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CARACTERIZAÇÃO BIOQUÍMICA DA PLANTA MEDICINAL DE *Ferula rutbaensis* NO DESERTO OCIDENTAL IRAQUIANO

BIOCHEMICAL CHARACTERIZATION OF *Ferula rutbaensis* MEDICINAL PLANT IN IRAQI WESTERN DESERT

التشخيص الكيموحيوي لنبات *Ferula rutbaensis* الطبي في صحراء العراق الغربية

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RESUMO

As plantas usadas na medicina popular não apenas representam fontes ricas de materiais terapêuticos, mas também desempenham um papel crucial no desenvolvimento de novas drogas sintetizadas completa ou parcialmente. A planta Mharut (*Ferula rutbaensis*) é parte integrante das práticas terapêuticas beduínas no deserto ocidental da província de Anbar-Iraque, mas, até o momento, este é o primeiro estudo que descreve seus constituintes fitoquímicos. A planta está crescendo perto da fronteira do Iraque com a Arábia Saudita e bem adaptada a uma ampla variedade de solos. Tradicionalmente, *F. rutbaensis* tem sido amplamente utilizado para tratar acne, distúrbios estomacais e intestinais, intoxicações alimentares e problemas respiratórios. Amostras frescas de plantas foram coletadas e caracterizadas morfologicamente. Da mesma forma, a técnica de codificação de barras de DNA baseada em ITS foi usada de forma eficiente para aprovar a identificação morfológica de *F. rutbaensis*. O espectro GC-MS foi adotado na caracterização fitoquímica de extratos aquosos e metanólicos de partes frescas e secas de plantas. O extrato aquoso de raízes secas foi a fonte mais rica de compostos bioativos em comparação com raízes frescas ou extratos metanólicos de partes frescas ou secas de plantas. Em geral, os fitoquímicos detectados caem em ácidos graxos, terpenos, hidrocarbonetos alcanos e ésteres. Notavelmente, os ácidos graxos na forma de ácidos oléico e palmítico foram os dois compostos bioativos mais abundantes em extratos aquosos e metanólicos de raízes frescas e secas de plantas. Aparentemente, os ácidos graxos insaturados detectados e / ou outros componentes bioativos estão por trás das propriedades terapêuticas de *F. rutbaensis* que podem ser ingredientes úteis para preparar cosméticos à base de Mharut, como sabonetes médicos, loções corporais, condicionadores de pele e protetores solares. Além disso, alguns outros componentes possuem propriedades anti-inflamatórias, antioxidantes e antimicrobianas. Mais investigações serão necessárias para confirmar a atividade antimicrobiana dos extratos de *F. rutbaensis*.

Palavras-chave: *Mharut, Ferula rutbaensis, DNA barcoding, ITS, espectro de GC-MS.*

ABSTRACT

Plants used in folk medicine not only represent rich sources for therapeutic materials, but it also plays a crucial role in developing completely or partially novel synthesized drugs. Mharut plant (*Ferula rutbaensis*) is an integral part of Bedouin therapeutic practices in the western desert of Anbar province-Iraq. Still, to date, this is the first study describing its phytochemical constituents. The plant was growing near the Iraq-Saudi Arabia borders and adapted to a wide range of soils. Traditionally, *F. rutbaensis* has been widely used to treat acne, stomach and bowel disorders, food poisoning and respiratory problems. Fresh plant samples were collected and morphologically characterized. Likewise, the ITS-based DNA barcoding technique was efficiently used to approve the morphological identification of *F. rutbaensis*. The GC-MS spectrum was adopted in the phytochemical characterization of aqueous and methanol extracts of fresh and dry plant parts. The aqueous extract of dry roots

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was the richest source for bioactive compounds than fresh or methanolic extracts of either fresh or dry plant parts. In general, the detected phytochemicals falling into fatty acids, terpenes, hydrocarbon alkanes, and esters. Notably, fatty acids in Oleic and Palmitic acids were the two most abundant bioactive compounds in both aqueous and methanolic extracts of plant fresh and dry roots. The detected unsaturated fatty acids and/or other bioactive components are laying behind the therapeutic properties of *F. rutbaensis* that can be useful ingredients to prepare Mharut-based cosmetics such as medical soaps, body lotions, skin conditioners and sunscreens. Additionally, some other components were found to have anti-inflammatory, antioxidants, and antimicrobial properties. Further investigations will be necessary to confirm the antimicrobial activity of *F. rutbaensis* extracts.

Keywords: Mharut, *Ferula rutbaensis*, DNA barcoding, ITS, GC-MS spectrum

المستخلص

لا تمثل النباتات التي تستخدم في الطب الشعبي مصادر غنية للمواد العلاجية فحسب، بل إنها تلعب دورًا مهمًا في تطوير عقاقير مصنعة جديدة كليًا أو جزئيًا. على الرغم من أن نبات المحروت (*Ferula rutbaensis*) جزءً لا يتجزأ من الممارسات العلاجية البدوية في الصحراء الغربية لمحافظة الانبار-العراق، إلا أن هذه هي الدراسة الأولى التي تصف مكوناته الكيميائية. ينمو النبات بالقرب من الحدود العراقية مع العربية السعودية وهو متأقلم جيدا مع مدى واسع من الترب. تقليدياً، استخدم نبات *F. rutbaensis* بشكل واسع في علاج حب الشباب واضطرابات المعدة والامعاء والتسمم الغذائي ومشاكل التنفس. تم جمع النماذج الطرية للنبات وتشخيصها مظهرياً. فضلاً عن ذلك، تم استخدام تقنية ترميز الحامض النووي المستندة إلى ITS بكفاءة في اثبات التشخيص المظهري لنبات *F. rutbaensis*. أعتمد تحليل GC-MS الطيفي في تشخيص المكونات النباتية الفعالة للمستخلص المائي والميثانولي للأجزاء النباتية الطرية والجافة. كان المستخلص المائي للجذور الجافة هو المصدر الأغنى للمركبات النشطة بيولوجيًا، مقارنة بالجذور الطرية أو المستخلصات الميثانولية لأجزاء النبات الطرية أو الجافة. بشكل عام، فإن المواد الكيميائية النباتية المكتشفة تقع ضمن الأحماض الدهنية والتربينات والألكانات الهيدروكربونية والإسترات. تجدر الإشارة إلى أن الأحماض الدهنية بشكل حامضي الأوليك والبالمتيك، كانت أكثر المركبات النشطة بيولوجيًا وفرة في المستخلصات المائية والميثانولية لجذور النبات الطرية والجافة. وعلى ما يبدو، فإن الأحماض الدهنية المكتشفة و / أو المكونات النشطة بيولوجيًا الأخرى تكمن وراء الخصائص العلاجية لنبات *F. rutbaensis* التي يمكن أن تكون مكونات مفيدة في تصنيع مستحضرات التجميل المضادة لحب الشباب، كالصابون الطبي ومرطبات الجسم ومكيفات الجلد وواقي الشمس. بالإضافة إلى أن بعض المكونات الأخرى وجد أن لها خصائص مضادة للالتهابات وللأكسدة والأحياء المجهرية. هناك حاجة إلى مزيد من الفحوصات للتأكد من الفعالية المضادة للأحياء المجهرية لمستخلصات نبات *F. rutbaensis*.

الكلمات المفتاحية: المحروت، *Ferula rutbaensis*، ترميز الحامض النووي، ITS، تحليل GC-MS الطيفي

1. INTRODUCTION:

Plants are an integral part of human dietary and therapeutic habits, especially in Middle Eastern countries, whereby over 20000 different plant species habituate a wide geographical region (Othman *et al*., 2019). Historically, wild plant species have been the first and perhaps the only choice available for medication, particularly for rural and Bedouin communities (Alencar *et al*., 2010). More recently, wild plants have attracted extra attention to cope with the growing demands of the changing lifestyle towards natural resources, in which practicing green medicine become inherent (Ross, 2005; Ekor, 2014). About 80% of the developing countries residents are reliant on natural sources in their primary healthcare. On the other hand, there is a growing demand for natural drugs derived from wild plant species (Hamilton, 2004; Cole *et al*., 2007).

In Iraq, desert climate prevails most of the country areas, mainly in the west part where Anbar, the largest province is located (32% of the total country area; 600 m above the sea level; 31° and 35° latitude; 39° and 44° longitude), (Figure 1). This wide geographical expansion provides unrivaled natural biodiversity, including plant genera. Unfortunately, due to different reasons, there are no concrete efforts to explore the nutritive, industrial, or pharmacological importance of wild plants that inhabiting and discriminating these areas (Chen *et al*., 2016).

Ferula is one of the three major genera in the Apiaceae family. However, more than 175 species belong to this widely distributed genus across Asia and the Mediterranean (Zhou *et al*., 2017; Mohammadhosseini *et al*., 2019). The genus members had proven numerous remedial properties making them eligible for treating many epidemics and diseases for centuries (Pavlovic *et al*., 2015; Nguir *et al*., 2016; Bagheri *et al*., 2017; Upadhyay *et al*., 2017). In addition to the pungent odor, most Ferula members are being characterized as gum-resin producers.

"Mharut" is the local name of *Ferula rutbaensis* C.C. Towns., the Bedouin food plant growing in the southwest part of the western desert near the Iraqi-Saudi Arabia borderlines. *F. rutbaensis* is described as a perennial herb growing to 50-60 cm high and has bleached green leaves covered with a white veil (Mandaville, 2011). The edible part is a thick taproot (5-7 cm diameter, Figure 2) that typically extends to 60 cm depth and can be easily characterized via peculiar odor (Ghazanfer and Edmondson, 2013). The species designation "*rutbaensis*" is originated from the name of "Rutba" city (310 km to the west of Ramadi city) where it grows nearby. Like most of *Ferula* members, *rutbaensis* has many therapeutic aspects served as antioxidant, antispasmodic digestive, antiseptic, anthelmintic, anti-

inflammatory, carminative, analgesic, expectorant, and laxative (Yaqoob and Ahmad, 2016), which enabled it to be widely practiced in folklore medicine (Mohammadhosseini *et al*., 2019). Even though *Ferula* genus has received considerable attention and its extracts have been extensively studied, there is no available data describing the phytochemical composition of *F. rutbaensis*.
Characterization of active pharmaceutical constituents of medically valuable species will create alternative sources for natural therapies and limit the irrational use of certain medicinal species that may result in their extinction (Chen *et al*., 2016).

The environmental independency of molecular markers gives them an advantage over the morphological approaches that may reveal significant alterations in response to environmental effects (Dormontt *et al*., 2018). Under this, a wild type may show minor phenotypic modifications (ecotypes) reflecting on a biased evaluation (Zhao *et al*., 2018; Kevin *et al*., 2019). Taxonomic identification of plant species is a very delicate and time-consuming process. It requires competent experts who are able to distinguish between even closely related species based on their complex phenotypic characteristics (Oliveira *et al*., 2018). Unfortunately, like any other technique that relies on human skill, it may reflect in unreliable results.

DNA barcoding is a well-known molecular approach serving in biodiversity investigations (Costion *et al*., 2016; Babiychuk *et al*., 2017). This technique is mainly depending on a short conservative DNA sequence adequate to identify inter-and intra-variations of plant species (Hebert *et al*., 2003; Smith *et al*., 2005; Desalle, 2006). Although Internal Transcribed Spacer (ITS) have some disadvantages hindered their extensive embracing in assessing the genetic diversity of plant populations (Fusco and Minelli, 2010; Timpano et al., 2020), growing interest has been reported for applying the second internal transcribed spacer (ITS2) in DNA barcoding of various plant species (Sickel *et al*., 2015; Fahner *et al*., 2016; Moorhouse-Gann *et al*., 2018; Timpano *et al*., 2020). The ITS2 was found to be the most suitable molecular marker for standard DNA barcoding due to its high distinctive ability of intra- and/or inter-specific variation (Chiou *et al*., 2007; Chen *et al*., 2010).

Therefore, the present study aimed to characterize the *Ferula rutbaensis* species at phenotypic and molecular levels and the phytochemical constituents of aqueous and methanolic extracts of fresh and dry plant parts that may have medicinal importance.

2. MATERIALS AND METHODS:

2.1. Collection of plant material

A fresh sample of naturally growing *F. rutbaensis* was collected at the blooming stage from the Western Desert, 160 km west of Ramadi (Figure 1). Whole plants were uprooted and transferred directly to the lab in polyethylene bags. The identification and authentication of the collected plant were made by Dr. Mohammed Othman Mosa/Center of Desert Studies/University of Anbar/Iraq and the Iraqi National Herbarium according to the deposited voucher specimen no. 51513 (Figure 2).

2.2. Genomic DNA Extraction

Fresh roots were used for DNA extraction with aid of Wizard® Genomic DNA Kit (Promega, Madison, WI, USA), and the supplier instructions were followed literally. A total of 100 mg of fresh root tissues was transferred to a 1.5 ml microfuge tube. Nuclei lysis solution of 600µl volume was added and vortexed for 3 seconds. The mixture was then incubated at 65°C for 15 min. Then, 3µl of RNase solution was added to the cell lysate, and incubated at 37°C for 15 min. Protein was precipitated by adding 200 µl of protein precipitation solution and vortex vigorously at high speed for 20 seconds, followed by centrifugation at 13,000 × g. Supernatant that contains the DNA (the protein pellet was left behind) was carefully removed and transferred to a clean 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol. The solution was mixed gently by inversion until thread-like strands of DNA form a visible mass. The centrifugation step was performed at 13,000 × g for 1 min. The supernatant was carefully decanted, subsequently, 600 µl of room temperature 70% ethanol was added and gently inverted the tube several times to wash the DNA, then centrifuged at 13,000 × g for 1 min. Ethanol was carefully aspirated by using a sequencing pipette tip. The tube was inverted onto clean absorbent paper and the pellet was air-dried for 15 min. DNA was rehydrated by adding 100 µl of DNA rehydration solution, then incubated at 65°C for 1 hour, then stored at 2–8°C till used.

According to Nanodrop reads, the final DNA concentration was adjusted to 50 ng/µl. Two previously designed plant-specific primers (5′-ATGCGATACTTGGTGTGAAT-3′ as forward and 5′-GACGCTTCTCCAGACTACAAT-3′ as

Reverse) were applied to amplify the ITS2 region of *F. rutbaensis* genome (Chiou *et al*., 2007).

2.3. ITS amplification and Sequencing

A final volume of Polymerase Chain Reaction (PCR) was of 25 µl (12.5 µl of Green MasterMix (Promega, Madison, WI, USA), 1 µl of each primer, 3 µl of DNA template, and nuclease-free water was used to complete the volume) subjected to the following PCR thermal profile: Initial denaturation was at 94°C for 5 min, then subjected to 36 cycles of denaturation for 40 sec. at 94°, followed by annealing at 56°C for 45 sec. Extension and final extension steps were at 72°C for 1 and 7 min., respectively. The purified PCR product was sequenced following the Sanger sequencing technique on ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in Macrogen Inc. (Seoul, South Korea).

2.4. Phytochemicals extraction

The aqueous and ethanol extraction procedures were adopted alternatively to extract the phytochemicals from each of the fresh and dried leaves and roots of *F. rutbaensis*. For aqueous extraction, 25 g of fresh parts (leaves and roots) were washed thoroughly and separately macerated in 100 ml of sterilized distilled water for one week, next placed on a shaker for 24 hours. Subsequently, the extract was filtered with Whatman filter paper (no.1). two times, then filtrates were concentrated and dried using a rotary evaporator (ISOLAB Laborgeräte GmbH, Germany) at 45° C under vacuum.

For dry extraction, leaves and roots were left to dry at room temperature until a constant weight is achieved. The dried plant materials were milled separately with an electric mill. A weight of 25 g from each of plant leaves and roots was separately soaked in 100 ml of sterilized distilled water and 10% ethanol (v/v), subsequently placed on a shaker for 72 hours. Then, the aqueous and ethanolic extracts were centrifuged for 10 min at 6000 rpm, filtered with Whatman filter paper (no.1). Finally, filtrates were concentrated with a rotary evaporator at 45° C under reduced pressure. The crud aqueous and ethanolic extracts were separately dissolved in DMSO at a rate of 100 mg 5 ml-1 for GC-MS analysis.

2.4. GC-MS Conditions

The GC-MS profile was generated using GCMS-QP2010 plus instrument (Shimadzu, Kyoto, Japan) equipped with autoinjector and 5ms capillary column of 30×0.25 mm dimension with 0.25 µm film thickness. Helium served as the carrier gas at 1.15 ml/min. flow rate. Mass spectroscopic analysis was done with 70eV ionization system. The primary temperature was established at 80°C for 2 min. to be gradually elevated at a rate of 10°C per min. up to 280°C for 5 min. The sample injection was according to split mode at 250°C. Two mass spectral databases National Institute of Standards and Technology (NIST14), and Wiley 10th/NIST 2014 mass spectral library (W10N14) adopted in the characterization of the extracted components based on retention time and mass spectra.

3. RESULTS AND DISCUSSION:

3.1. ITS-based DNA barcoding

The used primers were successfully amplified the Internal Transcribed Spacer of *F.* rutbaensis was in roughly 600 bp (Figure 3). The resulted sequence was registered in the NCBI (National Center for Biotechnology Information) under the accession number LC570805.1 and that was identical in 452 out of 519 original hits with solitary previously registered *F. rutbaensis* (voucher Rechinger 12872) under the accession number of KJ660812.1 collected from Jordan by (Panahi *et al*., 2018), (Figure 4).

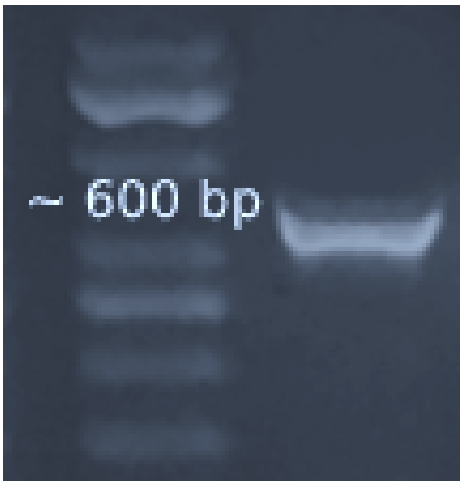

Figure 3. *PCR product of ITS region of F. rutbaensis electrophoresed on 2% agarose gel at 5 volt/cm2. 1x TBE buffer for 1:30 hours.*

3.2. Gas Chromatography-Mass Spectrometry (GC-MS)

3.2.1 Aqueous Extract

The combination between GC and MS techniques has many advantages over other traditional methods adopted to identify plant

phytochemicals for many reasons, mainly to achieve reliable qualification and quantification. Traditionally, roots of *F. rutbaensis* are the edible and remedially important part that has been widely used for folklore medication in Bedouin communities.

The GC-MS chromatogram indicated the presence of 12 phytochemicals in the aqueous extract of *F. rutbaensis* fresh roots, namely Tetradecane; Pentadecane; Hexadecane; 2-Methylhexacosane; 1-Heneicosyl formate; Nonadecane; Carbonic acid, decyl hexadecyl ester; n-Hexadecanoic acid; Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester; Eicosyl nonyl ether; Oleic acid and Octadecenoic acid (Table 1). The characterized phytochemicals are categorized into terpenes, hydrocarbon alkanes, esters, and fatty acids.

Nevertheless, Oleic and Palmitic (n-Hexadecanoic) fatty acids were the two main components of the aqueous extract occupied a peak area of 32.64% and 26.35%, respectively (Table 1). Interestingly, both active compounds are fatty acids that have been characterized with anti-inflammatory (Aparna, 2012; Korbecki and Bajdak-Rusinek, 2019), anticancer (Ismail, 2020), antioxidant (Vaithiyanathan and Mirunalini, 2015), antibacterial and antifungal activities (Ogunlesi, 2009).

Nona- and Pentadecane existed in smaller amounts of 4.34% and 3.25%, and both characterized with antioxidant, anti-inflammatory (Ali *et al*., 2015), and antimicrobial activity (Girija, 2014), respectively. From Table 1, the three alkanes, 2-methylhexacosane (2.52%), hexadecane (2.06%), and tetradecane (1.54%) were designated recently as effective inhibitors to microbial infections (Nair, 2019), decreasing blood cholesterol (Pandey *et al*., 2016) and antimicrobial diuretic, anti-tuberculosis (Girija, 2014; Kavitha and Mohideen, 2017), respectively.

From GC-MS analysis, the aqueous extract of *F. rutbaensis* dry roots was the richest and the most diverse compared to other extracts, whereby 19 bioactive compounds of terpenes, hydrocarbon alkanes and fatty acids, in addition to individual compound of each of alcohol, acetate, alkene, alkyl halide, and sulfolane were recognized (Table 2). Depending on the retention time (min.), the detected compounds were (3R)-1-Phenyl-1-pentyn-3-ol; 5-Acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol; Isolongifolen-5-one; 3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-; 11,13-Dimethyl-12-tetradecen-1-ol acetate; Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)-; 1-Methylbicyclo[3.2.1]octane; 1-Tetracosene; Cyclotetracosane; Heptadecane; n-Hexadecanoic acid; Hexadecanoic acid; 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-; Triacontane, 1-bromo; Cyclopentanol, 1-(1-methylene-2-propenyl); 4-n-Hexylthiane, S,S-dioxide; trans-Verbenol; 9-Octadecenoic acid and Octadecanoic acid.

However, n-Hexadecanoic and 9-Octadecenoic acids were the two most abundant components of the dry roots aqueous extract with a peak area of 27.69% and 27.28%, respectively (Table 2). Recently, several reports pointed to the substantial potent of such fatty acids, particularly as an anti-inflammatory (Aparna, 2012; Korbecki and Bajdak-Rusinek, 2019), anticancer (Ismail, 2020), antioxidant (Vaithiyanathan and Mirunalini, 2015), antibacterial and antifungal properties (Ogunlesi, 2009).

Diterpenoid in the form of 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E), (4.37 %) was detected in the dry roots aqueous extract known with various pharmacological activities like antimicrobial, anti-inflammatory, anticancer and diuretic activities (Devi and Muthu, 2015). Heptadecane (4.11%) and less abundant components such as 4-n-Hexylthiane S,S-dioxide (2.13%), and trans-Verbenol (1.12%) distinguished with antimicrobial activity (Girija, 2014; Mathew and Retna, 2016; Utegenova, 2018).

By contrast, GC-MS data of dry leaves reveals the existence of only 5 phytochemicals categorized into two groups; the first consisted of three fatty acids (n-Hexadecanoic acid (41.81%), 9-Octadecenoic (30.11%) and acid Octadecanoic acid (23.72%), while the second group was hydrocarbon alkane in the form of Nonadecane (4.35%), (Table 2). Both groups have distinctive bioactivity, as previously addressed by several reports.

3.2.2 Methanolic Extract

According to the GC-MS profile, methanol-based extraction was less efficient in extracting plant chemical constituents from each of fresh and dry roots compared to water-based extraction, where the screened methanolic extract of fresh roots included only 6 bioactive compounds namely, Nonadecane; n-Hexadecanoic acid; Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester; Sulfurous acid, 2-propyl tetradecyl ester; Oleic Acid and Octadecanoic acid. These compounds

can be categorized into fatty acids, esters, and hydrocarbon alkane (Table 3).

Likewise, screening of phytochemicals in the dry roots of *F. rutbaensis* showed the presence of almost the same bioactive compounds recognized in the fresh roots methanolic extract (Nonadecane; n-Hexadecanoic acid; Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester; Carbonic acid, decyl undecyl ester; Octadec-9-enoic acid and Octadecanoic acid (Table 3). However, Sulfurous acid, 2-propyl tetradecyl ester (2.32%) in the fresh roots methanolic extract was replaced by Carbonic acid, decyl undecyl ester (0.66%) in the dry roots counterpart.

In terms of peak area, Oleic acid and n-Hexadecanoic acid still representing the identified major constituents in the methanolic extract of fresh and dry roots having the largest peak area (%) of 38.58 and 27.73 in fresh roots and 35.65 and 32.02 in dry roots, respectively. Following the methanol-based extraction procedure, the dried leaves were the poorest source in respect of extracted phytochemicals. However, it showed distinct phytochemical profile by expressing acyclic alkane in the form of Tetracosane (57.55%) and organosilicon compound in the form of Methyltris (trimethylsiloxy) silane (42.45%) (Table 3). The former is reported to have antibacterial, antimicrobial (Panicker *et al*., 2019), and antioxidant properties (Boussaada, 2008).

4. CONCLUSIONS:

The wild plant types like *Ferula rutbaensis* are valuable sources for therapeutic and bioactive compounds, particularly as antioxidants, anti-inflammatory, antispasmodic digestive, and laxative. According to the GC-MS profile, a wide range of phytochemicals differed between plant parts. However, plant roots were the richest source for bioactive compounds. Under this, the aqueous extraction procedure exhibited a sophisticated ability to extract plant phytochemicals compared to methanolic extract, mainly from dry roots whereby nineteen biochemical constituents were recognized with remarkable bioactivities based on several reports.

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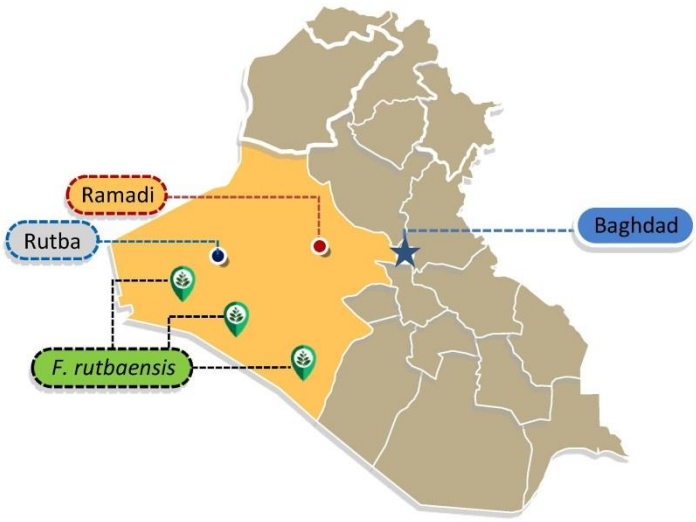

Figure 1. *Geographical origin of F. rutbaensis. Source: the author*

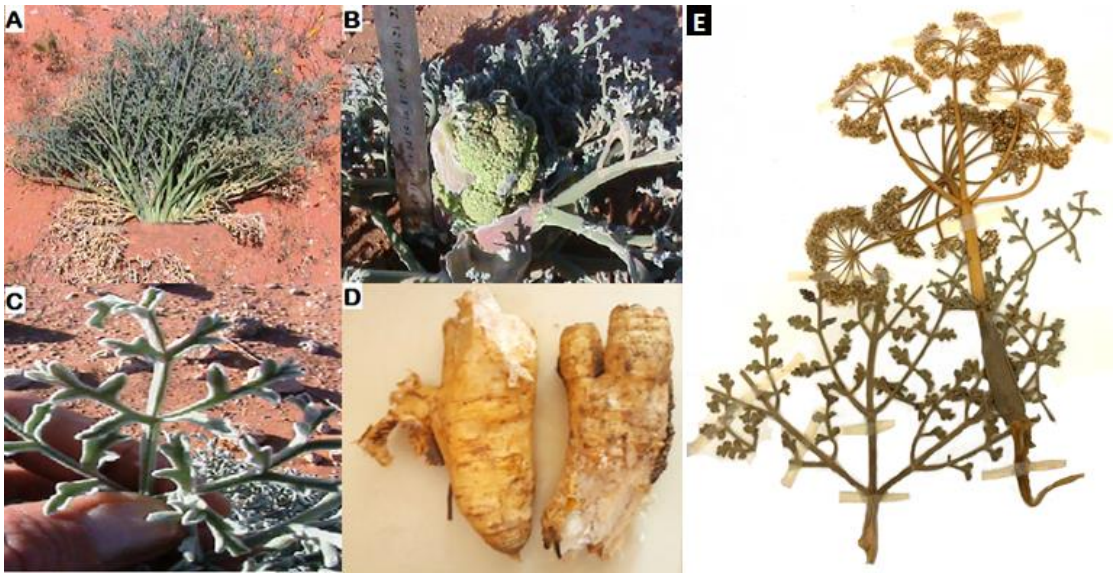

Figure 2. *The F. rutbaensis plant, (A) Arial shoot, (B) The emerged flower, (C) Leaf, (D) Root, (E) Deposited voucher specimen no. 51513. Source: the author*

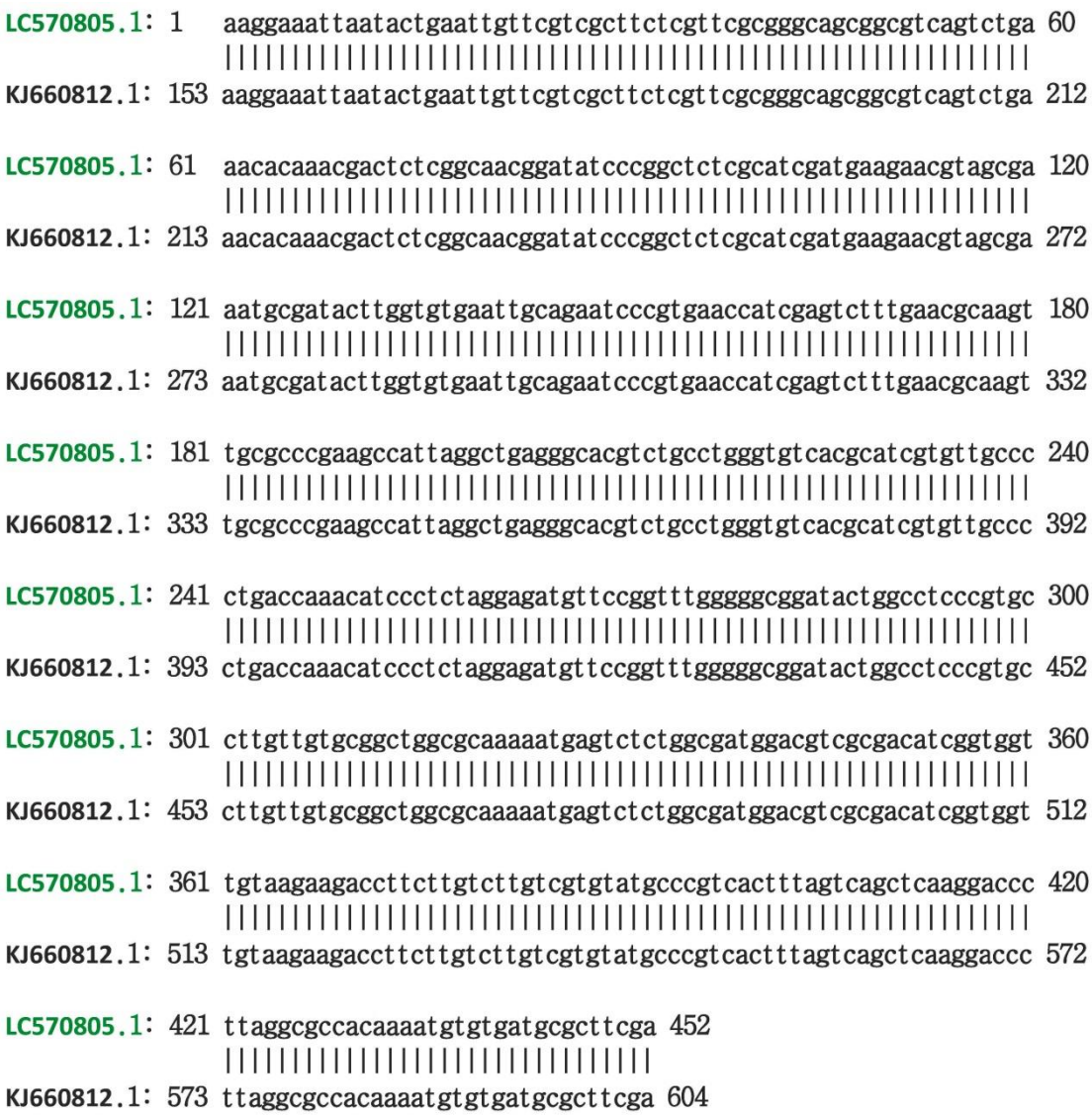

Figure 4. *Sequence alignment of ITS (internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence) of the studied F. rutbaensis (LC570805.1) and the previously registered voucher Rechinger 12872 (E) (KJ660812.1). Source: the author*

Table 1. *GC-MS analysis of aqueous extract of F. rutbaensis fresh roots. Source: the author*

Pea No.	RT (min)	Name of the compound	Molecular Formula	MW (g/mol)	Peak Area (%)	Nature of compound
1	14.13	Tetradecane	$C_{14}H_{30}$	198.4	1.54	Acyclic alkane
2	15.91	Pentadecane	$C_{15}H_{32}$	212.4	3.25	Acyclic alkane
3	17.61	Hexadecane	$C_{16}H_{34}$	226.4	2.06	Acyclic alkane
4	19.22	2-Methylhexacosane	$C_{27}H_{56}$	380.7	2.52	Branched alkane
5	21.91	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340.6	0.98	Monoterpene
6	22.24	Nonadecane	$C_{19}H_{40}$	268.5	4.34	Acyclic alkane
7	22.51	Carbonic acid, decyl hexadecyl ester	$C_{27}H_{54}O_3$	426.7	0.69	Carbonate ester
8	23.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	26.35	Saturated fatty acid
9	24.04	Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester	$C_2H_7NO_3S_2$	157.2	6.92	Thiosulfuric acid ester
10	25.01	Eicosyl nonyl ether	$C_{29}H_{60}O$	424.7	1.57	Dialkyl ether
11	26.14	Oleic Acid	$C_{18}H_{34}O_2$	282.5	32.64	Unsaturated fatty acid
12	26.35	Octadecenoic acid	$C_{18}H_{36}O_2$	284.5	17.14	Saturated fatty acid

(RT= Retention time) (MW= Molecular weight)

Table 2. *GC-MS analysis of aqueous extract of F. rutbaensis dry roots and leaves. Source: the author*

Plant part	Peak No.	RT (min)	Name of the compound	Molecular Formula	MW (g/mol)	Peak Area (%)	Nature of compound
Dry roots	1	10.57	(3R)-1-Phenyl-1-pentyn-3-ol	$C_{11}H_{12}O$	160.2	1.49	Propargylic alcohol
	2	18.29	5-Acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol	$C_{17}H_{30}O_3$	282.4	0.61	Monoterpene
	3	20.28	Isolongifolen-5-one	$C_{15}H_{22}O$	218.3	0.9	Sesquiterpene
	4	20.94	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-	$C_{15}H_{20}O_4$	264.3	1.76	Sesquiterpene
	5	21.12	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5	1.84	Acetate compound
	6	21.42	Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)-	$C_{10}H_{18}$	138.3	2.4	Monoterpene
	7	21.59	1-Methylbicyclo[3.2.1]octane	C_9H_{16}	124.2	1.1	Cyclic hydrocarbon
	8	21.92	1-Tetracosene	$C_{24}H_{48}$	336.6	2.02	Acyclic olefin
	9	22.06	Cyclotetracosane	$C_{24}H_{48}$	336.6	1.41	Cyclic alkane
	10	22.24	Heptadecane	$C_{17}H_{36}$	240.5	4.11	Acyclic Alkane
	11	23.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	27.69	Saturated fatty acid
	12	24.05	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	5.7	Saturated fatty acid
	13	24.82	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-	$C_{16}H_{26}O$	234.4	4.37	Diterpenoid
	14	25.01	Triacontane, 1-bromo	$C_{30}H_{61}Br$	501.7	7.07	Alkyl halide
	15	25.46	Cyclopentanol, 1-(1-methylene-2-propenyl)	$C_9H_{14}O$	138.2	1.26	Monoterpene
	16	25.59	4-n-Hexylthiane, S,S-dioxide	$C_{11}H_{22}O_2S$	218.4	2.13	Sulfolane
	17	25.8	trans-Verbenol	$C_{10}H_{16}O$	152.2	1.12	Monoterpene
	18	26.16	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	27.28	Unsaturated fatty acid
	19	26.37	Octadecanoic acid	$C_{18}H_{34}O_2$	282.5	5.74	Saturated fatty acid
Dry leaves	1	22.24	Nonadecane	$C_{19}H_{40}$	268.5	4.35	Acyclic alkane
	2	23.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	33.07	Saturated fatty acid
	3	24.04	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	8.74	Saturated fatty acid
	4	26.14	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	30.11	Unsaturated fatty acid
	5	26.35	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	23.72	Saturated fatty acid

(RT= Retention time) (MW= Molecular weight)

Table 3. *GC-MS analysis of methanol extract of dry and fresh roots of F. rutbaensis. Source: the author*

Plant part	Peak No.	RT (min)	Name of the compound	Molecular Formula	MW (g/mol)	Peak Area(%)	Nature of compound
Fresh roots	1	22.24	Nonadecane	$C_{19}H_{40}$	268.5	3.58	Acyclic alkane
	2	23.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	27.73	Saturated fatty acid
	3	24.05	Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester	$C_2H_7NO_3S_2$	157.2	10.38	Thiosulfuric acid ester
	4	25.01	Sulfurous acid, 2-propyl tridecyl ester	$C_{16}H_{34}O_3S$	306.5	2.32	Sulfurous acid ester
	5	26.14	Oleic Acid	$C_{18}H_{34}O_2$	282.5	38.58	Unsaturated fatty acid
	6	26.35	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	17.41	Saturated fatty acid
Dry roots	1	22.25	Nonadecane	$C_{19}H_{40}$	268.5	7.06	Acyclic alkane
	2	23.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	32.02	Saturated fatty acid
	3	24.05	Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester	$C_2H_7NO_3S_2$	157.2	7.29	Thiosulfuric acid ester
	4	25.02	Carbonic acid, decyl undecyl ester	$C_{22}H_{44}O_3$	356.6	0.66	Carbonate ester
	5	26.15	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5	35.65	Unsaturated fatty acid
	6	26.35	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	17.32	Saturated fatty acid
Dry leaves	1	10.05	Methyltris (trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$	310.7	42.45	Organosilicon compound
	2	22.75	Tetracosane	$C_{24}H_{50}$	338.7	57.55	Acyclic alkane

(RT= Retention time) (MW= Molecular weight)