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GC-MS Analysis and Identification of Many Chemical Composition and antioxidant activity of *Ruta graveolens* L

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Abstract

This study aims to extract the terpene active compounds from the nodules of Ruta graveolens L. plant, using hexane solvent, and then characterize the extracted chemical components using GC-MS technology, with evaluation of the antioxidant activity of the resulting compounds. (14) terpene compounds were identified, including saturated and unsaturated fatty acids. It was observed that short-chain fatty acids appeared with a lower retention time of (L-Menthyl2-methylbutyrate5-Methyl-2-(1-methylethyl) cyclohexyl2-methylbutyrate) by 2.029/min at a concentration of 19.26%, while the retention time increased with increasing carbon chain length for the compound (Trichloromethane Chloroform Freon 20 Methane, trichloro-R 20 Trichloroform CHCl3 Formyl trichloride Meth) by 64.37/min at a concentration of 17.658%. The results of the current study also showed that the hexane extract of Ruta graveolens L. plant has an antioxidant effect. The highest effect was 77.08% at a concentration of 500 micrograms/ml, which is greater than the effect of ascorbic acid, which had an effect of 71.08% at the same concentration.

Keywords: GC MS Analysis; Ruta Graveolens L; Fatty Acids; Antioxidant

1. Introduction

Ruta graveolens L. is a medicinal and aromatic plant belonging to the Rutaceae family. It has garnered significant interest in pharmacological and chemical studies due to its active compounds, particularly phenolic compounds, alkaloids, terpenes, and fatty acids. (Mohannad *et al*., 2023) In addition to the antioxidant activity of extracts obtained from its flower buds, which are among the richest plant parts in active secondary compounds, it has the ability to inhibit free radicals and prevent the auto-oxidation of lipids. This is due to the high metabolic activity associated with the flowering stage. (Saeed *et al*., 2023)

2. Materials and Methods

2.1. Preparation of the plant material

The *Ruta graveolens* L. plant nodes were washed with water to remove dirt, then left to dry for a period of time at room temperature for about 12 days. After that, the dried samples were ground to obtain a fine powder, and then the extraction process was carried out using a sexoleite apparatus.

2.2. Fatty Acid Extraction

A hexane extract containing nonpolar compounds was prepared using a continuous extraction system based on the modified Najm and Sultan (2022) method. Hexane was used as the nonpolar solvent. The extract was then subjected to

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a greenhouse process to release the fatty acids, and the samples were analyzed using GC-MS to detect the natural chemical compounds. (Saadallah. *et al*. 2023)

2.3. Saponification process for releasing fatty acids

The separation process was followed in the following way: 2 grams of crude hexane extract was taken and 100 ml of 7.5 M potassium hydroxide solution was added to it and then the mixture was heated to 100 o C temperature and allowed to cool down to 25, o C temperature. Then distilled water 100 ml was added to give shape of an emulsion solution. The emulsified solution was divided in 2 incidences into the separation funnel and 25 ml of the ether solution added to extract the un saponified fats. The saponified fats were collected and 20 percent of the concentrated sulfuric acid H2SO 4 was added to it until the acidity became pH 2. The ether was added to saponified fat in the funnel and 25 ml of the saponified fat was removed using the funnel. This will lead to the development of two layers with the top layer being the ether one containing the fatty acids and the bottom layer which will be discarded. The fatty acids were separated and stored the refrigerator in the sterile and opaque glass bottles, which were labeled with the help of the GC-MC technology (Najm *et al*., 2022, Sultan *et al*., 2020)

2.4. Gas chromatography-mass spectrometry (GC-MS) is used to diagnose terpene compounds and fatty acids

The GC-MS QP-210 Ultra of the Japanese company Shimadzu is one of the modern analytical devices; therefore, the fatty acids were identified after performing the saponification procedure on the hexane extract of the rue plant of the University of Basra. It is applied in the sensitive and exact qualitative and quantitative segregation, detection and identification of fundamental and stable chemical compounds. The device is a combination of gas chromatography (GC) to isolate the types of components of the sample and mass spectrometry (MS) to ascertain the molecular fingerprint of each substance. The compounds are separated based on their level of volatility and reaction with the chromatographic column via injection and vaporization of the sample in a GC unit. The two components are then transferred to a mass spectrometer where they are ionized (typically using electron ionization EI technology) and then the ions are separated based on the mass-to-charge ratio (m/z). Unique mass spectra are taken and the outcome is compared with established spectral libraries to identify the compounds with very high accuracy and reliability.

2.5. Antioxidant Activity of Hexane Extract

2.5.1. Antioxidant Extract

To prepare a concentration of 400 micrograms/ml, 0.04 g of DPPH was dissolved in 100 ml of methanol; to prepare a 5000-ppm concentration of the standard solution, 0.5 g of vitamin C was dissolved with 100 ml of methanol and distilled water. Okunade,2002, The remaining concentrations were then prepared by dilution.The concentrations (30, 60, 120, 250, 500 ppm) of the standard compound and the sample were prepared by the dilution method. (Vaidyaratnam, 2002).

The mixture was left to stand for half an hour after being shaken well. The absorbance was measured using a UV-VIS Shimadzu device at a wavelength of 517 nm, (Ahmed, 2013**)**

Use the logarithmic dose inhibition curve by calculating the value of IC 50 which is the concentration required to inhibit 50% of free radicals, Koleva, I.I 2011

Equation (Percent inhibition = A0 - A 1 / A0 × 100) was used to calculate the effect of free radical DPPH removal as a percentage, The absorption value in the presence of the laboratory sample is A1 and the absorption value for the reference sample is A0. (Koleva, 2011)

3. Results and Discussion

3.1. Diagnosis of fatty acids using gas chromatography-mass spectrometry (GC-MS)

The results of the GC-MS analysis of the hexane extract from rue nodules showed high efficiency in separating and identifying the fatty acids that make up the extract after the saponification process. The compounds were identified based on the retention time and the resulting mass spectrum for each compound, which facilitates the identification of the chemical compounds of fatty acids with high accuracy. The resulting chromatogram showed several distinct and well-defined peaks, indicating the presence of saturated and unsaturated fatty acids within the hexane extract. Short-chain fatty acids were observed to appear with a lower retention time of 2.029/min for the compound (L-Menthyl2-methylbutyrate 5-Methyl-2-(1-methylethyl) cyclohexyl2-methylbutyrate) at a concentration of 19.26%.

While the retention time increased with increasing carbon chain length in the compound (Trichloromethane Chloroform Freon 20 Methane, trichloro- R 20 Trichloroform CHCl3 Formyl trichloride Meth) by 64.37/minute at a concentration of 17.658% as shown in Table (1) and Figure (1).

The findings indicated that the fatty acids synthesized by the hexane extract indicate the significance of the plant nodules of rue as a source of active compounds because most pieces of evidence have shown that fatty acids have antioxidant properties. The difference in ratios of fatty acids can also be attributed to a number of factors including the type of part of the plant that was sampled, the level of growth and the conditions in which the plant was grown.

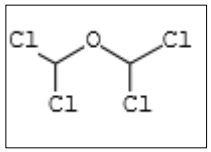
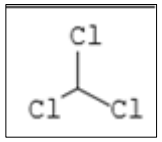
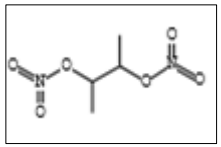
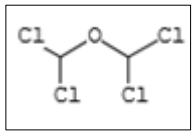
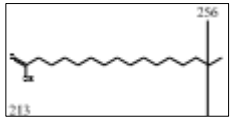
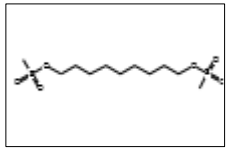
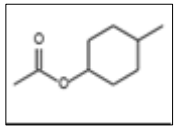
Findings of the study carried out by the researcher (Elshiekh *et al*., 2022) on the hexane extract prepared of the leaves of the rue plant proved that it contains a pool of natural chemical compounds, which were identified through the use of the gas chromatography-mass spectrometry (GC-MS) technology, and it also has a good antimicrobial and antioxidant activity in comparison to the other extracts like ethyl acetate and methanol, with a difference in biological activity noted among the extracts.

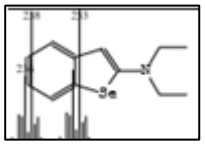
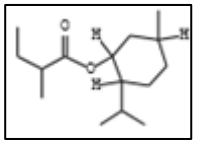
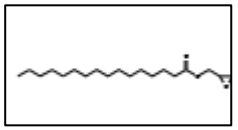
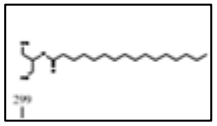
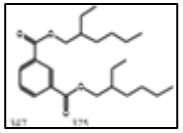
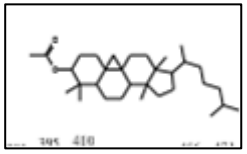
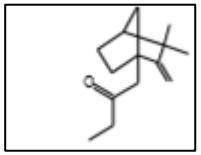
A study by Azalwork *et al*. 2017 established that the hexane extract of the flowering part and nodules of Rue (Ruta graveolens L.) produced, when analyzed by gas chromatography-mass spectrometry (GC-MS), contained a level of several nonpolar compounds with the most significant percentage being the saturated and unsaturated fatty acids including hexadecanoic acid, palmitic acid, octadecanoic acid, and stearic acid.Along with a few terpene hydrocarbons, the study.

Rue has numerous active compounds, such as terpenes, tannins, alkaloids, and saponins, as the researcher Álvarez *et al*. 2025 and Motamed *et al*. (2014) have identified using the chemical techniques. This is in line with our study findings.

Researcher Kathirvel *et al*. 2017 and researcher Mohannad *et al*. 2023 also used the GCMS device in identifying several active compounds in the rue plant, and the study established that the plant is rich in active compounds that demonstrate their antioxidant activity.

Table 1 Diagnosis of fatty acids separated from Ruta graveolens L. based on GC-MS technique

Peak	R.time	Area	Area%	Name	Type
1	2.029	36186036	19.26	:Methane, oxybis[dichloro- Ether, bis(dichloromethyl) Bis(dichloromethyl) ether Methane, oxybis*dichloro- Dichloro(dichlor	
2	2.245	120963922	64.37	Trichloromethane Chloroform Freon 20 Methane, trichloro- R 20 Trichloroform CHCl3 Formyl trichloride Meth	
3	3.319	3987885	2.12	Nitrogen dioxide Nitrogen oxide Nitrito Nitro Nitrogen peroxide Azote Rcra waste number P078 Stickstoffdioxid	
4	4.819	775417	0.41	Methane, oxybis[dichloro- Ether, bis(dichloromethyl) Bis(dichloromethyl) ether Methane, oxybis*dichloro- Dichloro(dichlor	
5	17.658	1544378	0.82	:n-Hexadecanoic acid Hexadecanoic acid n-Hexadecoic acid Palmitic acid Pentadecanecarboxylic acid 1-Pentadecanecarbo	
6	18.777	200995	0.11	:1,9- Nonanediol, dimethanesulfonate Nonane-1,9-dimethanesulfonate CB 2067 Methanesulfonic acid nonamethylene ester SK	
7	19.390	98387	0.05	:4- Methylcyclohexanol acetate 4-Methylcyclohexyl acetate #	

8	19.939	350470	0.19	:N,N-Diethyl-1-benzoselenophen-2-amine	
9	20.581	59699	0.03	:L-Menthyl 2-methylbutyrate 5-Methyl-2-(1-methylethyl)cyclohexyl 2-methylbutyrate	
10	20.708	420769	0.22	:Glycidyl palmitate Oxiran-2-ylmethyl palmitate Hexadecanoic acid, 2-oxiranylmethyl ester Hexadecanoic acid, oxiranylmethyl e	
11	22.727	2277553	1.21	:Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester Palmitin, 2-mono- Palmitic acid .beta.-monoglyceride 2-Hexadecan	
12	24.535	20357276	10.83	:1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester Bis(2-ethylhexyl) isophthalate Di-2-ethylhexyl isophthalate Dioctyl isopht	
13	25.787	451130	0.24	:9,19-Cyclolanostan-3-ol, acetate, (3. beta.)- 9,19-Cyclo-9. Beta. -lanostan-3. beta. -ol, acetate Cycloartanyl acetate 3-O-Acetyl-5a-d	
14	27.984	247120	0.13	4-Camphenylbutan-2-one 1-(3,3-Dimethyl-2-methylenebicyclo [2.2.1] hept-1-yl)-2-butanone	

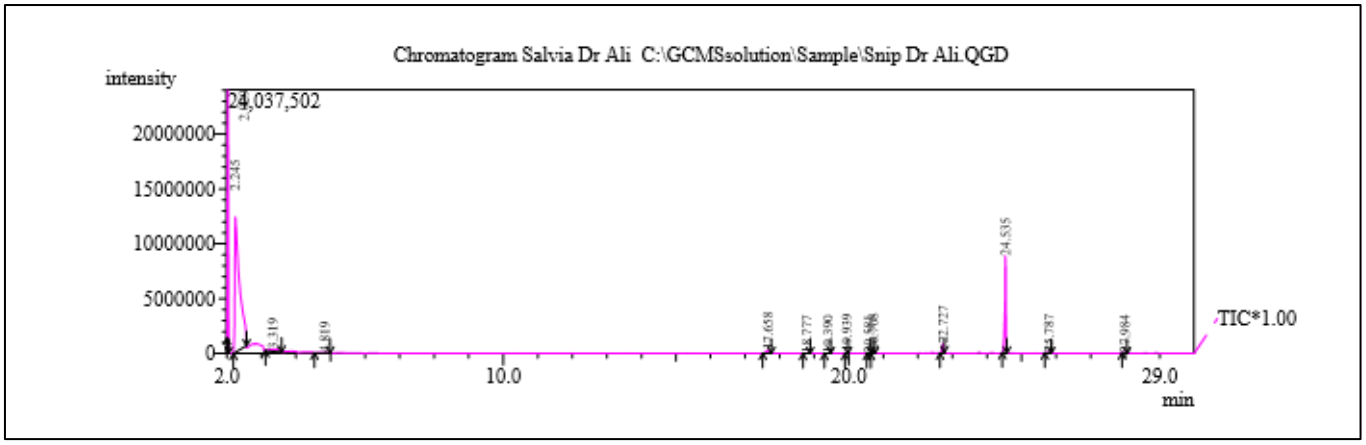


Figure 1 Gas chromatography-mass spectrometry (GC-MS) of fatty acids separated from hexane extract of Ruta graveolens plant

3.2. The effect of the antioxidant activity of the hexane extract produced from the nodule of the *Ruta graveolens* L. plant.

The findings of the present research demonstrated that yea extract of Ruta graveolens L., hexane, has an antioxidant effect, and the findings could be attributed to the properties of the used solvent as well as the chemical structure of the extract.

This possible activity has been explained by the presence of fatty acid compounds with antioxidant properties but Ruta graveolens is high in terpene compounds which are significant in antioxidant activity. These compounds also have a chemical composition that is able to prevent the formation of free radicals (Kathirvel *et al*. 2017).

Table 2 Antioxidant activity of the main compounds isolated from *Ruta graveolens* L. compared to ascorbic acid based on the DPPH test

Concentration	Ascorbic acid	Salvia (Hexsan)
Microgram per milliliter (µg/mL)	SD	SD
30	12.25	18.09
60	21.85	29.77
120	49.80	54.45
250	64.11	70.80
500	71.08	77.08
IC 50 (ppm)	120.4	110.2

Comparing the results of the antioxidant activity of hexane extract with ascorbic acid on Ruta graveolens nodules the nonpolar extracts usually gave a stronger effect at all concentrations when compared to ascorbic acid as indicated in Table (2). The reason is that the plant nodules are endowed with high contents of active fatty compounds unlike those in the leaves or stems. This is the clear indication of the existence of antioxidant activity in nonpolar extracts.

Researcher Merghem *et al*, 2019 provided a study, which affirmed that rue has active compounds, which help to enhance antioxidant capacity.

Another difference in the current study versus that confirmed by the researcher in their study (Molnar *et al*., 2017) was that ethanol demonstrates more activity in the inhibition of free radicals than the hexane extract of the same concentration applied in the DPPH test.This is because the activity of ethanol is higher than the hexane extract at the same concentration applied in the DPPH test.

The study by (Saeed *et al*. 2023) and the study by (Mohannad *et al*. 2023) showed that the extracts produced from Ruta graveolens showed the highest level of antioxidant activity, and their effect was similar to that of ascorbic acid when comparing the power of free radical capture.

4. Conclusion

In conclusion, this scientific research has shown that Ruta graveolens L contains many active terpene compounds, including saturated and unsaturated fatty acids, which play a vital role in serving society by serving as alternatives to chemical treatments that may have serious side effects on human health, in addition to the complications that humans may experience from them. By replacing these treatments with compounds extracted from the Ruta graveolens L , many diseases can be treated, including eliminating free radicals as antioxidants.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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