Original Article

GC/MS analysis of crude extract of Fig leaves naturally grown in Iraq

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ABSTRACT

This work detect bioactive compounds and their derivatives in fig Leaves found in Iraq, chromatography-mass spectrometry (GC-MS), an analytical technique for identifying the presence of many compounds in a given sample. The dried plant powder weighed 250 grammes, and it was soaked in Hexan, which was diluted to 1500 millilitres, with intermittent shaking, at room temperature. Filtration of the Hexan-soluble components occurred after 3 days. Utilizing a rotary evaporator, the filtrate was concentrated by evaporating it at a low temperature and high vacuum. A dark greenish residue was obtained. The residue evaporated to dryness and then submitted to gas chromatography-mass spectrometry. To differentiate the various physiologically active components, gas chromatography-mass spectrometry was used to detect many types of high and low molecular weight chemical entities in crude extracts of fig leaves hexane fraction. The presence of several distinct components was discovered using GC/MS. The results show fig leaves contain 7 compounds Major bioactive chemicals contained in fig leaves were discovered and described spectroscopically in the hexane fraction of the plant. As a result, the discovery of many physiologically active chemicals in plant leaf extracts calls for more biological and pharmacological research.

Keywords: Fig leaves, Bioactive compounds, Hexan, Gas chromatography-mass spectrometry

Introduction

Many powerful pharmaceuticals are derived from plants, and this is largely owing to the existence of bioactive chemicals in the pharmaceutical industry [1]. Various secondary metabolites, or phytochemicals, may be found in plants. Phytochemicals' beneficial effects on health may be independent, additive, or synergistic, making them a helpful tool in the treatment of certain conditions [2, 3]. The pharmaceutical industry relies heavily on phytochemicals in the creation of novel medications and the manufacture of therapeutic agents [4]. Finding natural sources of active ingredients is the first step in the creation of novel medications. Plant extract screening is a novel method for identifying plants with therapeutically active chemicals [1, 5]. Phytochemicals, including tannins, saponins, flavonoids, alkaloids, and terpenoids, have various biological effects, including anti-diarrheal, anti-ulcer, antioxidant, anti-inflammatory, and anticancer properties [5].

Over 800 species of tropical and subtropical shrubs, trees, climbers, hemiepiphytes, and creepers make up the family Ficus (Moraceae), which is one of the largest angiosperm families [6]. High economic and nutritional qualities make this species a valuable genetic resource, and its presence in the rainforest ecosystem is essential to the health of the ecosystem as a whole. Tropical fruit-eating animals may also benefit from it as a food source [7]. Based on early morphological data, the genus is split into six subgenera. There are roughly 280 species in the monoecious subgenus Urostigma, and they all have different hemiepiphytic characteristics. There are 23 different species of Ficus, and they range from hemiepiphytes to lithophytes, with aerial and creeping root systems [8].

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The fig tree, genus Ficus, and its citrus fruit are both members of the Moraceae family of plants. Although it originated in the western part of Asia and the Mediterranean, it has been cultivated for millennia and is now planted all over the globe for its fruit and decorative features [9]. Over 800 species of tropical and subtropical plants belong to the genus Ficus, of which Ficus carica is the type species. Smooth, white bark covers the trunk and branches of a fig tree or shrub, which may grow to a height of 7-10 meters (23-33 feet). Usually, the leaves are rather large, and they have anywhere from three to five lobes. The syconium, one of several types of fruits, has a teardrop shape measuring between 5 and 10 centimeters (1.2-2 inches) in length. The skin is green and becomes purple or brown when mature, while the red, juicy meat is sweet and juicy and speckled with crunchy seeds. Skin irritation may occur if contact is made with the milky sap found in green areas. It's late summer, too early fall in the Northern Hemisphere, the prime time for fresh figs. They can thrive in hot summer continental climes thanks to their tolerance to mild seasonal cold.

Figs are a versatile dessert ingredient, fresh, dried, or in the form of jam, rolls, biscuits, and other baked goods. Most commercial output is in the form of dried and processed fruit since ripe fruit does not move and is preserved well. Figs are mostly water and carbs (around 20%), with very little protein, fat, and micronutrients. They contribute little towards a healthy diet in the form of fiber. The 2018 global raw fig production was estimated at 1.14 million tonnes. Market share was highest in Morocco, Turkey, Algeria and Egypt [10].

Phytochemistry

Due to extensive phytochemical analysis, numerous bioactive substances have been identified in F. carica. These include phenolic compounds, phytosterols, organic acids, anthocyanins, triterpenoids, coumarins, and volatile molecules such as hydrocarbons and aliphatic alcohols [11-13]. Organic acids, Phenoxols, and volatile chemicals are common in F. carica species [14]. An aqueous extract of F. carica L. leaves has been used to isolate several phenolic and organic acids. These include 3-O- and 5-O-caffeoylquinic acid, ferulic acid, quercetin-3-O-glucoside, psoralen, quercetin-3-Orutinoside, and bergapten (oxalic, shikimic, malic, citric, quinic, and fumaric acids) [15-17].

Current and conventional uses

There are claims that F. carica may help with metabolic issues, cardiovascular health, lung function, spasms, and inflammation [18, 19]. The name "Fig" is the most common one for this fruit. F. Carica's leaves, fruits, and roots are used in traditional medicine to treat a wide range of conditions, including those related to the digestive system (indigestion, colic, loss of appetite, and diarrhea), the respiratory system (sore throats, cough, and bronchial problems), the inflammatory system, the cardiovascular system, and the immune system [20, 21]. Whether consumed fresh, dried, or cooked into jam, the fruits of the F. carica tree are delicious in any form. Figs are popular as a healthy snack since they are rich in protein and low in fat and cholesterol. Laxative, antispasmodic, circulatory, pulmonary, and anti-inflammatory are only a few of the ancient uses for figs in medicine [22]. Some people have found that a mixture of honey and the juice of the F. Carica fruit will stop bleeding. Various fruits are employed as mild laxatives, expectorants, and diuretics in traditional Indian medicine [23]. Liver and spleen disorders benefit from its usage. For people with diabetes, the dried fruit of F. carica might be a healthy addition to their diet. Since it contains a high sugar concentration, it is marketed to consumers as a tasty treat [24]. The localized use of fruit paste on painful cysts, tumors, and inflammatory conditions has been shown to be effective.

Biological activities

Activity of antioxidant

The phenolic chemicals found in F. carica serve several functions in plant physiology. Some of them are beneficial to human health because they have antioxidant properties. They might be reducing agents, hydrogen donors, scavengers of free radicals, quenchers of singlet oxygen, etc. The total polyphenol content, flavonoid content, antioxidant activity, and anthocyanin profile of six marketed F. carica fig cultivars were analyzed. The figs ranged in color from black to red to yellow to green. The ferric-reducing antioxidant technique was used to analyze the antioxidant qualities. The antioxidant potential and content of polyphenols, flavonoids, and anthocyanins were greatest in fruits [25-28].

Activity of anticancer

The in vitro inhibitory effects on the growth of several cancer cell lines led to the isolation of a combination of 6-O-acyl-d-glucosyl-sitosterols as an efficient cytotoxic agent from (F. carica) latex [29].

Activity of hepatoprotective

Biochemical, histological, and functional alterations induced by oral dosing with 50 mg/kg rifampicin in rats were significantly reversed after treatment with the petroleum ether extract from F. carica leaves, indicating possible hepatoprotective action [30].

Activity of hypoglycemic

Oral or intraperitoneal injection of the leaf extract had a considerable hypoglycemic effect in streptozotocin-diabetic rats. Treatment prevented weight loss in diabetic rats, and there was a significant change in the survival index related to plasma insulin levels. According to the findings, F. carica aqueous extract has hypoglycemic action [31].

Anti-Fungal activity and antibacterial activity

For oral bacteria, the F. carica methanol extract was highly bactericidal (MICs, 0.156 to 5 mg/mL; MBCs, 0.313 to 5 mg/mL). In vitro studies using a synergistic effect of methanol extract with ampicillin or gentamicin against oral bacteria [32, 33] suggest that figs may have antibacterial properties. Extracts of F. carica latex were tested for their ability to kill bacteria and fungi using the disc-diffusion technique. The results showed that hexane, chloroform, ethyl acetate, and methanol exhibited the highest antibacterial activity among the tested solvents. Microsporum canis was highly inhibited by both the methanolic extract (75%) and the ethyl acetate extract (85%) at a concentration of 750 g/mL; at this concentration, Candida albicans was totally inhibited by the methanol fraction [34].

Activity of antipyretic

An ethanol extract of F. carica at 100, 200, and 300 mg/kg doses substantially decreased baseline body temperature, whereas yeast-raised it. In comparison to the standard antipyretic medicine, paracetamol (150 mg/kg.b.wt., p.o.), the effect persisted for as long as five hours after drug administration [35].

Activity of antituberculosis

F. carica leaves were extracted with 80% methanol and examined for resistance to Mycobacterium tuberculosis H37Rv using a colorimetric microplate-based method. The outcome successfully treated TB with a MIC of 1600 g/mL [36].

The study aim

The analysis of the phytochemical components of F. Carica revealed the presence of a variety of bioactive substances, including phenolic compounds, coumarins, the composition of anthocyanins, phytosterols, organic acids, triterpenoids, and volatile substances like hydrocarbons and aliphatic alcohols.

Materials and Methods

Plant material

Fig leaves were collected from the Tikrit in November 2021. After being cleaned and dried in the shade, the leaves were crushed into a powder in a mechanical grinder.

Experimental work

The experimental work is categorized into:

Extraction method (cold method)

The dried plant powder weighed 250 grams and was soaked in Hexan, which was diluted to 1500 milliliters, with intermittent shaking, at room temperature. Filtration of the Hexan-soluble components occurred after 3 days. Utilizing a rotary evaporator, the filtrate was concentrated by evaporating it at a low temperature and high vacuum. A dark greenish residue was obtained. The residue evaporated to dryness for GC/MS analysis.

Preliminary phytochemical examination of crude extracts

Standard protocols for phytochemical analysis were applied to crude extracts, fractions, and powder specimens from the medicinal plants under investigation to screen and identify bioactive chemical ingredients.

Coumarins test: Each plant extract and fraction was measured to be 0.5 g in a test tube. We used filter paper that had been treated with a 1 N NaOH solution to seal the tube's opening. The filter paper was taken out of the test tube and examined under ultraviolet light for the presence of yellow fluorescence, a sign of coumarins, after being placed in a saucepan of boiling water for a short time.

Sterols and terpenes test: To get rid of the color, petroleum ether was shaken with 0.5 g of each plant extract and fraction. Anhydrous sodium sulphate was used to dry the chloroform layer after extracting the residue using 10 ml of chloroform. 0.5 ml of acetic anhydride and 5 ml of chloroform were combined, and two drops of strong sulfuric acid were added after that. Different hues were noticed to represent the presence of terpenes or sterols. The green tint identified sterols, whereas terpenes and triterpenes were shown by the pink to purple color.

Terpenoids test: Test of Salkowski 2 ml of chloroform and 5 ml of each plant extract and fraction were combined, and 3 ml of carefully diluted (H2SO4). At the contact, a layer of reddish-brown color appeared, providing evidence that terpenoids were present.

Flavonoids test: Petroleum ether and 0.5 g of each plant extract and fraction were agitated to remove the fatty components (lipid layer). 80% ethanol was used to dissolve the defatted residue before it was filtered. Further experiments were performed using the filtrate:

- A test tube containing 3 ml of the filtrate and 4 ml of 1% aluminum chloride in methanol was used to determine the color. Yellow color formation indicated the presence of flavonoids.
- In a test tube, 3 ml of the filtrate was combined with 4 ml of 1% potassium hydroxide to determine the color. The presence of flavonoids was indicated by a dark yellow tint.

Hexan fraction GC/MS

The SHIMADZU/Gas Chromatography GC 2010 Plus GC/MS equipment was utilized.

These are the settings for GC/MS analysis:

- The Injection Temperature: 240.0°C
- The Column Temperature: 70.0°C.
- The Sampling Time: 1 minute.
- The Injection Mode: splitless.
- Pressure: 100.0kPa.
- Flow Column: 1.53mL//min.
- Flow Purge: 3.0mL/min.
- The Flow control Mode: pressure.
- Linear velocity: 45.4cm/sec.
- Ratio of Split: 10.0

Results and Discussion

Methods of extraction

Separating the final products from the basic components begins with extraction. In order to successfully isolate a material, the method of extraction must be tailored to that substance. Two separate extraction procedures were performed, each at a different temperature, to get a sample of the plant's contents; the optimal solvent for extraction was chosen after taking into account the percentage yield from each method and the TLC examination of the crude extract for constituents [37]. According to the rule of similarity and impermissibility, when selecting a solvent for solvent extraction, selectivity, solubility, cost, and safety should all be taken into consideration (like dissolves). In general, the performance of a solvent improves when its polarity value is closer to that of the solute and vice versa.

Extraction method (cold methods)

This is a relatively basic extraction technique; however, it has the drawbacks of being time-consuming and inefficient. Thermolabile substances might be extracted using this method. Leaves are macerated for three days. In the context of GC/MS analysis, this strategy was favored.

Hexan fraction GC/ MS analysis

Ficus carica hexan extract GC-MS chromatogram revealed many peaks, each of which represented a unique chemical ingredient characterized by its characteristic limited retention time, peak area, and molecular formula. As showin in **(Figure 1)**. displays the findings, which showed that ni distinct natural chemical components were detected in the hexan extract of leaves.

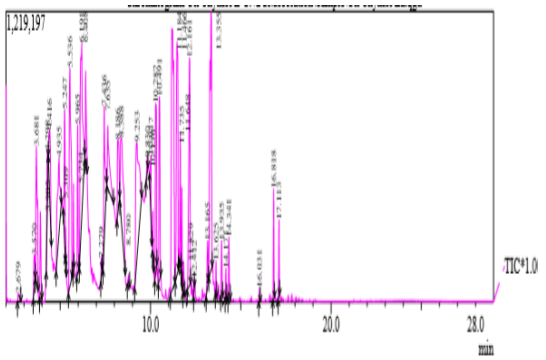

Figure 1. GC/ MS chromatogram of Hexan fraction

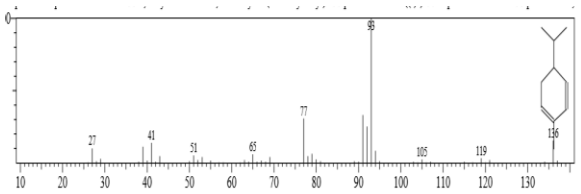

Figure 2. MS fragment interpretation of compound 1 of Hexan fraction

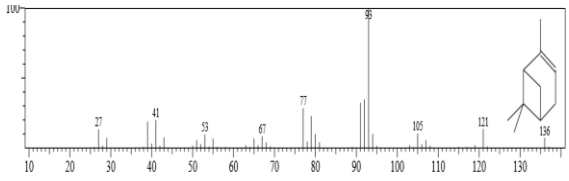

Figure 3. MS fragment interpretation of compound 2 of Hexan fraction

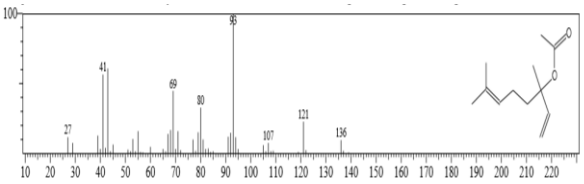

Figure 4. MS fragment interpretation of compound 3 Hexan fraction

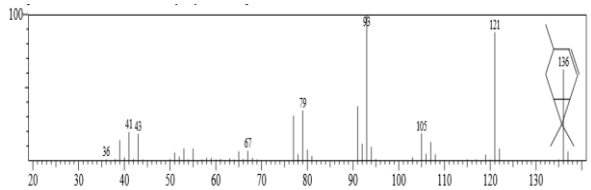

Figure 5. MS fragment interpretation of compound 4 of Hexan fraction

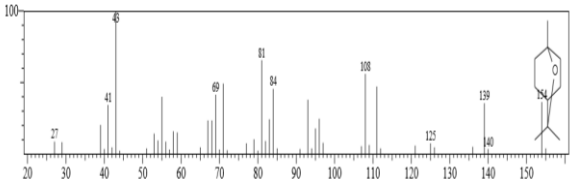

Figure 6. MS fragment interpretation of compound 5 of Hexan fraction

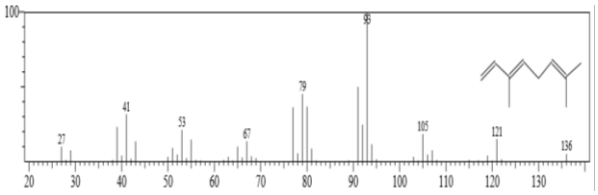

Figure 7. MS fragment interpretation of compound 6 of Hexan fraction

When the benefits of gas chromatography and mass spectrometry are combined, you get gas chromatography-mass spectrometry (GC-MS), an analytical technique for identifying the presence of many compounds in a given sample. GC-MS may be used for a wide variety of purposes, including drug detection, fire analysis, environmental analysis, explosives analysis, and the identification of unknown compounds. Because of its usefulness in separating volatile and semi-volatile organic molecules, gas chromatography (GC) is widely used in a variety of fields and industries, especially those concerned with ensuring the quality of food and the safety of the environment. GC-MS combines the specificity of GC with the sensitivity of MS to perform tasks such as separating mixtures, quantifying analytes, identifying unfamiliar peaks, and detecting very low concentrations of contaminants. Results show fig leaves contain 6 compounds that are shown in the **(Figures 2-7)**. above [38, 39].

- **Compound 1:** p-Mentha-1.
- **Compound 2:**. Alpha. -Pinene
- **Compound 3:** Linalool acetate
- **Compound 4:** (+)-4-Carene
- **Compound 5:** Eucalyptol $$ Cineole
- **Compound 6:** beta. -Ocimene

Conclusion

Phytochemical screening of fig leaves cultivated in Iraq demonstrates the presence of various phytochemicals which were separated from the leaves of plants according to differences in their chemical nature major bioactive chemicals contained in fig leaves were discovered and described spectroscopically in the hexane fraction of the plant. As a result, many physiologically active chemicals in plant leaf extracts calls for more biological and pharmacological research were discovered.

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Conflict of interest: None

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Ethics statement: None

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