Phytochemical and Anatomical Characterization of Newly Recorded Ranunculus species in Nassaf-Fallujah, Iraq

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Abstract

This study presents the first anatomical and phytochemical analysis of newly recorded *Ranunculus* species collected from the Nassaf-Fallujah region of Al-Anbar Province, Iraq. Detailed investigations were carried out on the stem and leaf structures, in addition to phytochemicals profiles via Gas Chromatography–Mass Spectrometry (GC-MS). Anatomical traits such as vascular bundle arrangement, epidermal thickness, and mesophyll differentiation were characterized and compared. Therefore, the two given species were different in these features. The GC-MS analysis revealed diverse bioactive compounds, highlighting the medicinal potential of these species. Where, there are some compounds were predominant and other were minor. These compounds belong to different classes such as glycosides, terpenoids, alkaloids and fatty acids derivatives. This research contributes new insights into the taxonomy and pharmacognosy *of Ranunculus* in Iraq and provides a baseline for future ecological and pharmacological studies. More studies are very important to extract the significance of these two species in taxonomy and as medicinal plants. R. acris was thicker than R. muricatus, but *R. muricatus* was chemically most various.

Keyword: Ranunculaceae, Anatomical properties, tall buttercup, Iraq

دراسة تشريحية وكيميائية لنوعين جديدين في مقاطعة النسافـ–الفلوجة، العراق

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المستخلص

يعد هذا البحث الأول بما يتعلق بالتحليل التشريحي والكيميائي النباتي لأنواع جديدة مسجّلة من جنس *Ranunculus* تم جمعها من منطقة نسّافـ–الفلوجة في محافظة الأنبار، العراق. شملت الدراسة فحصًا تفصيليًا لبنية الساق والأوراق، وترتيب الحزم الوعائية، وسمك البشرة، وتميّز النسيج المتوسط. لذا اختلف النوعان في هذه الصفات. كما أُجري التحليل الكيميائي النباتي باستخدام تقنية كروماتوغرافيا الغازية المدعمة بالمطياف الكتلي (GC–MS)، والذي كشف عن مجموعة واسعة من المركبات الفعّالة حيويًا، مما يبرز الإمكانات الطبية لهذه الأنواع. فقد وجد ان هناك مركبات سائدة واخرى متباينة. وقد اختلفت المركبات من حيث المجموعة فمنها كلايكوسيدات وتربينات وقلويدات ومنها مشتقات الاحماض الدهنية. تسهم هذه النتائج في إثراء المعرفة بالتصنيف وعلم العقاقير لجنس *Ranunculus* في العراق، وتوفر أساسًا لدراسات مستقبلية الخاصة بالجوانب البيئية والدوائية. لذا فان مزيد من الدراسات تعد مهمة جدا لاستخلاص أهمية هذين النوعين في مجال التصنيف والنباتات الطبية. فقد كان النوع *R. acris* اسمك من النوع *R. muricatus* الا ان النوع الأخير كان أكثر تباينا من ناحية التركيب الكيميائي.

الكلمات المفتاحية: عائلة شقائق النعمان، الصفات تشريحية، الحوذان الطويل، العراق

Introduction

The genus *Ranunculus* (family Ranunculaceae) comprises over 600 species globally, many of which are known for their ecological diversity and pharmacological significance (Tobe & Raven, 2011; Tamura, 1993). Members of this genus are widespread in temperate and subtropical regions and are recognized for their traditional uses in folk medicine, including anti-inflammatory, antimicrobial, and wound-healing applications (Ahmad et al., 2015). Some bioactive constuents were identified in buttercup as characterized by Qualitative analysis such as saponins, phenolics and alkaloids. Saponins were present in high amount (Khan et al., 2016), carioactively glycuside (Aslam et al., 2013). A novel natural hydrazine derivative, muricazine had isolated from *R. muricatus* which had antiradical (DPPH) activity (Raziq et al., 2020). Two other compounds were isolated from this taxon from methanolic extracts of the above-ground parts of this taxon: caffeoyl-β-d-glucopyranoside and 1,3-dihydroxy-2-tetracosanoylamino-4-(E)-nonadecene. Both possessed antioxidant efficacy (Azam et al.,2019

The observed anatomical features on *Ranunculus auricomus* L. var. *biformis* L. showed that the species grows in flooding conditions of Vlasina Lake, Serbia (Atanacković et al., 2013; Tobe & Raven, 2011). *Ranunculus arvensis* var. *spinosus* was found to have larger basic epidermal cells and larger stomata. These plants are similar in anatomical features to those in Ahtopol, where the climate is more humid than the Northeastern Bulgaria (Dochev et al., 2014; Tobe & Raven, 2011). The mitosis of root cells in meristematic zone of *Allium cepa* (L.) had been depressed by aqueous floral extracts of buttercup and caused chromosomal

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abnormalities (Smirnova et al., 2023; Tobe & Raven, 2011) due to its content of flavonoids and polyphenols. Some of these compounds may determine other organisms' communities like volatile organic compounds especially that released from *R. acris* (Gaube et al., 2023).

In Iraq, the flora is rich in native and naturalized species, yet the diversity and distribution of Ranunculus species remain incompletely documented. Most botanical studies in the country have focused on floristic surveys or basic taxonomy, with limited attention to internal anatomical traits or secondary metabolite profiles. Specifically, regions like Nassaf-Fallujah in Anbar Province have received little botanical scrutiny due to past security concerns and under limited exploration. However, there exists a significant gap in the literature concerning the anatomical and phytochemical characterization of Ranunculus species growing in Iraq. Despite the ecological importance and medicinal potential of this genus, no prior studies have detailed the internal vegetative anatomy or conducted GC-MS-based phytochemical profiling for species newly recorded in the Nassaf-Fallujah area. Understanding anatomical traits such as vascular arrangement, mesophyll differentiation, and protective tissue structure can support taxonomic differentiation and adaptive ecology (Metcalfe & Chalk, 1979). Likewise, phytochemical profiling provides valuable insight into the presence of bioactive compounds that may have pharmaceutical or ecological significance (Harborne, 1998). This study addresses the aforementioned gap by: (i) providing a detailed anatomical description of the stem and leaf tissues of two newly recorded *Ranunculus* species, (ii) identifying and analyzing phytochemical constituents through GC-MS technique, and (iii) establishing baseline data that can inform future taxonomic, ecological, and pharmacological investigations of *Ranunculus* in Iraq.

Materials and Methods

Plant Collection and Identification

Specimens of *Ranunculus* spp. were collected from the Nassaf-Fallujah region (33°21'N, 43°47'E) during the spring of 2024. Morphological identification was conducted using regional floras (Townsend & Guest, 1985) and confirmed by the second author. Voucher specimens were pressed, dried, and deposited in the Herbarium of University of Anbar, Center of Desert Studies. [Your Institution Name] Separate samples were preserved in Formalin acetic acid (FAA) for anatomically studying.

Anatomical Trial:

Free-Hand Sectioning

Freshly stems and petioles were cut into 4–6 cm segments and thin transverse sections were prepared using a razor blade following Al-Hajj (1998). To remove chlorophyll, sections were handled with 0.5% sodium hypochlorite for 5 min, then stained with 1% safranin (1–2 h). Excess stain was removed with 70% ethanol, followed by sequential dehydration in 90%, 95%, and absolute ethanol. Sections were cleared in a graded ethanol–xylene series and finally in xylene, mounted in DPX, tested underwith a light microscope, and photographically imaged using a digital camera.

Paraffin (Wax) Sectioning

Plant segments (0.5–2 cm) were fixed in Formalin–Acetic Acid (FAA) for 20–24 h at room temperature. After washing with distilled water, samples were dehydrated through a graded series of tertiary butyl alcohol (TBA) as described in (Alrawi et al., 2023), cleared in xylene, and infiltrated with paraffin (four changes, three days each). Embedding was carried out in paraffin molds, and ribbons (8–12 µm thick) were sectioned using a sliding microtome (Leica SM 200R).

Sections were immersed in a warm water bath (40–45 °C) containing 0.5% gelatin, transferred to slides, and dried on a hot plate. Paraffin was removed by sequential immersion in xylene and descending ethanol grades. Staining was performed with 1% safranin (12–24 h), followed by brief washing in 70% and 95% ethanol, counterstaining with fast green, and final clearing in clove oil/ethanol/xylene mixtures. Slides were mounted in DPX and examined with an Olympus light microscope, and images were captured using an Olympus CH3 camera that uses specialized imaging software (Olympus Viewer 3). The pre-staining and staining protocol were conducted out as described in Alrawi et al., (2023), Al- Masoudi & Al-Dobaissi (2022) and Hutchinson (1954). Moreover, measurements of tissue layers were taken using calibrated ocular micrometers. Anatomical features recorded included: Thickness of epidermis and cuticle, Arrangement of vascular bundles, Presence of sclerenchyma and collenchyma tissues and Leaf mesophyll type (palisade and spongy tissues). Standard plant anatomical terminology and procedures followed Metcalfe and Chalk (1979).

Phytochemical Analysis:

Extraction Procedure

Fresh, healthy leaves of both *Ranunculus* species were collected, washed completely with distilled water to elute surface contaminants, and air-dried in a shaded, ventilated place for 10–14 days to preserve thermolabile compounds. The dried plant material was then grinded using a mechanical grinder to obtain a uniform fine powder. Approximately 50 grams of powdered foliage materials were undergone to extract using 70% ethanol, a hydroalcoholic solvent known for its efficiency in extraction wide spectra of both polar and semi-polar phytochemicals, involved flavonoids, phenols, alkaloids, and terpenoids (Sasidharan et al., 2011). Maceration was performed in an amber glass bottle for 72 hours at room temperature with intermittent concussion to enhance dissolution of constituents. The extraction solutions were filtered via Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to elute the solvent, yielding a thick, semi-solid crude extract. The extract was stored at 4°C in a sealed bottle until GC-MS analysis.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

For identifying the volatiles and semi-volatile constituents, three runs GC-MS analyses for the ethanolic extract were conducted via an Agilent 7890B Gaseous Chromatography equipped with a 5977B Mass Selective Detector (MSD). Its system coupled with an HP-5MS capillary column (30 m length × 0.25 mm ID × 0.25 µm film thickness), which is widely used for phytochemical and essential oil profiling due to its polarity balance and thermal stability.

Instrumental parameters were as follows:

The carrying gas was high-purity Helium (99.999%), used at a constantly flowing rate of 1.0 mL min^{-1}. Injection volume: 1.0 µL of the concentrated ethanol extract diluted in analytical-grade ethanol (1:10 v/v). Injection mode: Splitless to maximize detection sensitivity. Oven temperature program was: Initial temperature 50°C, held for 2 minutes; then elevated using a rate of 10°C per min to an ending temperature of 280°C, held for 10 minutes. Ion source temperature: 230°C. Quadrupole temperature: 150°C. Transfer line temperature: 280°C. Mass scan range: m/z 50–600. Electron ionization (EI): 70 eV. Total run time for each sample was about 30 minutes. Chromatograms were acquired and processed using Agilent MassHunter software. The spectra of unknown compounds were compared on those in electronic library (NIST) embedded in this device (Alrawi et al., 2023).
Comparative abundance of major compounds in *R. muricatus* and *R. acris* was done using histogram plot.

Results and Discussion

Stem Cross Sections

Both studied *Ranunculus* species exhibited circular stem shapes in cross-section (Figs. 1–5). Based on growth stages, R. *muricatus*, *R. acris* species were grouped in one. Both *R. muricatus* and *R. acris* exhibited secondary growth and are dicotyledonous. The cuticle thickness ranged from 2.5 µm in *R. acris* to 4.0 µm in *R. muricatus* (Table 3).

Table 1. Stem-cross sections features of *Ranunculus acris* and *R. muricatus* wildly grown in Iraq (µm).

Species	Thickness of cuticle	Thickness of epidermis	Thickness of periderms	Thickness of cortex	Pith thickness	vascular bundle diameter
Ranunculus acris	2.5-4.5 (3.75)	33.2-38.4 (36.5)	-	384.5-388.6 (386.2)	-	124.5-128.6 (125.5)
Ranunculus muricatus	3.25-4 (3.75)	19.8-26.4 (25.4)	-	111.2-116.4 (115.3)	-	58.4-61.3 (59.3)

The epidermis in species with primary growth was simple, with thickness ranging from 19.8 µm (*R. muricatus*) to 38.4 µm (*R. acris*). In species with secondary growth, the epidermal region comprised cork, cork cambium, and secondary cortex. In primary growth stems, the cortex included collenchyma, chlorenchyma, and ordinary parenchyma. Cortex thickness ranged from 111.2 µm in *R. muricatus* to 388.6 µm in *R. acris*. Vascular tissues were arranged in opening immanent vascular bundles resulting in a continual vascular cylinder. The vascular bundle diameter ranged from 58.4 µm in *R. muricatus* to 128.6 µm in *R. acris*. The central region of the stem contained a parenchymatous pith with polygonal cells, and pith thickness ranged from 59.3 µm in *R. muricatus* to 125.5 µm in *R. acris*. In stems with secondary growth, cortex became narrower and formed the first annual vascular ring.

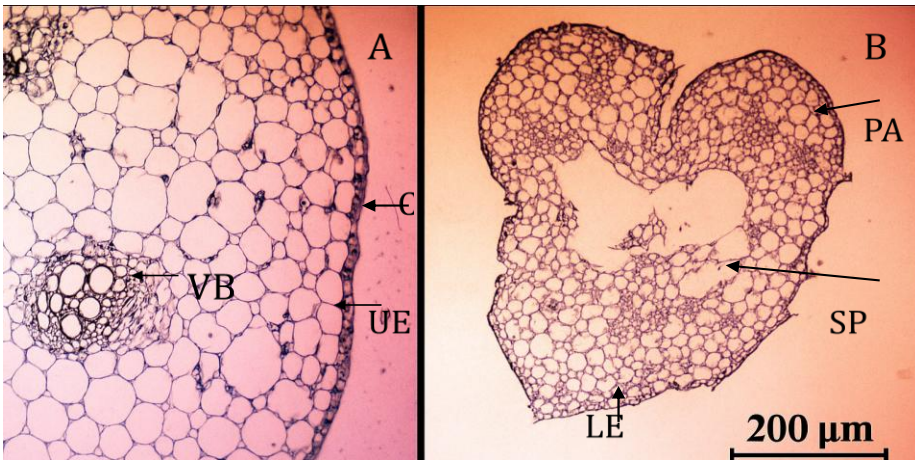

Plate 1: cross section of stem in the species, A: *R. muricatus*, B: *R. acris*. where are the C: cuticle, UE: upper epidermis, LE: lower epidermis, PA: palisade layers, Sp: spongy layers, 10X (200 µm)

Leaf Blade Cross Sections

Dermal Tissue System

All studied species had an adaxial and abaxial epidermis covered by a cuticle. Blade thickness ranged from 84.5 µm in *R. acris* to 108.2 µm in *R. muricatus*. Cuticle thickness varied from 2.6 µm (*R. acris*) to 3.9 µm (*R. muricatus*). Upper epidermis thickness ranged from 12.5–24.5 µm and lower epidermis from 10.5–18.4 µm (Table 1).

Table 2: the quantities characters of leaf blade for the species under study (µm).

Species	Cuticle thickness	Upper epidermis thickness	Lower epidermis thickness	Palisade thickness upper epidermis	Spongy layer thickness	Blade thickness	Vascular bundle thickness
R. acris	2.6-3.2 (2.9)	12.5-16.5 (14.8)	10.5-15.4 (13.2)	24.3-29.4 (26.1)	28.4-33.1 (30.5)	84.5-86.3 (85.4)	30.5-33.1 (31.2)
R. muricatus	3.4-3.9 (3.8)	19.4-24.5 (22.5)	16.5-18.4 (16.8)	38.5-40.1 (39.4)	25.6-29.4 (27.5)	106.7-108.2 (107.4)	27.2-36.4 (35.5)

Ground Tissue System

Mesophyll was bifacial in both species (*R. acris* and *R. muricatus*), composed of palisade and spongy parenchyma. Palisade thickness ranged from 24.3–40.1 µm, with R. muricatus having the greatest thickness. Spongy parenchyma ranged from 25.6 µm (*R. muricatus*) to 33.1 µm (*R. acris*).

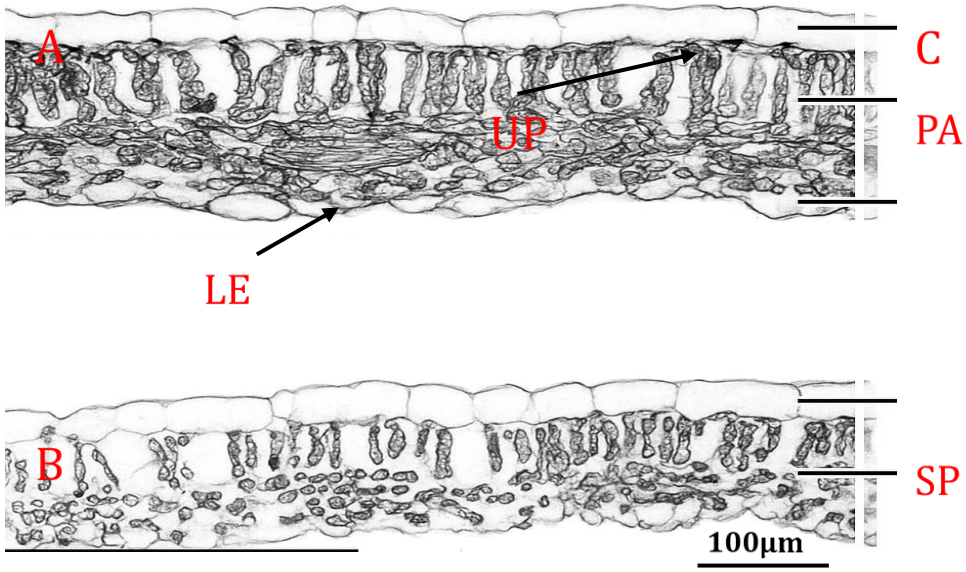

Plate 2. Figure: cross section of leaf blade in the species, where are the A: *R. acris*, B: *R. muricatus*, UE: upper epidermis, LE: lower epidermis, PA: palisade layers, Sp: spongy layers. 10X (100 µm)

Vascular Tissue System

Vascular bundles were of the open collateral type without Kranz anatomy, confirming the C_3 photosynthetic pathway. The midrib appeared crescent-shaped with an ovoid vascular bundle. Vascular bundle thickness ranged from 27.2 µm (*R. muricatus*) to 36.4 µm (R. muricatus) in the central bundle (Table 1).

Leaf Petiole Cross Sections

All species had petiolate leaves. Petiole cross-sectional shape varied which gave two groups; Group 1: Circular (*R. muricatus*) and Group 2: Cordate (*R. acris*). The petiole structure included a single-layered epidermis with ovoid cells and outer cuticle, followed by a cortex comprising collenchyma (2–3 layers) and parenchyma. Central vascular bundles matched the petiole shape.

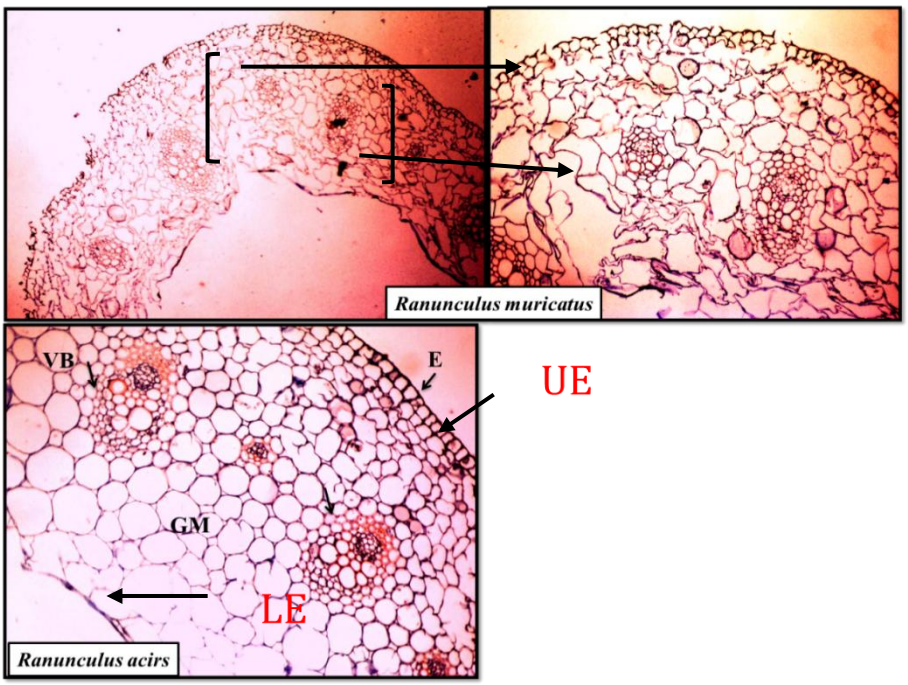

Plate 3: Leaf Midrib-Cross sectioning of *R. muricatus* and *R. acris*, where are the UE: upper epidermis, LE: lower epidermis, VB: vascular bundle, GM: granular material 10X (100 µm).

GC-MS Phytochemically Profiles

Chromatographic Gas coupled to Spectrometrically Mass characterization of ethanol leaf extracts from *R. muricatus* and *R. acris* revealed distinct qualitative and quantitative differences in their phytochemical compositions. A total of 35 compounds were detected in *R. muricatus*, whereas 25 compounds were identified in *R. acris*. These compounds belong to diverse chemical classes, including lactones, fatty acids, alkaloids, glycosides, terpenoids, esters, and alcohols (table 3). Where, Lactones: 27.41% (mainly 5-Hydroxymethylfurfural, 17.17%), Fatty acids: 22.43% (dominated by Palmitic acid, 15.77%), Fatty esters + fatty alcohols: ~9.7% combined, Alkaloids: 7.08% and Others (amino acids, pyrans, sugars, terpenes, steroids, etc.) in smaller amounts.

Furans and Furfural Derivatives included 5-Methylfurfural – Aromatic aldehyde; flavor compound, 5-Hydroxymethylfurfural (HMF) – Derived from sugar degradation and 5-Hydroxymethyldihydrofuran-2-one – Lactone derivative. Aldehydes & Ketones involved Phenylacetaldehyde – Aromatic aldehyde; found in flowers/fruits, 2-Hydroxy-5-methylacetophenone – Aromatic ketone and Megastigmatrienone – Norisoprenoid, contributes to aroma. Pyrazoles & Related Heterocycles represented as Methyl 1H-pyrazole-3-carboxylate – Nitrogen heterocycle and 5,7-Dimethylpyrazolo[1,5-a]pyrimidine-2-carboxylic acid – Condensed heterocycle. Amino Acids & Derivatives concluded dl-Citrulline – Non-proteinogenic amino acid and Methyl 5-oxo-L-prolinate – Proline derivative. Pyrans & Pyranones included 3,5-Dihydroxy-6-methyl-2H-pyran-4(3H)-one – Sugar degradation product. Carbohydrates & Sugar Derivatives, ⌊-Glucosan – Anhydrosugar from cellulose and α-Methylglucofuranoside – Sugar derivative. Fatty Acids & Esters such as Myristic acid which is saturated C_{14}-fatty acid, Palmitic acid considered as a saturated C_{16}-fatty acid, Methyl palmitate / Ethyl palmitate – Fatty acid esters, Elaidic acid – Trans-unsaturated C_{18}-fatty acid, Stearic acid – Saturated C_{18}-fatty acid and Methyl 17-methyloctadecanoate – Branched fatty acid ester. Alkenes, Alkanes & Alcohols involved 1-Nonadecene – C_{19} monoalkene and 1-Tetracosanol (appears twice) – Long-chain fatty alcohol (C_{24}). Ketones, Lactones & Related Compounds represented as Hexahydrofarnesyl acetone – Terpenoid ketone, 10-Undecenyl cyclobutanecarboxylate – Unsaturated cyclobutane ester and 1-Monopalmitoylglycerol – Monoacylglycerol. Phthalates (Plasticizers/Pollutants) combined from two compounds like Diethyl phthalate and Di-n-2-propylpentylphthalate. Steroids & Terpenoids consisted of two compounds: Stigmasta-4,7,22-trien-3β-ol – Phytosterol and (6R,9R)-Vomifoliol – Apocarotenoid (oxidized terpenoid). Finally, Other Notables such as 2,4,7-Trihydroxypteridine (Violapterin) – Pteridine derivative, possibly with biological pigment function, Barbituric acid – Barbiturate core; sometimes found in plant or microbial extracts, Heptadecyl perfluorobutyrate – Synthetic or artifact compound and 6-Methylthieno[2,3-b]pyridine – Sulfur-containing heterocycle.

Table 3. Phytochemical classes and compounds of *R. muricatus* detected by GC–MS.

Class	Compound	Area (%)	RT (min)
Lactones	5-Methylfurfural	4.21	4.116
	5-Hydroxymethyldihydrofuran-2-one	6.03	7.622
	5-Hydroxymethylfurfural	17.17	8.118
Aldehyde	Phenylacetaldehyde	2.69	5.194
Ketones	2-Hydroxy-5-methylacetophenone	2.18	9.316
	Hexahydrofarnesyl acetone (terpene ketone)	3.42	16.469
Alkaloids	Methyl 1H-pyrazole-3-carboxylate	3.25	5.766
	Methyl 5-oxo-L-prolinate	0.97	10.351
	2,4,7-Trihydroxypteridine (Violapterin)	1.30	12.854
	5,7-Dimethylpyrazolo[1,5-a]pyrimidine-2-carboxylic acid	0.77	14.138
	6-Methylthieno[2,3-b]pyridine	0.79	16.717
Amino acid	dl-Citrulline	1.65	6.521
Pyrans	3,5-Dihydroxy-6-methyl-2H-pyran-4(3H)-one	5.38	6.791
Monosaccharide	⌊-Glucosan	3.46	12.207
Glycoside	α-Methylglucofuranoside	2.13	14.678
Fatty acids	Myristic acid	1.24	15.465
	Palmitic acid	15.77	18.022
	Elaidic acid	2.69	20.007
	Stearic acid	2.73	20.288
Fatty esters	Methyl palmitate (FAME)	1.56	17.472
	Ethyl palmitate	2.63	18.313
	Methyl 17-methyloctadecanoate (FAME)	1.18	20.611
Fatty alcohols	1-Tetracosanol	3.56	23.762
	1-Tetracosanol (isomer/duplicate)	0.77	25.704
Other lipids	1-Monopalmitoylglycerol	0.87	23.945
Alkanes/Alkenes	3-Acetoxytridecane (alkane)	1.46	11.527
	1-Nonadecene (alkene)	1.41	15.875
Esters	10-Undecenyl cyclobutanecarboxylate	1.11	6.209
	Di-n-2-propylpentylphthalate (benzoate ester)	1.23	24.118
Monoterpenoids	Diethyl phthalate	1.63	13.221
	Megastigmatrienone	0.80	13.739
	(6R,9R)-Vomifoliol	0.94	13.966
Fluorinated substances	Heptadecyl perfluorobutyrate	1.29	19.587
Phytosteroids	Stigmasta-4,7,22-trien-3β-ol	0.77	29.242
Pyrimidine derivatives	Barbituric acid	0.98	30.159

Major Compounds in *Ranunculus muricatus*

The predominant compound identified in *R. muricatus* was 5-Hydroxymethylfurfural (HMF), accounting for 17.17% of the total extract area. HMF, a furan-derived lactone, is widely recognized for its potent antioxidant and anti-inflammatory activities (Qu et al., 2025; Zhao et al., 2013). Another major component was palmitic acid (15.77%), a saturated fatty acid known for antimicrobial and cytotoxic effects (Ahmed et al., 2023; Bajpai et al., 2013; Aparna et al., 2012). Additionally, moderate amounts of 3,5-dihydroxy-6-methyl-2H-pyran-4-one (5.38%) and hexahydrofarnesyl acetone (3.42%) were detected, compounds that have been associated with antioxidant and antibacterial properties (Prasad et al., 2009; Naz & Bano 2013). The presence of several alkaloids and esters further suggests enhanced therapeutic potential.

Major Compounds in *Ranunculus acris*

In *R. acris*, Glycoside was the most abundant (β-D-Glucopyranoside, methyl, 31.97%). Fatty alcohols + fatty acids + fatty esters also contributed a significant portion. Minor fractions include alkaloids, aldehydes, ethers, and sugars (table 4). the most abundant compound was methyl β-D-glucopyranoside (31.97%), a glycoside reported to exhibit strong antioxidant and antidiabetic properties (Tran et al., 2025). The volatile ester isoamyl acetate (18.51%) was also prominent, known for its antimicrobial activity and characteristic fruity aroma (Musyimi & Neema, 2019). Furthermore, cis-vaccenic acid (9.55%), a monounsaturated fatty acid, has documented anti-inflammatory and cholesterol-lowering effects (Lopez-Huertas et al., 2010). Notably, *R. acris* showed higher proportions of glycosides and sugar derivatives compared to R. muricatus, suggesting differences in biosynthetic pathways and ecological adaptations.

Table 4. Phytochemical classes and compounds of *Ranunculus acris* detected by GC–MS.

Class	Compound	Area (%)	RT (min)
Lactones	5-Methylfurfural	4.21	4.116
	5-Hydroxymethyldihydrofuran-2-one	2.49	7.978
	2-Butyne-1,4-diol bis(tetrahydropyranyl) ether	8.52	8.323
	D-Glucuronic acid lactone	0.74	18.572
Aldehyde	Phenylacetaldehyde	2.69	5.194
Oxide	2,3-Epoxybutane	0.91	5.292
Monosaccharide	Butane-1,2,3,4-tetrol	0.77	5.799
Cyclopropane	Cyclopropyl carbinol	0.34	6.101
Pyran	3,5-Dihydroxy-6-methyl-2H-pyran-4(3H)-one	6.58	6.813
Fatty alcohols	Isoamyl acetate	18.51	7.503
	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.84	20.665
	2-Methyl-Z,Z-3,13-octadecadienol	2.05	25.822
Sugar alcohol	2,4-Methylene-D-epirhamnitol	0.86	8.776
Ether	3-Acetylanisole	1.14	9.381
Alkaloids	Methyl 5-oxopyrrolidine-2-carboxylate	0.39	10.675
	Trifluorothymine	0.35	12.930
Glycoside	β-D-Glucopyranoside, methyl	31.97	14.904
Trisaccharide	D-(+)-Melezitose	0.36	16.069
Fatty methyl esters	Methyl palmitate	1.04	17.548
	Methyl elaidate	5.68	19.597
	trans-13-Octadecenoate	0.53	19.705
Fatty acids	Palmitic acid	1.27	18.022
	cis-Vaccenic acid	9.55	20.104
	cis-13-Octadecenoic acid	1.07	20.396
	Linoleic acid	0.35	21.032

Comparative Insights

Both species share several common phytochemicals such as phenylacetaldehyde, methyl palmitate, and 3,5-dihydroxy-6-methyl-2H-pyran-4-one, yet their dominant metabolites vary considerably. *R. muricatus* is characterized by a higher abundance of fatty acids and lactones, while *R. acris* is richer in glycosides and esters. These variations may reflect species-specific ecological roles or evolutionary adaptations, supporting their differing ethnobotanical uses. The distinct phytochemical profiles provide a biochemical basis for the therapeutic potential of each species within different pharmacological contexts (table 5).

Table 5. Comparative Summary of Major Phytochemicals of Ranunculus muricatus and Ranunculus acris

Compound	RT (min)	*R. muricatus* Area (%)	*R. acris* Area (%)	Compound Class	Reported Pharmacological Activities
5-Hydroxymethylfurfural (HMF)	8.118	17.17	—	Lactone	Antioxidant, anti-inflammatory, anticancer (Qu et al., 2025; Zhao et al., 2013)
Isoamyl acetate	7.503	—	18.51	Ester	Antimicrobial, flavoring agent (Naz & Bano 2015)
β-D-Glucopyranoside, methyl	14.904	—	31.97	Glycoside	Antioxidant, antidiabetic (Musyimi & Neema, 2019)
Palmitic acid	18.022	15.77	1.27	Fatty acid	Antimicrobial, cytotoxic (Thai et al., 2017)
Hexahydrofarnesyl acetone	16.469	3.42	—	Terpene ketone	Antibacterial, anti-inflammatory (Bajpai et al., 2013)
cis-Vaccenic acid	20.104	—	9.55	Fatty acid	Anti-inflammatory, lipid-lowering (Lopez-Huertas et al., 2010)
3,5-Dihydroxy-6-methyl-2H-pyran-4-one	~6.8	5.38	6.58	Pyran	Antioxidant (Ahmed et al., 2023)
Phenylacetaldehyde	5.194	2.69	2.69	Aldehyde	Antifungal, flavor/aroma compound (Medeiros et al., 2020)
Methyl palmitate	~17.5	1.56	1.04	Fatty methyl ester	Antimicrobial, anti-inflammatory (Prasad et al., 2009)

Pharmacological Implications

The major bioactive compounds identified in both species possess a range of pharmacological properties syuch as anti-oxidant, anti-inflammatory, anti-microbial, anti-diabetic, and anti-cancer efficacies (table 5). The predominance of fatty acids, lactones, glycosides, and alkaloids in these extracts underlines the need for further in vitro and in vivo trials to explore their modes of action, particularly regarding oxidative stress, inflammation, and microbial infections. Such research may pave the way for developing novel plant-based therapeutics derived from Ranunculus species.

Phytochemical Constituents (GC-MS Analysis) Comparison

Major Identified Phytochemicals are compared between *R. muricatus* and *R. acris* (table 6). While the shared compounds were Methyl Palmitate, Methyl Oleate, Methyl stearate, Phytol and Ethyl palmitate.

Table 6. Comparison of Major Identified Phytochemicals in two species

Phytochemical Compound	*R. muricatus* (Area %)	*R. acris* (Area %)
Methyl Palmitate	20.15	7.87
Methyl Oleate	18.22	6.33
Methyl stearate	7.63	1.88
Phytol	6.38	13.17
1-Heptacosanol	2.48	—
Ethyl palmitate	3.91	3.12
2-Methyloctacosane	4.65	—
Nonacosane	1.49	—
Dasycarpidan-1-methanol acetate	—	7.69
Stigmasterol	—	5.90
1-Hexacosanol	—	3.93
Methyl linoleate	—	3.41

The unique compounds to *R. muricatus* were 1-Heptacosanol, 2-Methyloctacosane and Nonacosane. Whereas, the compounds unique to *R. acris* were Dasycarpidan-1-methanol acetate, Stigmasterol, 1-Hexacosanol and Methyl linoleate (figure 1).

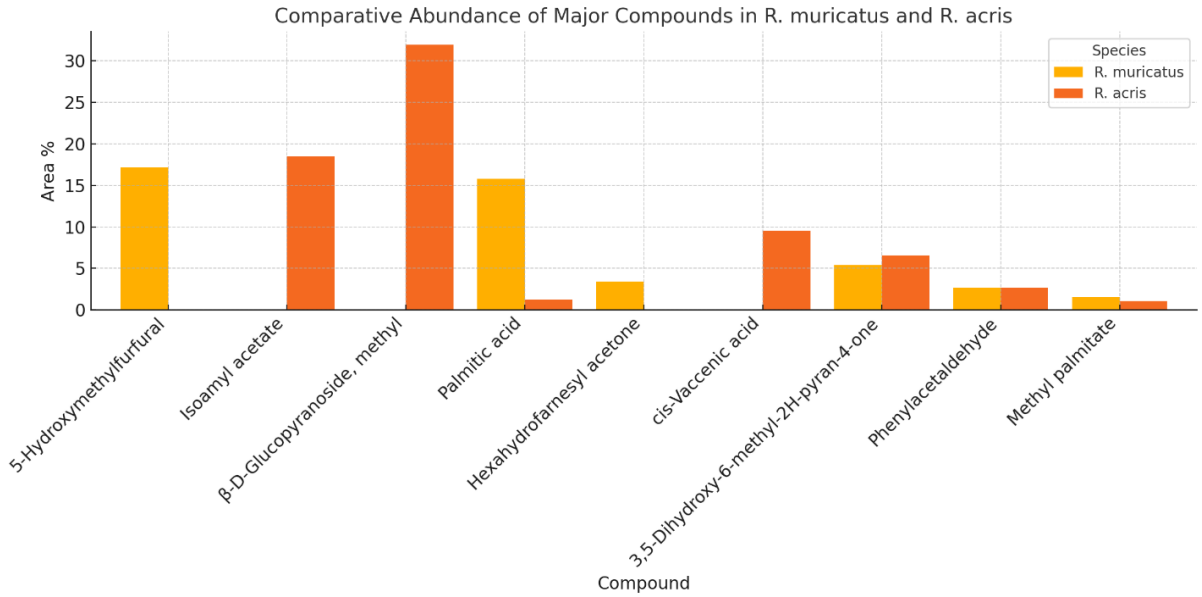

Figure 1. comparative abundance of major compounds in *R. muricatus* and *R. acris*.

In Magnoliopsida, the epidermis, which the exterior leaf layer, is combined of flat and intertwined cells (Tobe & Raven, 2011; Fahn, 1990; Mauseth, 1988). Contrarily, the primary sensitive zone is popular petioles base ensuring that the lower cortex is more sensitive than the upper cortex. Furthermore, the spindles and leaflets are the ordinally sensitive zones (Ming-Lin et al., 2013; Tobe & Raven, 2011). The transversal mesophyll is consisted of a palisade with two or three layers of elongated cells under the adaxial surface and a palisade of one or two layers of adjacent cells on the abaxial surface, corresponding to ¾ of the mesophyll. The lacunar parenchyma is made up of 2-3 layers of rounded and thin-walled cells. The intercellular spaces vary in size and have uneven borders, making them relatively small. A sclerenchymal sheath made up of one or two cells surrounds the entire bundle of vascular collateral bundles, which have vascular elements up to four times larger in diameter than phloem cells. With palisade

tissue, reduced lacunar, and sparse, tiny intercellular meatus, the species exhibits xeromorphic traits (Tobe & Raven, 2011; Simões *et al*., 2003). These results are continent with Alrawi et al. (2023). The anatomical adaptations of *Ranunculus auricomus* L. var. *biformis* L., such as those observed under the flooding conditions of Vlasina Lake in Serbia, highlight the ability of certain buttercup taxa to tolerate hydrophilic environments (Atanacković et al., 2013; Tobe & Raven, 2011). Similarly, *R. arvensis* var. *spinosus* exhibits enlarged basal epidermal cells and stomata, features comparable to those reported in populations from Ahtopol, where the relatively humid climate promotes similar structural modifications (Dochev et al., 2014; Tobe & Raven, 2011). Such anatomical plasticity may represent an adaptive response to local ecological pressures, particularly variations in water availability.

In addition to their structural traits, species of *Ranunculus* are also characterized by distinct phytochemical properties. For instance, aqueous extracts of buttercup flowers exert a mitotic inhibitory effect on Allium cepa root meristem cells and induce chromosomal abnormalities, most likely due to their high content of flavonoids and polyphenols (Smirnova et al., 2023). Beyond their cytogenetic effects, these compounds—especially volatile organic compounds (VOCs) such as those emitted by *R. acris*—may further influence ecological interactions by shaping the composition of associated microbial and insect communities (Gaube et al., 2023). Taken together, the evidence suggests that both anatomical and phytochemical features of Ranunculus species not only contribute to their environmental adaptability but also play a potential role in mediating interactions within their ecosystems.

The GC-MS phytochemical profiling of *Ranunculus muricatus* and *Ranunculus acris* highlights both shared and species-specific bioactive compounds, providing important insights into their potential medicinal applications. The predominance of 5-Hydroxymethylfurfural (HMF) and palmitic acid in *R. muricatus* aligns with previous reports that emphasize the antioxidant and antimicrobial potential of these compounds (Qu et al., 2025; Ahmed et al, 2023; Zhao et al., 2013; Aparna et al., 2012). The significant presence of lactones and fatty acids supports the traditional use of *R. muricatus* in treatments for inflammation and infections, as these classes are well-documented for such bioactivities (Ayllón-Gutiérrez et al., 2024; Shukla, 2018; Bajpai et al., 2013). Conversely, *R. acris* is distinguished by its high content of glycosides and esters, particularly methyl β-D-glucopyranoside and isoamyl acetate. Glycosides are widely studied for their antioxidant, cardioprotective, and antidiabetic effects, which may underlie the ethnomedicinal applications of *R. acris* in metabolic disorders, these results align with (Tran et al., 2025). Isoamyl acetate, aside from contributing a characteristic aroma, exhibits notable antimicrobial activity that could support its therapeutic use in infections (Ando et al., 2015). The higher abundance of sugar derivatives in R. acris also suggests distinct biosynthetic adaptations that may confer ecological advantages such as herbivore deterrence or pathogen resistance. The contrasting phytochemical profiles reflect divergent metabolic strategies and evolutionary pathways between the two species, possibly driven by differences in habitat, pollinator interactions, or stress responses. The shared compounds, such as phenylacetaldehyde and 3,5-dihydroxy-6-methyl-2H-pyran-4-one, may represent conserved metabolites essential for basic physiological functions or generalized defense mechanisms (Medeiros et al., 2020).

Pharmacologically, the identification of multiple bioactive classes—including alkaloids, terpenoids, fatty acids, and lactones—underscores the complexity and therapeutic potential of these species. For instance, alkaloids are well-known for their wide range of biological efficacies, which include antimicrobial and anticancer effects (Thai et al., 2017), while terpenoids contribute to anti-inflammatory and antioxidative mechanisms (Ayllón-Gutiérrez et al., 2024; Shukla, 2018). This phytochemical richness justifies further bioassay-guided fractionation and in vivo studies to isolate and evaluate specific compounds or synergistic combinations for drug development. Overall, the results advance the phytochemical understanding of Ranunculus species in Iraq and highlight the need for integrative studies combining ethnobotany, phytochemistry, and pharmacology. This will help validate traditional uses and potentially uncover novel natural products with significant health benefits.

Conclusion

Conclusively, anatomical studies are regarded as important and additional tools for classifying species. Where there were notable differences between the two speciesanatomical leaf and stem markers. Consequently, anatomical charactristics could be used to describe these species. An effective method for classifyng these species was the analysis of anatomical image. Numerical characteristics were also very useful for studying particular specis. Additionally, these taxa may be beneficial to the environment by reducing desertification and boosting biodiversity in the area, which includes western Iraq. In *R. acris* Glycoside was the most abundant (β-D-Glucopyranoside, methyl, 31.97%). Fatty alcohols + fatty acids + fatty esters also contributed a significant portion. Minor fractions include alkaloids, aldehydes, ethers, and sugars. The lactones were abundant in *R. muricatus*.

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