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Anatomical Characteristics, Active Chemical Constituents and Oxidative Activity of *Rubus ulmifolius L.* (Rosaceae) Fruits and Seeds

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Abstract

This study described the structure of the fruits and seeds of *Rubus ulmifolius*. Sectional studies of the seed coat and pericarp revealed differences in the thickness of their constituent layers. The thickness of the layers of the exocarp, mesocarp and endocarp in this species was found to be 17.4 µm, 20.5 µm and 26.7µm, respectively. Some tissues are characterized by the presence of thin, loose parenchymal tissue containing numerous secretory ducts. The active chemical compounds were also analyzed and the species showed differences in their compound content. The results showed that *R. ulmifolius* contains tannins, saponins, glycosides and phenols, while flavonoids could not be detected. The oxidative activity of the fruits also proved to be high, exceeding that of vitamin C at high concentrations of 81%. The plant extract of *R. ulmifolius* showed DPPH inhibition values of approximately 71% and 83% for the two samples tested, compared to ascorbic acid, which showed DPPH inhibition values of approximately 75% and 80%.

Keywords: Anatomical, fruits and seeds, chemical content

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Introduction

The Rosaceae family is a widespread family of economic and nutritional importance. They occur in the form of trees, shrubs, or herbs. Their leaves are simple, alternate, and rarely opposite, and are simple or compound. Their flowers are bisexual (Mehiou,2024, Hamood,2022). They are divided into 31 subfamilies containing more than 80 genera and 2,600 species widely distributed in temperate climates. Plants in this family produce a range of environmentally important secondary metabolites, including saponins, plant steroids, sterols, flavonoids, polyphenols, and essential oils. This family is an important food source for humans, especially its fruits, which contain important chemicals, including phenols with significant health benefits (Hameed *et al.*,2021). Many leaves of the Rosaceae family also have anti-cancer properties (Martins *et al.*,2024). Cranberry (*Rubus ulmifolius* Schott) (Rosaceae) The perennial shrub, often called cranberry or ashberry, grows widely across Asia, North Africa, and Europe, particularly on the Iberian Peninsula, both naturally and in cultivated areas. (Issa and Mohammed,2020., Mehiou,2024). The blooming season occurs between May and June, followed by the ripening and development of the fruit, that is characterized as an aggregate of several fleshy drupelets, that during ripening change their colour from green to black (Sánchez-Velázquez *et al*,2024). These fruits are consumed fresh or as derivate products such as jams, juices, liqueur and marmalades, due to their delicious flavour and taste. Furthermore, their bioactive compounds have been the main focus of many scientific studies, generating a large number of dietary supplements and food products fortified with phytochemicals. therapeutic properties are assigned to the high concentration of biologically active compounds such as phenolic compounds, ellagitannins and ascorbic acid (Chwil *etal*,2023, Martins *etal*,2023). Several studies have been conducted on various species of Rubus, such as *R. idaeus*, *R. tachymensis*, and *R. suavessimis* (Yang *etal*,2024., Ollani, & Sottile,2024). More in-depth studies have been conducted on blackberries (Yadav *etal*,2020), on the flower buds and blossoms of *R. ulmifolium* (Kamnev *etal*,2020), and on the fruits. The study aimed to evaluate the anatomical and morphological differences of the seeds and fruits of *R. ulmifolius* and to study .the specific content of active compounds

Materials and Methods

Sample Collection

Fresh samples were collected directly from various areas of Anbar Governorate, western Iraq, during 16 field trips to different regions in the spring, summer, and fall of 2023–2024. Plant samples containing ripe fruits were collected for seed and photographed using a Canon A4500 camera. Each collection was accompanied by a record of information by placing a label with each sample, indicating the sample number, the place, and the date of collection. Information about plant characteristics important for diagnosis was also recorded.

Anatomical Study

Plant samples were studied in detail, including all plant parts, using a compound microscope and a dissecting microscope. Sample families were identified using several sources, including Iraqi flora books (Kamnev *etal*, 2020). Samples were identified at the herbarium in Baghdad. Plants were directly pressed using a plant press to obtain dry samples that retained their characteristics.

Chemical Study

25g of dried powder prepared from the fruits was weighed into 250ml of 75% solution, and placed in a Soxhlet extractor at 40°C for 6 hours. The alcoholic extract was subsequently used for qualitative detection by (Abubakar Haque, 2020). Qualitative tests were conducted to identify active groups in the raw alcoholic extract of each plant according to the method by (Kamnev and Molkanova, 2021)

Antioxidant Activity

The antioxidant profile of rhodium was determined according to method (Niero R and Filho,2008) by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Ascorbic acid was dissolved in methanol to prepare 50 ml of stock solution in concentration of 500 ppm that served as standard antioxidant. In the same manner, 50 ml of separate base solutions were prepared for each test sample of ruthenium, chromium, and rhodium. Final concentrations of 40, 60, 80, 100 and 200 ppm (5 ml each test samples) were prepared in test tub es through serial dilutions. Final concentrations of 40, 60, 80, 100 and 2 00 ppm (5 ml each test samples) were prepared in test tubes through serial dilutions. The test tubes were incubated in dark for 30 min at room temperature. The OD value of the supernatant was measured at a wavelength of 517 nm using ultraviolet spectrophotometer (OD sample). The inhibition or removal of DPPH free radicals was determined using the following formula:

Inhibition % DPPH = [$A_{DPPH} - A_{sample} / A_{DPPH}$] x 100…………

Results and Discussion

Morphological characteristics of R. ulmifolius

The fruit was a capsule-like fruit with a light greenish-brown color, a smooth wall, and no hairs. The seeds were small, elongated, brown, and had a smooth wall**.**

Fig 1: Morphological characteristics of fruits and seeds of R. *ulmifolius*

Table 1: Estimation of the thickness of the fruit coat, seed coat and endosperm layers

Endosperm thickness (µm)	Seed thickness (µm)	Endocarp thickness (µm)	Mesocarp thickness (µm)	Exocarp thickness (µm)
44.4-49.1 (47.3)	21.9-25.5 (23.6)	24.3-28.5 (26.7)	18.5-22.3 (20.5)	16.6-19.4 (17.4)

The results in Table (1) and Figure (1) show differences in the thickness of the layers of the pericarp, seed coat (sprout), and endosperm. The fruit in all plants usually consists of two parts: 1. The pericarp layer and 2. The seeds. The pericarp has three layers: Exocarp or Epicarp: This is the outermost layer of the pericarp that forms the skin. The pericarp has three layers: Exocarp or Epicarp: This is the outermost layer of the pericarp that forms the skin., e is the innermost laye r of the fruit which often develops into the seeds.

Anatomical Characteristics

Figure2 shows the anatomical characteristics of the fruit of the species R. ulmifolius. The cross-section of the fruit wall is ellipsoidal, consisting of three layers. The outermost layer, the exocarp, is very thin, consisting of the epidermis, which consists of oval-shaped cells arranged in a single row, covered by a waxy cuticle layer and consisting of a few stomata. Further inside the epidermis is a thin layer of parenchyma tissue. Its thickness in this species is 17.4 mm.

The middle layer, the mesocarp, is composed of loose parenchyma tissue and contains numerous secretory ducts. Its thickness in the species reached (20.5) micrometers. The endocarp layer, the deepest layer of the fruit peel, is juicy and fragile and consists of an inner epidermis layer and above it, forming ventral meristem tissue. Its thickness in the species reached (26.7) micrometers.

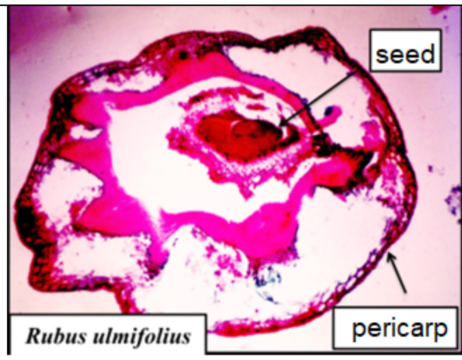

Fig. 2: Cross section of the seed coat and fruit coat of R. ulmifolius where Exo = Exocarp, Meso = Mesocarp, Endo = Endocarp, E = Endosperm, T = testa,

The cross-section of the seeds was oval in shape, and each seed typically consists of a seed coat, also known as the testa. The outer coat is thick and solid, consisting of two layers of cells: the epidermal cells and the innermost layer of the inner coat, composed of parenchyma tissue. Inside lies the endosperm, composed of parenchyma tissue that stores nutrients and essential nutrients for the embryo. (Figure2), Fruit characteristics, including type, color, decoration, and hair shape, are: capsule, greenish-brown, light, with a smooth wall, free of hairs. Seed characteristics: small, elongated, light brown, with a smooth wall.

Chemical Testing

In table 2 *R. ulmifolius* was found to contain: tannins, saponins, flavonoids, glycosides, and phenols, glycosides, However, flavonoids and saponins were not detected.

Table 2: Chemical testing of the active compounds of R. *ulmifolius***.**

Plant	Phenol	Glycosides	Flavonoid	Sapanin	Tannins
Rubus ulmifolius	+	+	-	_	+

needed to isolate and characterize the active compounds and evaluate their Possible reasons for the absence of flavonoids include the fact that the extraction method used is ideal for extracting flavonoids. can affect the extraction efficiency of certain compounds. Flavonoids may be present in other parts of the plant (such as flowers and fruits) but not in the part examined (presumably the leaves or stems, although the table does not specify this). Flavonoid content may vary depending on the season or growth stage of the plant. The chemical screening method used may not have been sensitive enough to detect low concentrations of flavonoids. Plant extracts contain many active ingredients with concentrations that differ from one plan t to another, which made the tests related to the use of chemical reagents to investigate them in the plant a. The results of the chemical discoveries of plant extracts were similar to previous studies on the containment of some study plants including (Hummer ,2010), which relied on qualitative and quantitative chemical discoveries in separating the species and dividing them into approximate hypothetical groups based on this characteristic. Study (Rocabado *etal*,2008) agreed that the species E. globulus contained all the active compounds, while the species C. colocyn this contained all the mentioned active substances except for the two compounds tannins and saponins, and this did not agree with (Ahmed, 2013). Likewise, the species R. communis did not contain saponins and glycosides, and this did not agree with (Ahmed, 2013) .

The table shows the results of a chemical test to determine the presence of specific classes of compounds in *R. ulmifolius*. The chemical compounds tested for the presence of: tannins, saponins, flavonoids, glycosides, and phenols. Blackberry was found to contain tannins, saponins, glycosides, and phenols, while Flavonoids, with their antioxidant, anti-inflammatory, and other health promoting properties, are and diverse biological activities, including antioxidant and antimicrobial effects. Saponins have soap-like properties and can exhibit diverse pharmacological effects, such as anti-inflammatory and cholesterol-lowering effects. Glycosides are a wide group of compounds that may perform various functions depending on their sugar and

aglycone (non-sugar) components. Some glycosides are known for their medicinal properties. Phenols are a large class of compounds with antioxidant effects and other benefits. Flavonoids are a subclass of phenols, known for their antioxidant, anti-inflammatory, and other health-promoting properties. The absence of flavonoids in this assay is notable, and this may be due to several reasons. The presence of tannins, saponins, glycosides, and phenols suggests that *R. ulmifolius* may possess diverse medicinal properties. Further research is value. The discovery, isolation and purification of these compounds has an effective impact on employing their results for the purpose of using them to control many diseases that affect humans and enhance immunity.

Antioxidant activity of *R. ulmifolius*

The results in Figure 3 show the antioxidant activity measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition test for R. ulmifolius fruit extract. This test is a common and accepted method for assessing the ability of substances to scavenge free radicals, as a decrease in the absorbance of the DPPH solution indicates increased antioxidant activity. The results are expressed as a percentage of the Vitamin C is considered to be a powerful antioxidant. The graph shows DPPH inhibition values of approximately 75% and 80% for the two tested samples.

The content of secondary metabolite s in plants of the same species can vary due to several factors, including the degree of maturity, extraction methods, and preservation. harsh climatic and environmental conditions, especially high temperatures and drought, as well as soil chemical composition, pH levels, and trace minerals, significantly influence the production an d concentration of these compounds within the plant. Therefore, comparing the results with the various results of researchers remains impossible, as it requires identifying many other factors related to the environment, plant species, functional activity, genetic and hereditary factors, origin, and formation (United States ,2024). In general, the presence of these active compounds in these plants indicates their great importance, and explains the reason for their use in plant diagnosis and classification, in addition to their medicinal value. This calls for us to pay attention to these bushes because of their importance and medicinal and therapeutic

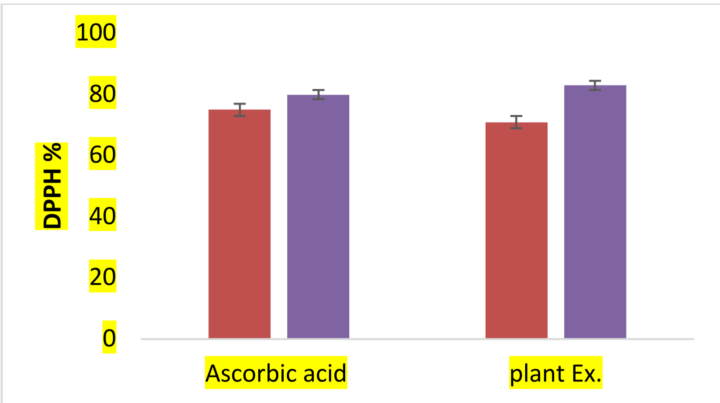

Fig. 3: Antioxidant activity of *R. ulmifolius*. All values are taken as mean ± SEM ($n = 3$), $*P < 0.05$

relatively small error bars indicate that the data are fairly close, increasing confidence in the average values measured for each substance. However, it is important to note that the presence of two values for each substance suggests that different concentrations or possibly replicates of a different experiment were tested, which requires further clarification to fully understand the experimental design. The marks below the figure (**P < 0.05, ***P < 0.01) indicate statistically significant differences between some of the groups compared. However, the text accompanying the figure does not indicate the specific comparisons made. To understand the significance of these statistical

These values indicate the high efficacy of ascorbic acid in inhibiting free radicals under the experimental conditions used. *R. ulmifolius* extract: The plant extract showed DPPH inhibition values of approximately 71% and 83% for the two tested samples. These results suggest that the *R. ulmifolius* extract also possesses significant antioxidant activity, and in one case (the higher value) appears to exhibit activity similar to or even slightly higher than the highest value obtained for ascorbic acid. There is a slight discrepancy between the measured values for both ascorbic acid and the plant extract, as reflected by the error bars (SEM - standard deviation of the arithmetic mean). The

Conclusion

Studying the development of fruit and seed structure is a valuable tool in plant classification and determining relationships between different genera and species. In this context, this study describes the structure of the fruits and seeds of *R. ulmifolius*, analyzing histological sections of the perianth and pericarp. The results revealed differences in the thickness of the layers comprising the fruit and seed coats, highlighting the importance of these traits in plant classification. Disintegrated parenchyma and secretory ducts were also observed in some tissues. Chemical analysis revealed the presence of various active compounds in the plant, including phenolics, glycosides, resins, and tannins. However, flavonoids and saponins were not detected. The fruits of *R. ulmifolius* demonstrated high antioxidant activity, surpassing vitamin C at high concentrations. The plant extract was observed to exhibit remarkable antioxidant activity, and in some cases, it exhibited activity comparable to or even slightly higher than vitamin C.

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significances, it is necessary to know whether the comparison is between different ascorbic acid values, between different plant extract values, or, more importantly, between ascorbic acid values and plant extract values.

Overall, the results indicate that *R. ulmifolius* extract possesses potent antioxidant properties and may be a promising source of bioactive compounds. If one of the plant extract values (83%) is statistically significantly higher than the ascorbic acid values, this may indicate that this extract, under the specified experimental conditions, possesses a greater capacity to inhibit free radicals. In basic proc ess of oxidation, the most important step is the formation of free radicals (having unpaired electrons) in living systems, drugs and even food (United States ,2024). Free radicals, which are extremely unstable, causes many diseases of kidney and liver, Alzheimer's disease, fibrosis, cardiac reperfusion abnormalities, atherosclerosis, cancer, arthritis, neurodegenerative disorders and aging (Food and Agriculture,2024). It is reported that antioxidants prevent the oxidative damage which is caused by reactive oxygen species (ROS) and free radicals. Antioxidants act by interfering with the oxidation process by chelating and catalyzing metals and free radicals as oxygen scavengers (Chen *etal*,2010). Our findings confirm reports that Rubus species are extremely abundant with phenolic contents like flavonoids, phenolic acids and anthocyanins. These phenolic compounds have been reported to possess antioxidant, anti-inflammatory and anticancer properties (Er-Rahmani ,2024). Among the different species of the genus Rubus, R. ellipticus has shown antioxidant activities (Venkidasamy *etal*,2024., Silva2025). Rubus has been reported to have antioxidant properties, and phenolic compounds such as flavonoids have been reported to exhibit prominent antioxidant activities (Bramki ,2024., Kumar2024). Based on reports for importance of phenolic components as antioxidants, the observed antioxidant activity may be attributed to the occurrence of flavonoids like active constituent s which require further research for isolation of antioxidant components from the Rubus *ulmifolius*.

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