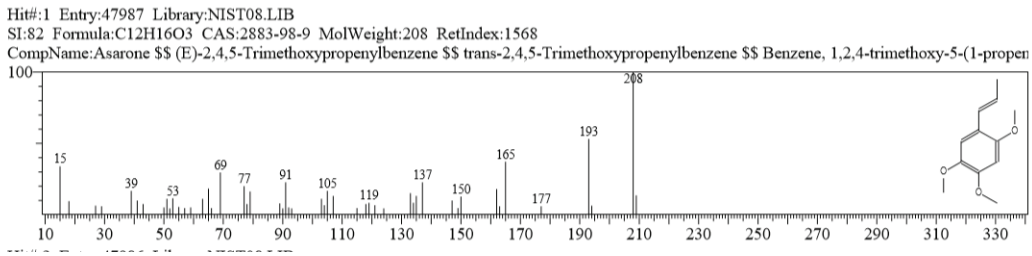


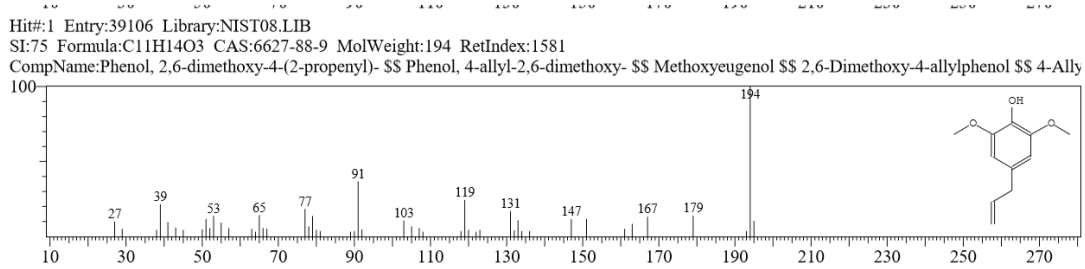
Figure (1): TIC of the GC-MSS of methanol: acetone (1:1) seeds extract of Iraqi *M.oliefera*



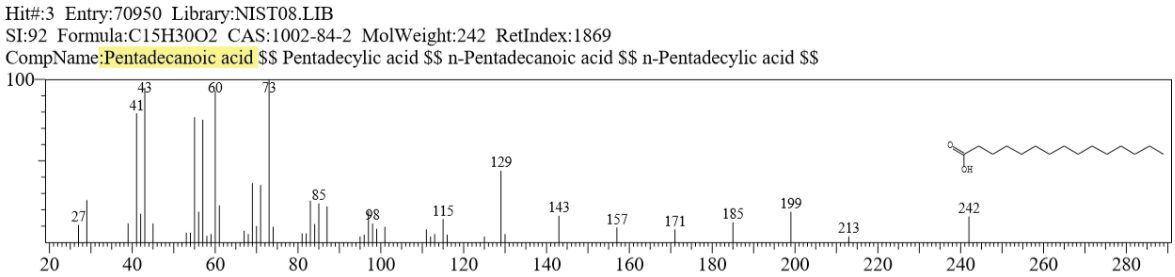
A- Myristicin



B- Asarone



C- Methoxyeugenol



D- Pentadecanoic acid

Figure 2 : Some important compounds in methanol: acetone (1:1) seeds extract of Iraqi *M.oliefera*

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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