DETECTION OF BIOACTIVE CHEMICAL COMPOUNDS IN THE METHANOLIC EXTRACT OF *AZOLLA FILICULOIDES* LAMARK FERN BY GC-MS TECHNIQUE

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ABSTRACT

The phytochemical analysis of *Azolla filiculoides* Lamark in the methanolic whole plant extract was undertaken and published for the first time in Iraq in this article. The existence of twenty-four bioactive phytochemical compounds were discovered using GC-MS analysis. , such as Ethyl Formate, 1,3-Cyclohexanedione, Butanal, 2-Nonenal,9-Octadecenal,2,4,6-Trimethyl-1-nonene, 3-(Prop-2-enoyloxy) tetradecane, Carbonic acid, 9-Oxabicyclo [6.1.0] nonane. Oleic Acid, 13-Docosenoic acid, Z-10-Pentadecen-1-ol, Oxirane, Diethyl carbonate. Hydroperoxide, 2-Butene ozonide. These various active phytochemicals of alkenes, alkanes, esters, ethers, and carboxylic acids were found in the plant with great potential to bind with other compounds, this plant is great to be used as a filter to waste water.

Keywords: GC-MS, phytochemicals, *Azolla filiculoides* Lamark, pteridophytes

مجلة العلوم الزراعية العراقية -2022 :53(4):922-930 الخفاجي وأخرون

الكشف عن المركبات الكيميائيه الفعاله في المستخلص الميثانولي لسرخس *Azolla filiculoides* Lamark بواسطة جهاز GC-MS ال

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المستخلص:

تم إجراء التحليل الكيميائي النباتي للمستخلص الميثانولي للنبات الكامل *Azolla filiculoides* Lamark ونشر لاول مره في العراق في هذه المقاله. تم اكتشاف اربع وعشرين مركب كيميائي نشط بواسطة استخدام تقنية الـ GC–MS، مثل Ethyl Formate, 1,3-Cyclohexanedione, Butanal, 2-Nonenal,9-Octadecenal,2,4,6-Trimethyl-1-nonene, 3-(Prop-2-enoyloxy) tetradecane, Carbonic acid, 9-Oxabicyclo [6.1.0] nonane. Oleic Acid, 13-Docosenoic acid, Z-10-Pentadecen-1-ol, Oxirane, Diethyl carbonate. Hydroperoxide, 2-Butene ozonide. تم العثور على هذه المواد الكيميائيه النباتيه النشطه المختلفه مثل الالكينات، الالكانات، الاسترات، الايثرات، الاحماض الكربوكسيليه في النبات مع امكانيه كبيره للأرتباط بمركبات اخرى، وهذا النبات رائع لأستخدامه لترشيح مياه الصرف.

الكلمات المفتاحية: GC-MS, Phytochemicals, *Azolla filiculoides* Lamark, Pteridophytes.

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INTRODUCTION

Azolla filiculoides Lamark is one of the Pteridophytes species that belong to Salviniaceae (4). Contained around 16 species found in the tropical and warm temperate regions (8). Only one species is found in Iraq (13), float as mats that can be up to 20 cm thick on the water surface (16), small size about 5cm broad and 20cm long, green or dark-red in color, the leaves are bilobate, arranged tile-like in two rows, rhizome root, trichome are unicellular, Reproduce by spores. In ponds, lakes, ditches, water bodies, and streams, *A.filiculoides* inhabit stagnant or slow-moving water. Additionally, it's used as a supplement to phytoremediators for wastewater with high phosphate levels, and it's good at absorbing the water's trace element content (10, 17). Also, as a nitrogen fixation due to the existence of cyanobacteria inside the dorsal leaf lobe of *Azolla* (5,19). Because of its ability to fix nitrogen at both a high and low cost, Azolla is used as a bio-fertilizer, especially in the paddy fields. *Azolla* is often used as a green fertilizer to reduce ammonium volatilization after chemical nitrogen application, livestock food and medication, water purifier, hydrogen fuel, biogas manufacturer, weed and insecticides monitor. By eliminating excess nitrate and phosphorus, the consistency of *Azolla* improves water (20). Plant growth promoters are found in *Azolla* plants, according to experiments (6), hepatoprotective (12), antioxidant (18), bioremediation (22), and antimicrobial activity (18). The aim of this research is to investigate the GC-Ms technique for the most important chemical compounds that may act as an antioxidant, antibacterial activity, and bioremediation.

MATERIALS AND METHODS

ollection of plant and extraction preparation: Fresh plant samples were collected from the Euphrates River, which passes through the Musayyib region in Babylon during the period from November 2020 to January 2021 (Fig.1). The whole plant was washed several times, then dried in the shade for 3-4 days. The dry plant is ground for 5 minutes by an electric grinder to obtain a well-grounded powder (3). Then make a plant extract of 20 gm of plant powder, soaked for 24 hours in 100 ml of methanol (11). The soaked plant is first filtered with filter paper, then in syringe filter, its diameters are 25mm and pore size is 0.22µm directly to the GC-MS device to analyze (2).

Phytochemical screening by GC-MS Analysis: *Azolla filliculoides* Methanol extract was analyzed using a GC-MS analyzer (Agilent Technologies). 1µl methanol sample was injected into a column at 250°C injector temperature, and the oven temperature was started at 60°C and held for 2 minutes before being increased at a rate of 5°C per minute to 310°C without keeping. The Holding was authorized for 2 minutes at a 5Co per minute program pace. The ion sources were held at 230°C. The injector temperature was set to 250 degrees Celsius, and the detector temperature was set to 310°C. The mass spectrum of compounds present in samples was obtained using electron ionization at 70 eV and a detector that scans from 50 to 600 Da atomic units. The scan period was held at 0.5 seconds, with fragments ranging from 50 to 600 Da. The total running time was 31 minutes.

Method for analyzing chemical compounds with MS-GC technology: The active compounds were diagnosed and quantified by the GC-MS device type (Agilent Technologies,7820A GC system) American-made. Separation column type HP-5ms ultra–Inert By dimensions (30m×250µm×0.25µm) which compound of which works in Electron Effect Mode 70-EV (Electron fixed Detector). At a continuous flow rate of 1.2ml per min, helium gas (99.999%) was used as a carrier gas. The pumped fluid volume is 1 with a split ratio of 1:20. The temperature in the injector is 250 Cº. The oven temperature is automatically on 60Cº within an increase of 10Cº per min until 280Cº, then 6Cº per min to 300Cº, hold for 2min then increase to 310Cº by 5Cº per min. Settle in 310Cº. The device pressure is 10.7 PSI with an average of 1.2ml per min. The total time to start and end operation of the device for the sample is 31 min.

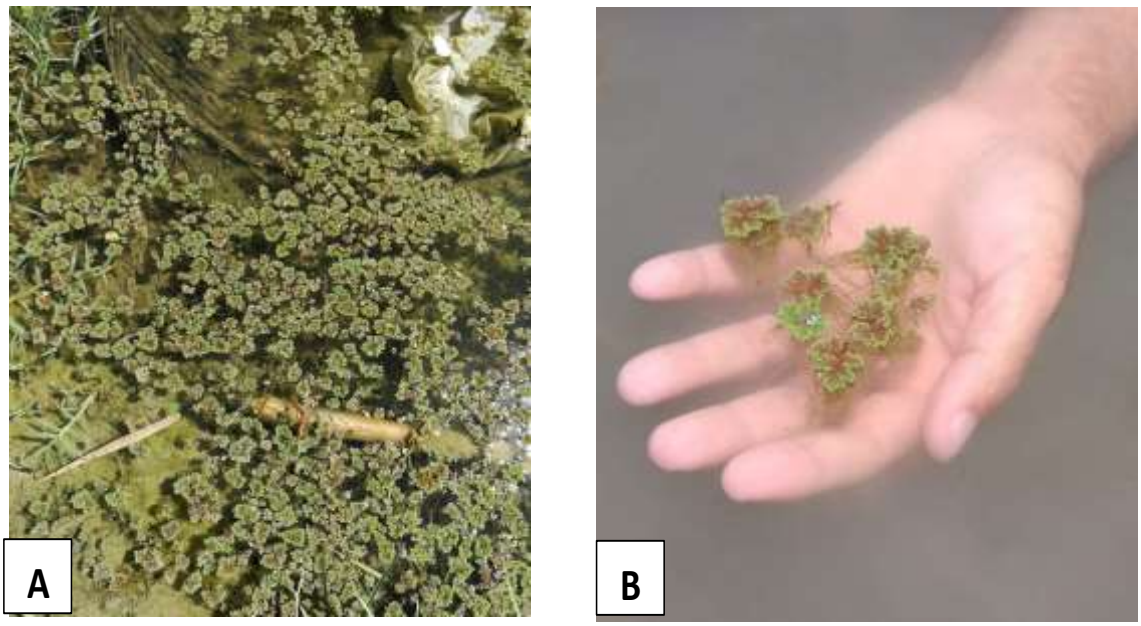


Figure.1. *Azolla filiculoides* (A- Natural Habitat, B- Collection of plant from water)

RESULTS AND DISCUSSION

From the GC-MS analysis of the methanolic extract, the presence of twenty-four bioactive compounds were evident in the chromatogram (Fig. 2). Chemical profiles of the identified compounds were ascertained together for their Time of Retention (RT), chemical structure, molecular formula, molecular weight, and percentage of peak area, CAS, classification number, and using (Table 1). GC-MS study of *A.filiculoides* phytochemicals indicated the existence of twenty-four compounds, as follows with RT as min in the order, 4.853, 6.947, 8.693, 7.280, 7.537, 8.693, 9.740, 10.270, 10.777, 11.655, 12.119, 15.299, 15.398, 15.523, 15.676, 16.052, 17.552, 18.273, 23.499, 25.965, 26.417, 26.619, 26.826, 27.290, and 30.127min, respectively with their names Ethyl formate; Oxirane, (ethoxymethyl)-; Ethanol, 1-ethoxy-2,2,2-trifluoro; Acetic acid, oxo-, methyl ester; Diethyl carbonate; Hydroperoxide, heptyl, Oxirane, (butoxymethyl)-; 1-Butanol, 2-methyl-, (S)-; Valeric acid hydrazide; Aminocyanoacetic acid; 1,3-Cyclohexanediol; Butanal, 3-hydroxy-; 2-Nonenal, (Z)-; 9-Octadecenal; 2,4,6-Trimethyl-1-nonene; 3-(Prop-2-enoyloxy)tetradecane; 3-(Prop-2-enoyloxy)dodecane; Cyclopentane, 1-ethyl-3-methyl-; 9-Oxabicyclo[6.1.0]nonane, cis-; Oleic Acid; 13-Docosenoic acid, methyl ester; 13-Octadecenal, (Z)-; Z-10-Pentadecen-1-ol, and 9-Tetradecenal, (Z)-. These results compatible with Maswada and *et al*., (2020) (15), when pointed to the *Azolla* genus includes several important phytochemicals such as flavonoids, hormones, alkaloids, phenols, triterpenoid derivatives, amino acid, and fatty acid kinds (secondary metabolites). These bioactive components contribute to a wide range of useful and therapeutic properties, including antioxidant, anticarcinogenic, anti-inflammatory, antidiabetic, hepato- and gastro-protective, antiviral, neuroprotective, cardioprotective, and anti-hypertensive properties, also Veerabahu *et al.* (2015)(21) study revealed the presence of steroids, coumarins, tannins, saponins, flavonoids, anthraquinones, phenols, proteins, and lipids in methanolic extract of *A.microphylla*. The *Azolla* lipid fraction can be used to make high-quality biodiesel, but this requires an additional fractionation stage (7). The initial binding and exchange of mineral ions by the Azolla biomass could be supported by surface chemical groups (such as carboxyl and phosphate) (9). *Azolla* has a higher crude protein content (19–30%) than most green forage crops and aquatic macrophytes, as well as a more suitable essential amino acid (EAA) composition for animal nutrition. Plants under stress, such as Azolla ferns, are known to produce phenolic compounds, which have antioxidant properties (14). From Table (1) the results of the research revealed the presence of phenolic substances, flavonoids, and fatty

acids and thus consistent with the study of (1) who indicated phenolics, flavonoids, tannins, anthraquinone, glycosides, and fatty acids in methanolic extraction of *Azolla filiculoides*, Bioactivity against a variety of pathogens has been identified for these compounds, Plants with phenolic compounds, such as *Azolla* ferns, are known to have antioxidant properties. Polyphenols have been shown to inhibit microbial growth by forming complexes with microbial enzymes or proteins, and iron depletion is one of the recognized inhibition mechanisms. Flavonoids have previously been shown to inhibit bacterial development. Purification of active components from Azolla extracts and determination of their inhibitory activity against cariogenic bacteria will be studied further.

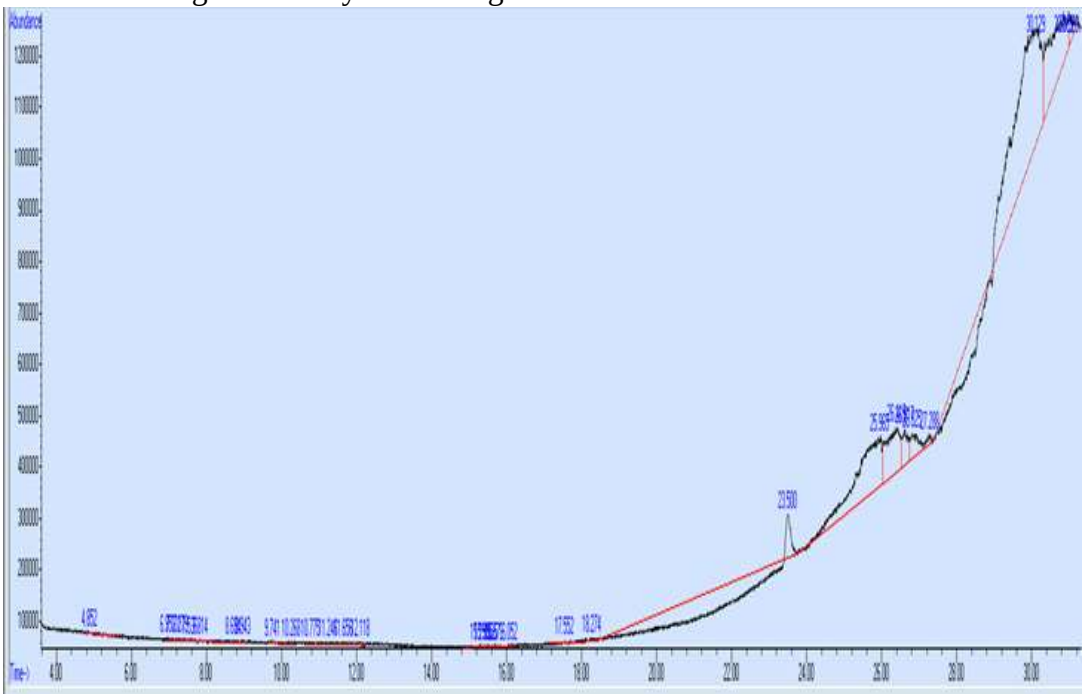
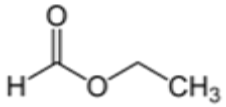
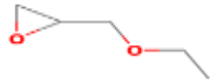
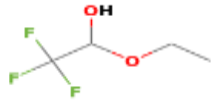
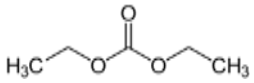
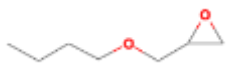
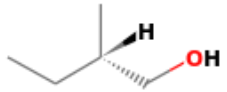
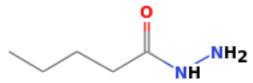
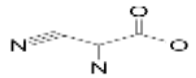
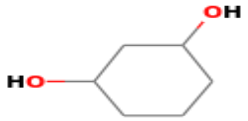
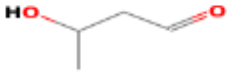
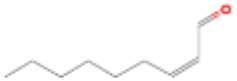
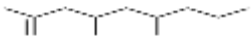
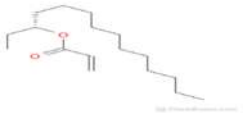
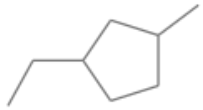
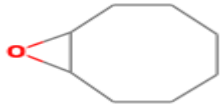
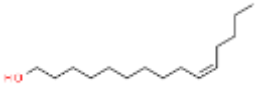
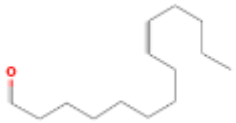


Figure 2. *Azolla filiculoides* GC-MS chromatogram profile

Table 1. Components found in Azolla filiculoides methanolic extract

No.	Name of compound	Retention time	Area	Cas. No.	Molecular Weight g/mol	Molecular Formula	Class	Structure	Application or Uses
1	Ethyl formate	4.853	0.44	000109-94-4	74.08	$C_3H_6O_2$	ester		It's used as a solvent in industry, as well as a fungicide and larvicide in processed foods including dried fruits and cereals.
2	Oxirane, (ethoxymethyl) -	6.947	0.05	004016-11-9	102.1317	$C_5H_{10}O_2$	An epoxide and ether		Ethylene glycol (antifreeze), textiles, detergents, polyurethane foam, solvents, medication, adhesives, and other goods use this chemical intermediate.
3	Ethanol, 1-ethoxy-2,2,2-trifluoro	7.280	0.14	000433-27-2	144.0924	$C_4H_7F_3O_2$	trifluoroacetaldehyde		used to prepare α-trifluoromethylated alcohols for antifungals, antitumor, and chemotherapeutic agents
4	Acetic acid, oxo-, methyl ester	7.537	0.16	000922-68-9	88.0621	$C_3H_4O_3$	ester		No activity reported
5	Diethyl carbonate	8.693	0.17	000105-58-8	118.13	$C_5H_{10}O_3$	a carbonic acid and ethanol ester		a solvent used in intramuscular injections of erythromycin It can be used as an electrolyte component in lithium batteries.
6	Hydroperoxide, heptyl	9.740	0.32	000764-81-8	132.2007	$C_7H_{16}O_2$	organic hydroperoxides.		as found in many paints and varnishes, function via the formation of hydroperoxides.
7	Oxirane, (butoxymethyl) -	10.270	0.25	002426-08-6	130.1849	$C_7H_{14}O_2$	Alkyl epoxides; glycidyl ethers		As a reactive diluent for epoxy resins, a chemical intermediate, and an acid acceptor for chlorinated solvent stabilization
8	1-Butanol, 2-methyl-, (S)-	10.777	0.40	001565-80-6	88.1482	$C_5H_{12}O$	amyl alcohol		It's used as a solvent and a step in the production of other chemicals. 2-Methyl-1-butanol is a part of many amyl alcohol mixtures marketed commercially.
9	Valeric acid hydrazide	11.655	0.70	038291-82-6	116.1616	$C_5H_{12}N_2O$	alkyl carboxylic acid		Because of the fruity taste of the esters, they're used in perfumes and cosmetics, as well as plasticizers and pharmaceuticals.
10	Aminocyanoacetic acid	12.119	0.34	6232-21-9	100.0272	$C_3H_4N_2O_2$	Alpha amino acids		can be used as a reagent, and used in other reactions such as cyclizations, syntheses of coumarins and other heterocycles

11	1,3-Cyclohexanediol	15.299	0.24	504-01-8	116.1583	$C_6H_{12}O_2$	Alcohol		used in organic synthesis, pharmaceuticals, agrochemicals and dyestuff fields.
12	Butanal, 3-hydroxy-	15.398	0.05	107-89-1	88.1051	$C_4H_8O_2$	acetaldehyde		used in medicine as a hypnotic and sedative
13	2-Nonenal, (Z)-	15.523	0.09	60784-31-8	140.226	$C_9H_{16}O$	aldehyde		important aroma component of aged beer and buckwheat
14	9-Octadecenal	15.676	0.15	005090-41-5	266.5	$C_{18}H_{34}O$	fatty aldehydes		Antimicrobial
15	2,4,6-Trimethyl-1-nonene	16.052	0.08	055771-40-9	168.32	$C_{12}H_{24}$	Alkane (Ketones)		Antimicrobial
16	3-(Prop-2-enoyloxy)tetradecane	17.552	0.04	1000245-67-1	268.4	$C_{17}H_{32}O_2$	Hydrocarbons		on the various different situations of industrial applications
17	3-(Prop-2-enoyloxy)dodecane	18.273	0.40	1000245-66-6	240.387	$C_{15}H_{28}O_2$	Hydrocarbons		No activity reported
18	Cyclopentane, 1-ethyl-3-methyl-	23.499	45.69	003726-47-4	112.2126	C_8H_{16}	hydrocarbon (cycloalkane)		No activity reported
19	9-Oxabicyclo[6.1.0]nonane, cis-(cyclooctene oxide)	25.965	29.48	004925-71-7	126.2	$C_8H_{14}O$	Cyclic hydrocarbon		For businesses and individuals involved in the industry, it is a valuable source of advice and direction.

20	Oleic Acid	26.417	13.66	000112-80-1	282.47	$C_{18}H_{34}O_2$	fatty acid		As an emulsifying agent, the sodium salt is a major component of soap. It's also used as a moisturizer. Oleic acid is used as an excipient in pharmaceuticals and as an emulsifying or solubilizing agent in aerosol products in small quantities.
21	13-Docosenoic acid, methyl ester	26.619	4.15	1120-34-9	352.5943	$C_{23}H_{44}O_2$	methyl ester of a fatty acid		is suitable for biological studies and as a normal. Anti-microbial and anti-oxidant properties
22	13-Octadecenal, (Z)-	26.826	4.41	58594-45-9	266.46	$C_{18}H_{34}O$	fatty aldehydes		is a sex pheromone component of the rice stem borer *Chilo suppressalis* (Lepidoptera).
23	Z-10-Pentadecen-1-ol	27.290	1.05	1000245-48-5	226.4	$C_{15}H_{30}O$	Alcohol		No activity reported
24	9-Tetradecenal, (Z)-	30.127	62.26	053939-27-8	210.3556	$C_{14}H_{26}O$	fatty aldehydes		Surfactant and emulsifier are two industrial applications. Membrane stabilizer, source of energy, energy storage, and nutrient

Conclusion

Azolla filiculoides is one of the species of *Azolla* ferns, which are distributed in the water of Iraq. For the first time, GC-MS techniques were used to conduct phytochemical screening, which resulted in the isolation and detection of twenty-four structurally chemical compounds in methanolic extract, which are responsible for a variety of biological activities such as phenolic substances, flavonoids, and fatty acids, which can be used as antioxidants, antibacterial agents, and bioremediation.

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