Evaluation of chemical composition, some elements and antioxidants to various solvent extracts from *Ruta chalepensis* L. plant growing in al-jabal al- khadar region, Libya

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Abstract: This study was conducted to evaluate the active substances and antioxidants of *Ruta chalepensis* L. in two types of different polar solvents (ethanol - hexane*).* It is one of the medicinal plants of commercial importance. It was collected in May 2023 in the city of Al-Qubba, located in northeastern Libya in the al-jabal al-khadar region, drying it and preparing extracts (flowering tops/seeds, leaves, stems). The results of detecting the active ingredients showed the superiority of the solvent hexane over ethanol, especially in leaves, while the highest values of mineral elements(sodium/potassium and copper) recorded for the leaves in the solvent ethanol, respectively (9.14 / 5.9 / 42), whilst the highest value for iron was recorded in the flower tips and seeds in hexane, about 2.2 ,This demonstrated the superiority of ethanol over hexane in extracting mineral elements and in addition to estimating the percentage of antioxidants for each studied parts, and they showed the highest value in the leaves and flowering tops 0.3µg/g, and the lowest value in the stems, 0.09µg/g. This supports that the higher the percentage of phenolic content of the plant, the greater its antioxidant activity, which gives it great potential for use as a natural antioxidant.

Keywords: *Ruta chalepensis* L., Medicinal plants, Polar solvents, Flavonoids, Mineral elements.

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المستخلص: أجريت هذه الدراسة لتقييم المواد الفعالة و مضادات الأكسدة لنبات الفيجل في نوعين من المذيبات مختلفة القطبية (الايثانول/ الهكسان) وينتمي النبات إلي عائلة Rutaceae وهو من النباتات الطبية ذات الأهمية التجارية ويستخدم لعدة أغراض أهمها مطهر للأمعاء و المعدة و اضطرابات الجهاز التناسلي و البولي و مجهض و مسكن للألآم واضطرابات العضلات ضد الروماتيزم كما أثبتت بعض الدراسات أن له خصائص مضادة للسرطان لاحتوائه على القلويدات والفلافونويدات و الفينولات والأحماض الأمينية و الفورانوكومارين و الصابونيات والتي لها خصائص دوائية مستهدفه بشكل جيد حيث تم جمع النبات في مايو 2023 من مدينة القبه التي تقع شمال شرق منطقة الجبل الأخضر وتم تجفيفها و تحضير المستخلصات للقمم الزهرية والبذور والأوراق والسيقان وأظهرت نتائج الكشف عن المكونات الفعالة بأن تفوق مذيب الهكسان على الإيثانول خاصة في الأوراق بينما سجلت أعلى قيم للعناصر المعدنية (الصوديوم/البوتاسيوم/ النحاس) في الاوراق لمذيب الإيثانول على التوالي (42/5.9/9.14) بينما سجلت أعلى قيمه للحديد في القمم الزهرية والبذور للهكسان حوالي 2.2 مما يدل على تفوق الإيثانول على الهكسان في استخلاص العناصر المعدنية، بالإضافة إلى تقدير نسبة مضادات الأكسدة لكل الأجزاء المدروسة وأظهرت أعلى قيمه في (الأوراق/ القمم الزهرية/ البذور) 3.0 ميكروجرام/ جرام وأقل قيمه في السيقان 0.09 ميكروجرام /جرام وهذا يدل على أنه كلما ارتفعت نسبة المحتوى الفينولي للنبات زاد نشاط المضاد للأكسدة مما يمنحه إمكانية كبيره لاستخدامه كمضاد طبيعي للأكسدة.

الكلمات المفتاحية: نبات الفيجل، نباتات طبيه/ مذيبات قطبيه، الفلافونويدات، مضادات الأكسدة، العناصر المعدنية.

Introduction:

Plants constitute a valuable source of natural antioxidants such as vitamins, phenolic compounds, and flavonoids (El-Ghorab, *et al.*, 2007). Moreover, chemical treatments cause side effects, for order to minimize that, research is increasingly developing natural processes based on extracts and active ingredients of plant origin, where this trend is concomitant with an increasing interest valorization of natural antioxidants of plant origin (Karmakari, *et al.*, 2011).

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Ruta is a genus of Rutaceae family and features primarily shrubby plants that are native to the Mediterranean region and usually grow on rocky slopes. The three most diffused species are *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L., were this plant is commonly used as a traditional medicinal plant. It is protective against various disorders such as rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsions, and other bleeding and nervous disorders (Pollio, *et al.*, 2008 & Aguilar, *et al.*, 1995). However, data on the bioactivities of R. *chalepensis* are scarce.

R. *chalepensis* herb contains secondary metabolites from various chemical groups: coumarins: simple coumarins (coumarin, scopoletin, umbelliferon), coumarin dimers (daphnorin), furanocoumarins (bergapten, chalepensin, chalepin, isopimpinellin, xanthotoxin), dihydrofuranocoumarins (rutamarine, isorutarin); alkaloids: acridine (arborinin, rutacridone), quinoline (graveolin, graveolinin, 3-hydroxygraveoline), furoquinoline (dictamine, isotaifine, cocusaginine, maculosidine, skimmianine, taifine, fagarine, 8-methoxytaifine); flavonoids (hesperidin, rutoside); and essential oil (approximately 0.7%) (Kacem, *et al.*, 2015)

In this work we aimed to evaluate the phenolic composition and the antioxidant for (ethanolic/hexane) extracts of R. *chalepensis* as function of the plant parts (flowering tops/seeds, leaves, stems) In addition, the objective of this study was to establish differences between the two solvents and to evaluate which is the most interesting. The results of this work highlighted the possibility of using R. *chalepensis* organs as a potential source of natural antioxidants.

Materials and Methods:

Collection and Preparing Plant: The *Ruta chalepensis* L. plant was collected on May 2023 in the city of Al-Qubba, which is located in the northeast of Libya in the Jabal Al-Akhdar region. The city of Al-Qubba is about 40 km west of the city of Derna and about 50 km east of the city of Al-Bayda

( ) Figure showing a map of Libya and identifying the Qubba area

The whole *R. chalepensis* plant (leaves - stems - flower tops and seeds) was collected and carefully separated, then washed twice with water, then dried for 10-14 days in the shade, stirring, at room temperature to avoid damage and contamination. After the drying process, the plant parts are separated individually and partially ground, and the resulting product is stored in bags for use. (Jarada *et al.*, 2016)

- **Preparation of extracts:** Weights were taken from the previously prepared samples (leaves

- stems - flower tops and seeds) in order to prepare extracts in solvents (ethanol - hexane). They were 30 g of samples in 300 ml of solvent and left for 48 hours, then filtering the samples and then extracting them and disposing of the solvents using... Rotary evaporator. (Zirihi, *et al.*, 2003)

- **Chemical detection:** Chemical reagents were prepared in cooperation with the Department of Chemistry at the College of Science, University of Derna. Chemical tests were carried out on each of the plant parts separately and included: Tannins, alkaloids, flavonoids, phenols, saponins and terpenes. (Harborne,1973 & El-Hefnawy, *et al.*, 1992)

- **Estimating the concentration of some mineral elements:** The concentration of some mineral elements (iron (Fe) - sodium (Na) - potassium (K) - copper (Cu)) was measured using an atomic absorption spectrophotometer in cooperation with the Department of Chemistry, College of Science, Omar Al-Mukhtar Al-Bayda University.

- **Estimation of antioxidant activity:** 1 gram of plant powder was added to it, adding 5 ml of diethyl ether, then the mixture was shaken for a few minutes and filtered to take the remaining part of the sample, adding 10 ml of methanol with shaking, then adding 10 ml of hydrochloric acid (HCl), adding 100 ml of methanol, then filtering where the remaining extract was. From the filtrate, it is placed in a rotary extraction flask until the quantity is reduced to 10 ml. Take 5 ml of this filtrate, plus 3 ml of distilled water with 3 ml of iron cyanide K_3 [Fe(Cn)6],

concentration 0.008 M, plus 3 ml of hydrochloric acid 0.1 M HCl and a solution of ferric chloride, $FeCl_3$, and leave it. Solution for 10 minutes, then measured at two wavelengths of 720nm using an atomic absorption spectrophotometer using the method (Wangensteen *et al.*, 2004).

Results:

1. Chemical detection of active substances in different parts of the plant: Table No. 1 shows the results of chemical detection of the active substances of the parts (leaves - stems - flower tops and seeds) of the *Ruta chalepensis* plant that were detected (alkaloids, flavonoids, tannins, terpenes, phenols and soaps) to compare the presence of the active compound in the different parts of the plant. Depending on the type of solvent (ethanol - hexane).

The results showed that the plant parts contained alkaloids in equal quantities in the solvent (ethanol), while in the solvent (hexane), the highest concentration was in the leaves, followed by the stems, and then the flower tops. In addition, saponins were present in the flower tops and seeds in a noticeable concentration than in the rest of the plant parts in (the solvent). Ethanol) and quite the opposite in (hexane solvent), where it showed its absence in the tops and its presence in the rest of the plant parts.

As for astringent substances (tannins), upon detection, they showed their absence in both leaves and stems, and their presence was limited to the flower tips and seeds in the ethanolic solvent, while they appeared in high and equal quantities in the leaves and stems, and their absence in the flower tips and seeds in the hexane solvent.

The results showed the presence of terpenes in all parts of the plant in equal quantities in the ethanolic solvent, while in the hexane solvent they were absent in the flower tips and seeds, while their concentration was high in the leaves and stems.

The results of detection of phenols and soaps were somewhat similar, as they appeared in noticeable concentrations in both leaves and stems in the ethanolic and hexane solvents, while they were absent in the hexane solvent of the flower tips and seeds.

Active compound groups	Type of solvent	plants parts		
		Flower tops and seeds	Leaves	The stems
Alkaloids	Ethanol	+	+	+
	Hexane	+	+++	++
Flavones	Ethanol	++	+	-
	Hexane	++	++	++
Tannins	Ethanol	+	-	-
	Hexane	-	+++	+++
Terpenes	Ethanol	+	+	+
	Hexane	-	+	-
Phenolic compounds	Ethanol	+	++	+
	Hexane	-	++	+
Saponins	Ethanol	++	+	+
	Hexane	-	++	++

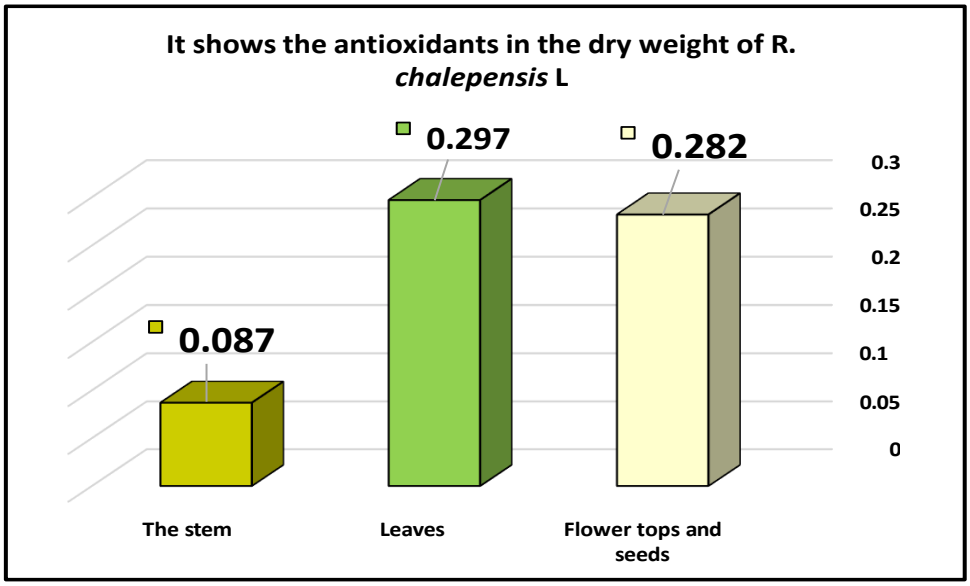
- The sign (+) indicates the presence of the substance upon detection, the sign (-) indicates its absence, while the signs (++) and (+++) indicate the concentration and clarity of the substance upon detection.

2. Determining the mineral elements present in the Ruta chalepensis plant: Table No. 2 shows the mineral elements detected in plant parts (leaves - stems - flower tops and seeds) in both solvents (ethanol - hexane) using a spectrophotometer, in three replicates for each sample.

The highest percentage of iron was recorded in the leaves, which was 0.6 for the ethanolic solvent, while it was at a noticeable concentration in the flower tips and seeds in the hexane solvent, at about 2.2, and the lowest concentration in the leaves, at about 0.03. While the sodium element recorded the highest value in the leaves, about 9.14, and the lowest, about 3.43, in the flower tips and seeds in the ethanolic solvent, while the results were equal in the hexane solvent, while the potassium element recorded the highest value, 5.9, in the leaves, followed by the stems, with a value of 1.71 in the ethanolic solvent, and its absence in the hexane solvent for all parts. While the results were for copper in the ethanolic solvent in varying concentrations, they recorded values respectively (42 - 25 - 2.31) (leaves - flower tops and seeds - stems), while it was recorded only in the leaves with a value of 1.29 and its absence in the rest of the parts in the hexane solvent.

Metal elements	Type of solvent	plants parts		
		Flower tops and seeds	leaves	The stems
Fe^{+3}	Ethanol	0.4	0.6	0.1
	Hexane	2.2	0.03	0.2
Na	Ethanol	3.43	9.14	5.14
	Hexane	1.83	1.94	1.94
K	Ethanol	0	5.9	1.71
	Hexane	0	0	0
Cu	Ethanol	25	42	2.31
	Hexane	0	1.29	0

Estimation of antioxidants in Ruta chalepensis: Figure (3) shows the detection of antioxidants in the dry weight of R. chalepensis as a comparison between plant parts (leaves-stems - flower tops and seeds). The results showed the highest value of antioxidants in the leaves, flower tops and seeds, about 0.3 (µg/g).) after rounding, while it was lower in the stems by about 0.09 (µg/g) after rounding.



Discussion:

1. Chemical detection of *Ruta chalepensis* L: The results showed, in general, for the aforementioned materials in the table, the presence of the active substances in the leaves, followed by the flower tips, seeds, and some in the stems. This is consistent with the study of (Fakhfakh, *et al.*, 2012), which confirmed the connection between the presence of the active substances in the leaves as secondary metabolites. Also mentioned that most alkaloids and saponins vary in quantities in different parts of the same plant, and the differences may be related to the morphological differentiation that occurs during the phenological cycle (Ouerghemmi, *et al.*, 2016).

As (Kasimala, *et al.*, 2014) indicated that the R. *chalepensis* plant contains (alkaloids, flavonoids, tannins, terpenes, phenols, and saponins), the total phenolic content is affected by the polarity of the solvent used for extraction, and it is maximum in polar solvents and minimum in non-polar solvents, so The phenolic content of R. *chalepensis* is polar and extracts well with polar solvents. Ereifej *et al.*, 2015), and (Al-Ismail, e*t. al.*, 2022) indicated that more polar solvents will produce a greater extraction yield, as the higher the percentage of phenolic content, the greater the antioxidant activity.

2.Determination of mineral elements of *Ruta chalepensis* L: The detection results showed (iron, sodium, potassium, copper) in varying degrees and concentrations, where Cu recorded the highest values (42 and 25), and compared with the study (Ereifej, et al., 2015) in which Cu recorded a value of 0.14, while the values of the elements were (Fe / K / Na) respectively (2.1 / 52 / 9.4) and may be due to the distribution and accumulation of minerals in the leaves of R. chalepensis L., a reflection of the mineral composition of the soil and the environment in which R. chalepensis L. grows. The plants accumulate Metals from soil and the environment in their different parts (Solomon, et al., 2018). In addition, these compounds are synthesized during secondary metabolism and their production and accumulation may vary according to species and environmental conditions (Da Silva et al., 2014)

3.Estimation of antioxidants in *Ruta chalepensis* L: The results of estimating antioxidants

for plant parts (leaves - stems - flower tops and seeds) showed the highest concentration in the (leaves, flower tops and seeds) about 0.3µg/g. and the lowest value in the stems 0.0 9 µg/g.

where the biological activity of the plant may depend on factors such as the part of the plant, the geographical source, soil conditions and time. Harvest, moisture and post-processing methods. Therefore, when harvested, Ruta chalepensis leaf extract has high phenolic content and antioxidant activity as well as moderate antimicrobial activity, so this plant can be useful as a medicinal plant. (Abdel Aziz et al., 2020) As the study clearly indicated, R. chalepensis L. has great potential to be used as a natural antioxidant as well as an anti-diabetic agent (Al-Ismail et al., 2022).

Conclusion: This study concluded that the difference in the polarity of the solvent has an effect in extracting the active substances of medicinal plants, and it was clear in *Ruta chalepensis*, where it was clear that some of them were present in ethanol and their absence in hexane. In addition, their presence in hexane and their absence in ethanol, and this supports the theory of extraction, as the solvent spreads in the plant material and dissolves the compounds. Which has a similar polarity to it. Addition, the results showed that the higher the percentage of phenolic content, the greater the activity of antioxidants. The results also indicated the presence of most of the active substances, antioxidants, and mineral elements in noticeable concentrations in the leaves, while they varied in the rest of the plant, which reinforces previous studies that reported that an increase in solvent polarity follows. High extraction productivity.

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