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RESEARCH ARTICLE

Phytochemical extraction and analysis of phenolic compounds and alkaloids from selected *Medicago* species (Fabaceae) in Central and Northern Iraq

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Abstract

Medicago yields several food and medicinal values due to the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids, carotenoids, sterols, phytoestrogens, flavones and isoflavonoids. Because of the *Medicago* plant's rich and beneficial chemical components and its various benefits, this study was conducted to extract phenolic and alkaloid compounds from the plant in central and southern regions of Iraq. A total of nine species of the genus *Medicago* (Fabaceae) from central and northern Iraq were concluded from this study: *M. polymorpha*, *M. monspeliaca*, *M. lupulina*, *M. rigidula*, *M. radiata*, *M. noeana Boiss*, *M. orbicularis*, *M. sativa* and *M. monantha*. Extraction was performed by a 72 hr maceration with ethyl acetate (10 g/10 mL) and resulted extracts were filtered, concentrated in vacuum and prepared for high-performance liquid chromatography (HPLC) analysis. The investigated compounds included rutin, gallic acid, quercetin, kaempferol, caffeine and ergotin, and the species showed different endogenous accumulation levels. (R)-rutin was found at the highest concentration compared to the other phenolic compounds, in the following sequence: *M. sativa* with a concentration of 598.863 ppm, *M. radiata* with 272.2776 ppm, *M. lupulina* with 235.089 ppm, followed by *M. orbicularis* at 177.148 ppm, then *M. polymorpha* at 172.969 ppm, *M. monspeliaca* at 169.786 ppm, *M. noeana Boiss* at 136.3892 ppm, then *M. rigidula* at 129.6292 ppm and finally *M. monantha* at 122.2005 ppm. Despite this, the phenolic compound Quercetin was observed in the *M. radiata* species at a high concentration of 343.7141 ppm, while it did not appear in any of the other plants this suggests perhaps both chemical diversity and potential utility in providing therapeutically useful compounds from these species. This study found that nine plant samples from different locations of Iraq have identical phenolic and alkaloid contents, indicating that they belong to the same genus. Rutin is found in all plant species in high amounts relative to other phenolic and flavonoid chemicals, indicating that they are from the same genus.

Keywords: chemical diversity; ergotin; fabaceae; HPLC; kaempferol; *M. polymorpha*; phenolic compound

Introduction

The Iraqi environment contains many wild species of the genus *Medicago*, which are distributed throughout the Iraq regions, particularly in northern Iraq, characterized, on the one hand, by inter-specific diversity and climatic variety in the systems of mountains and plains (the temperate climate) (1). Exploring these species within a taxonomic, anatomical, molecular and genetic framework is an important step coupled with exploring local adaptation mechanism and genetic patterns of economic significance that can aid efforts in plant breeding programs for lasting sustainable (2). The Fabaceae family, which is believed to be the third largest plant family after Asteraceae and Poaceae families, comprises more than 19000 species dispersed in diverse environments globally (3). This family is agronomically and ecologically important due to the ability to form symbiotic association with *Rhizobium* bacteria which fix atmospheric nitrogen resulting in soil fertility and improved soil nitrogen status, reducing needs for chemical fertilizer (4). Genus *Medicago* was the most important forage genera in the Fabaceae family boasts more than 80 species, mainly occurring in temperate and semi-arid regions, particularly within the Mediterranean basin and West Asia (5). *Medicago* yields several food and medicinal values due to the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids, carotenoids, sterols, phytoestrogens, flavones and isoflavonoids (6).

Species such as alfalfa (*M. sativa*) are among the most important members of this genus. It is defined by high contents of bioactive compounds (flavonoid, phenolic acid, saponins and organic acids) that demonstrate antioxidant and anti-inflammatory properties in addition to its antimicrobial and dermo protective effect (7, 8). *Medicago* provides a wide range of alkaloids and therefore it is priceless as far as drug discovery and therapeutics area often concerned (9). Because of the *Medicago* plant's rich and beneficial chemical components and its various benefits, this study was conducted to extract phenolic and alkaloid

compounds from the plant in central and southern regions of Iraq.

Materials and Methods

Sample collection

The samples of *Medicago* were gathered through a geographical survey in the regions of central and northern Iraq (Fig. 1), then the method of preparation was done as follows.

Preparation of dry samples (herbarium specimens)

The plant samples were dried by placing them between absorbent papers and pressing them using a special plant press. The papers were replaced periodically until the samples were completely dry. The samples were then mounted and attached to cardboard paper, wrapped in clear nylon and stored in special folders in the herbarium for use in future taxonomic studies. The plant samples were collected during the flowering stage, dried in the shade at room temperature and then crushed for use in the chemical study.

Extraction process

Samples were prepared after grinding and weighing 10 g in 10 mL of ethyl acetate for 72 hr. The sample was then filtered using a 0.45 µm syringe filter and prepared for injection into the device.

Diagnosis and purification using high-performance liquid chromatography (HPLC) technology

A Shimadzu HPLC device (Japan) was used with a Phenomenex-C18 column, 100 A, 250 x 60 mm. The mobile phase was prepared from a mixture of (Water: Methanol) in a volumetric ratio of 80:20 (v/v). The samples were separated from the column after filtration and degassing using an ultrasonic device (Sonicator).

A flow rate of 1 mL/min was adopted at a wavelength of 280 nm. A volume of 20 µL of the samples to be measured was injected and the retention time of the standard compounds (phenols and alkaloids) was compared to the retention time of the compounds separated from the extracts dissolved in ethyl acetate (10). Analysis of chemical composition by injecting 20 µL of the phenolic extract of each sample in high performance liquid chromatogram for identification. The peaks were detected by UV detector in 30 °C. Compound concentration might determined by the following equation:

Results and Discussion

Identification of standard phenolic compounds using high -performance liquid chromatography

The retention time for several standard compounds including (R)-rutin, kaempferol, eriocitrin, quercetin, gallic acid and the alkaloid caffeine was determined, as shown in Fig. 2. These were then compared to the retention times of the compounds from nine species of the *Medicago* within the Fabaceae family, specifically: *M. polymorpha*, *M. monspeliaca*, *M. lupulina*, *M. sativa*, *M. radiata*, *M. monantha*, *M. rigidula*, *M. noeana Boiss* and *M. orbicularis* (Fig. 3 and Table 1). Table 2 shows the concentrations of phenolic and alkaloid compounds identified in 9 plants of *Medicago*, using HPLC. Fig. 4 compares the concentrations of the various phenolic compounds extracted from nine different plants belonging to the same (*Medicago*). The results showed the presence of the compounds (R)-rutin and gallic acid in all nine plant species, with slight differences among the individual phenolic compounds across all plants. (R)-rutin was found at the highest concentration compared to the other phenolic compounds, in the following sequence: *M. sativa* with a concentration of 598.863 ppm, *M. radiata* with 272.2776 ppm, *M. lupulina* with 235.089 ppm, followed by *M. orbicularis* at 177.148 ppm, then *M. polymorpha* at 172.969 ppm, *M. monspeliaca* at 169.786 ppm, *M. noeana Boiss* at 136.3892 ppm, then *M. rigidula* at 129.6292 ppm and finally *M. monantha* at 122.2005 ppm.

Fig. 1. Species of the genus *Medicago* used in the current study, which were collected from various regions in central and northern Iraq.

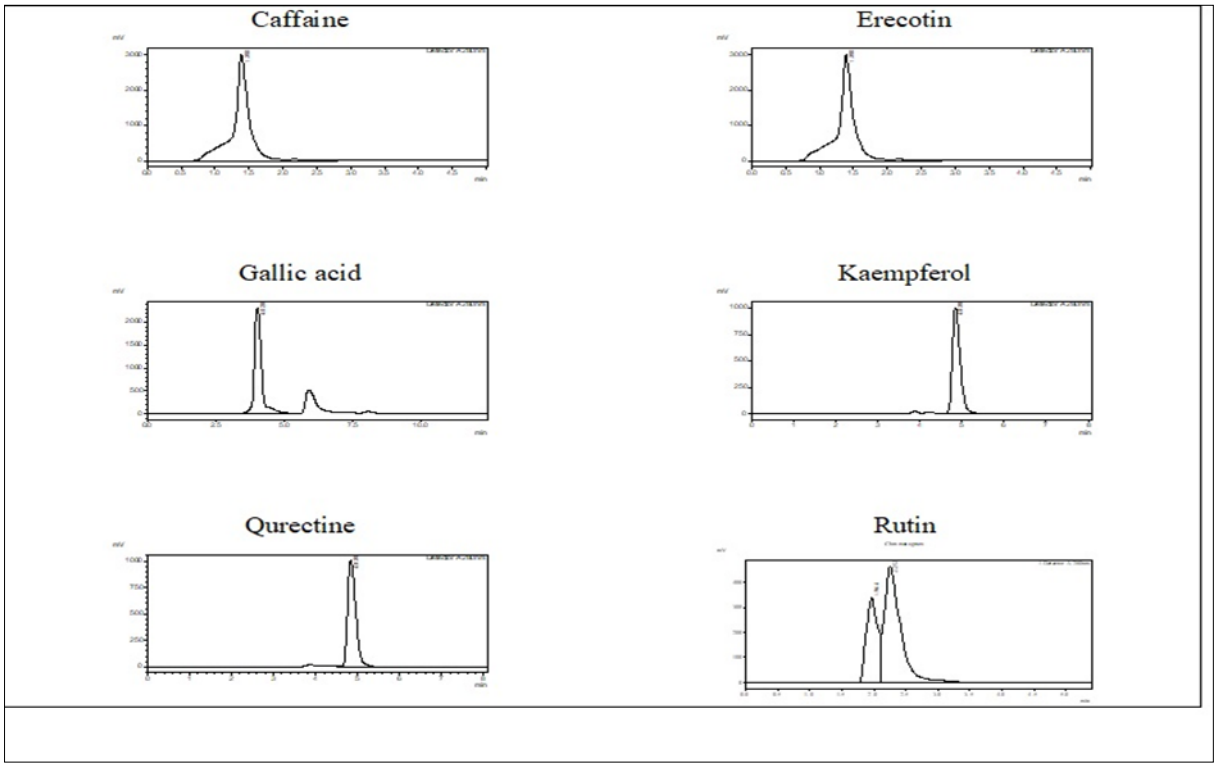

Fig. 2. Standard chromatogram of the phenolic and alkaloid compounds (rutin, gallic acid, kaempferol, quercetin, eriocitrin and caffeine) identified by HPLC.

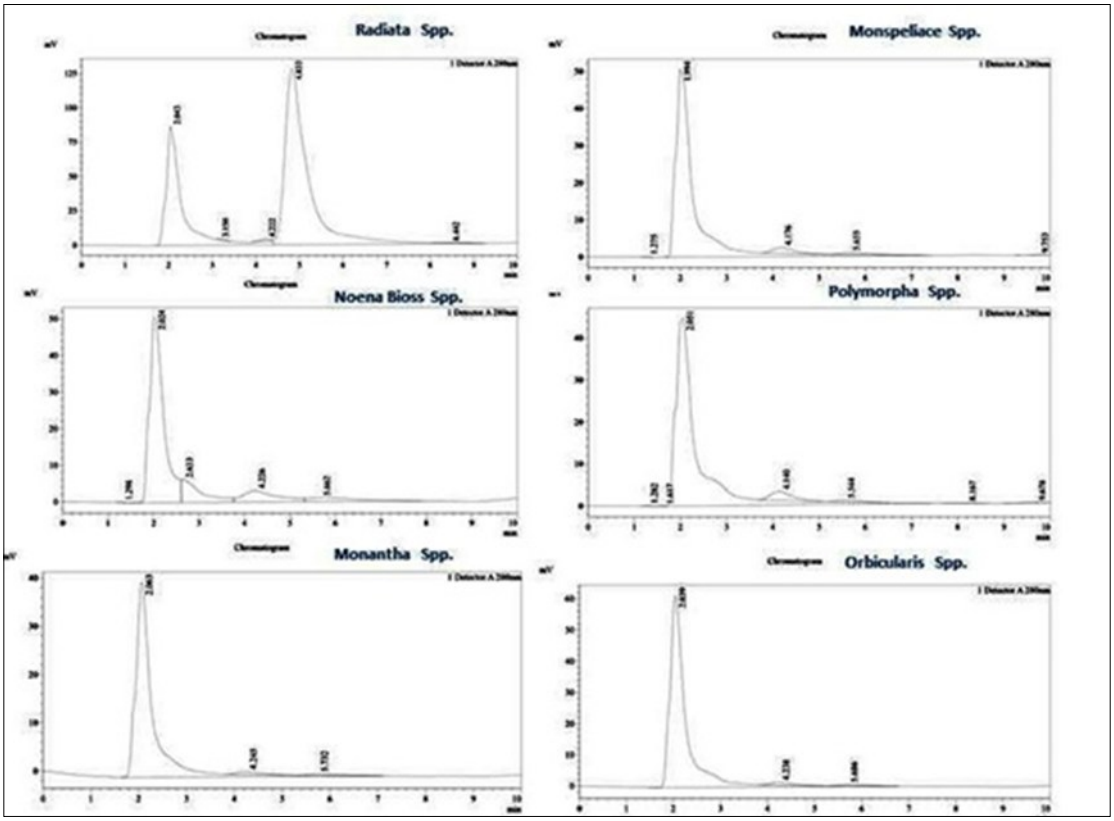

Fig. 3. HPLC chromatogram of extracts from nine different *Medicago* species, showing different curves that were compared to the standard curves of phenolic and alkaloid compounds, as in Table 1, based on the retention time of the curve in the sample.

Despite this, the phenolic compound quercetin was observed in the *M. radiata* species at a high concentration of 343.7141 ppm, while it did not appear in any of the other plants. The other plants showed low concentrations of phenolic compounds compared to (R)-rutin. As for the alkaloids, the study showed the presence of Caffeine in varying proportions and at a lower concentration than some of the phenolic compounds in most of the plants, as illustrated in Fig. 5. The results showed a significant variation in the accumulation of phenolic compounds in plants found in different regions of Iraq (north and semi-south) but belonging to the same genus. The first group was shown to contain five compounds, the second group four compounds and the third group three or fewer compounds.

The current study demonstrated the variation in total phenolic and alkaloid content among *Medicago* extracts from

Table 1. Retention times of phenolic and alkaloid compounds in nine *Medicago* species identified by HPLC and compared with those of standard compounds

Identified phenolic compounds/ alkaloids		Retention time (minutes) for nine plant species								Standard retention time (min)
		Sativa	Orbicularis	Noenaboiss	Radiata	Rigidula	Lupulina	Monspeliace	Polymorpha	
(R)-Rutin	2.063	2.001	2.039	2.024	2.043	2.052	1.979	1.994	2.051	2.252
Gallic acid	4.245	4.315	4.238	4.226	4.222	4.207	4.191	4.176	4.140	4.028
Qurecetin	-	-	-	-	4.833	-	-	-	-	4.839
Kaemferol	-	-	-	-	8.442	8.350	8.381	-	8.167	7.925
Caffeine	5.732	-	5.686	5.662	-	5.637	5.658	5.655	5.544	5.790
Eriocitrin	-	1.315	-	1.298	-	-	1.282	1.275	1.282	1.390

Table 2. Concentrations of phenolic and alkaloid compounds in nine *Medicago* species extracted with ethyl acetate and identified by HPLC at a wavelength of 280 nm

Identified phenolic compounds/ alkaloids	Standard substance concentration (ppm	Concentration of standard substances for nine identified plants (ppm)								
		Monaatha	Sativa	Orbicularis	Noenaboiss	Radiata	Rigidula	Lupulina	Monspeliace	Polymorpha
(R)-rutin	1000	122.2005	598.863	177.148	136.3892	272.2776	129.6292	235.089	169.786	172.969
Gallic acid	1000	0.4748	0.5606	0.71288	3.8898	0.51435	4.5944	2.8879	1.1257	1.592
Qurecetin	1000	-	-	-	-	343.7141	-	-	-	-
Kaemferol	1000	-	-	-	-	0.5722	0.30622	0.4866	-	0.2063
Caffeine	261.709	1.585	-	0.49913	2.0049	-	1.7831	0.5242	0.6122	0.392
Eriocitrin	1000	-	0.0830	-	0.0219	-	-	0.0258	0.0238	0.03352

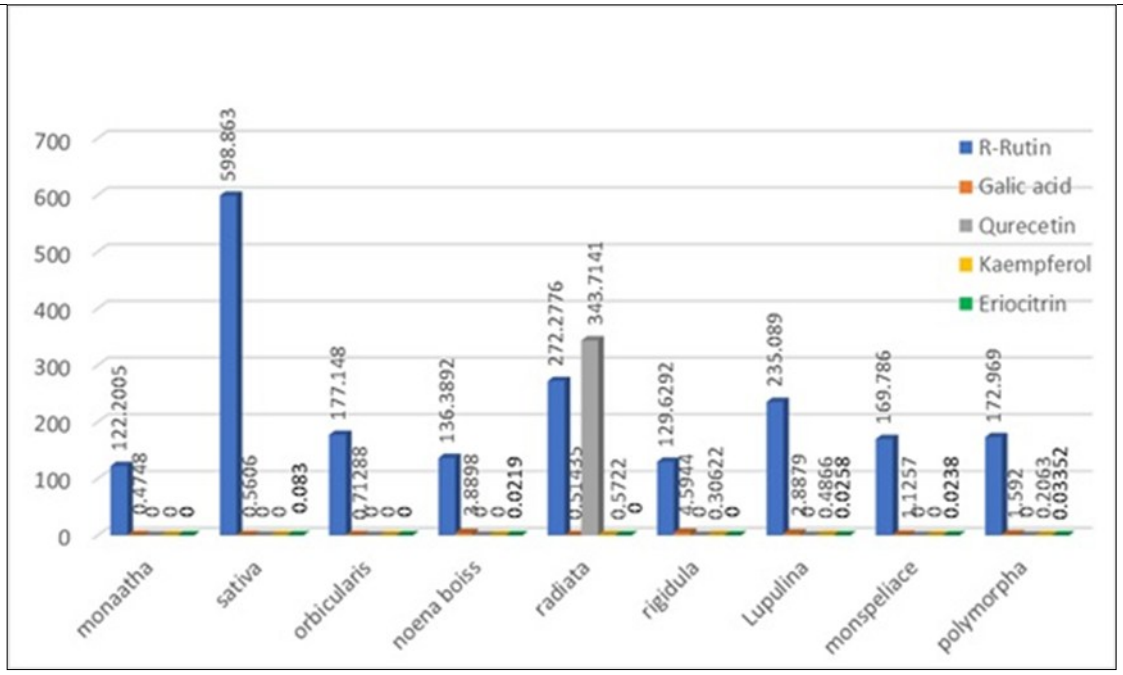

Fig. 4. Comparison of the concentrations of multiple phenolic compounds (5 standard compounds) in nine plants extracted with ethyl acetate, belonging to a single genus (*Medicago*) of the Fabaceae family, using HPLC technology.

nine types in relation to their antioxidant capabilities. Upon comparison with recent studies, the extraction yield of methanol from *Medicago* spp. was shown to surpass that of vetch seeds, which varied from 6.15 % to 9.70 % in the study (11). *In vitro* phytochemical analysis was performed on Medicago sativa raw and germinated seed extracts (12). The phytochemical investigation found alkaloids, carbohydrates, flavonoids, glycosides, saponins, phytosterols, tannins, anthraquinone, phenols and proteins. Ethanol extracted phytochemicals are best from *M. sativa* seeds, both raw and germinated. In the ethanolic extract of *M. sativa* raw seeds and germinated seeds, 2,2-diphenyl-1-picryl hydrazil (DPPH), hydrogen peroxide and hydroxyl radicals, superoxide anion radicals and nitric oxide radicals were compared to ascorbic acid. The ethanolic extract of *M. sativa* raw seeds and germinated seeds scavenged free radicals concentration-dependently. Ethanolic extract of germinated *M. sativa* seeds had a stronger antioxidant effect than ascorbic acid.

An analytical study to determine the biochemical substances found in a single species, *M. sativa*, which grows in Iraq, using the classical method and the technology of plant tissue culture (13). With the aid of HPLC techniques, the study described could meaningfully link the changes of phenolic and flavonoid compounds with the proposed, suggesting that only the phenotype and chemical profile could lay the groundwork for their potential use in taxonomic and pharmaceutical purposes. This is consistent with the current investigation, which found alkaloids, flavonoids and phenolic substances in nine *Medicago* plant samples.

M. lupulina and *M. radiata* had the highest rutin concentrations in this investigation. Other research shows that it includes anti-inflammatory (R)-rutin and caffeine, which are utilised in medicine (14). This is also supported by

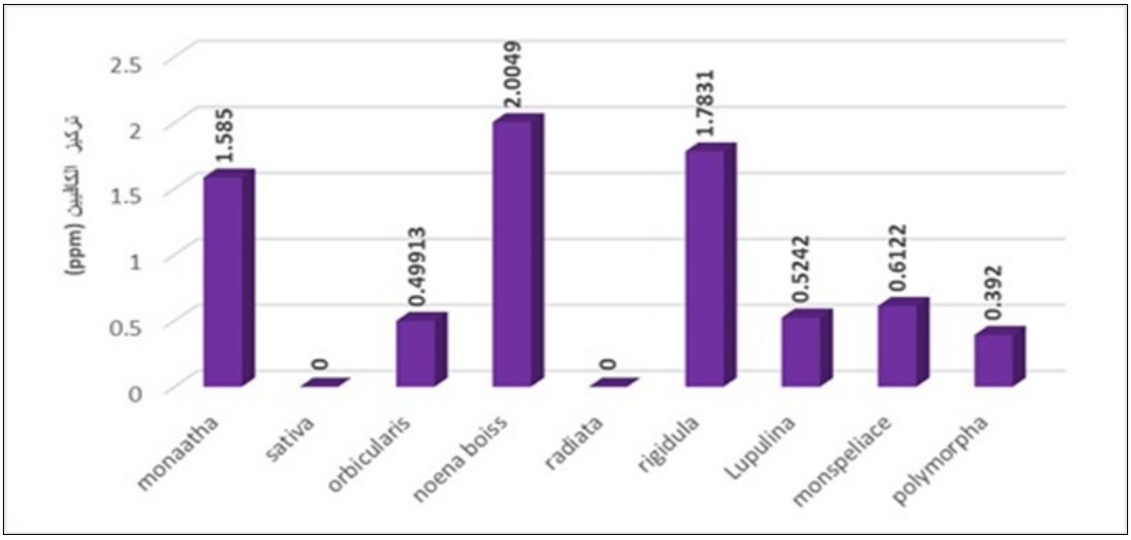

Fig. 5. Comparison of the concentrations of alkaloids in nine plants extracted with ethyl acetate, belonging to the single genus *Medicago* of the Fabaceae family, using HPLC technology.

the phenolic and flavonoid content of two *Medicago* species, *M. rigidula* and *M. sativa*, which contain four of each. Quercetin was found in *M. radiata* plant seeds that were acquired from Mount Azmar and had a high concentration of flavonoids and phenolic compounds (15). This finding was consistent with the current investigation and could lead to regional variance. Alfalfa in Turkey showed that its extract's capacity to scavenge free radicals and inhibit enzyme activity could help create pharmaceutical formulations to treat a variety of chronic diseases linked to oxidative stress, diabetes mellitus linked to α-glucosidase and brain degenerative diseases linked to cholinesterase inhibition (16).

The antioxidant potential of the seeds of fifteen different alfalfa types grown in Turkey was compared in order to identify variations among the kinds. Alfalfa seed methanol extracts were tested for ferric-reducing antioxidant power (FRAP), DPPH• and ABTS•+ scavenging activities, as well as total phenolic (TP) content. The extract's TP concentrations ranged from 37.03 to 54.04 mg GAE/g. The FRAP changed from 389.90 to 791.02 µmol Fe^{2+}/g extract, whereas the ABTS test results varied from 0.100 to 0.158 mmol Trolox/g extract. According to the results, there were significant differences ($p < 0.01$) in the studied features between the alfalfa cultivars (17). Research has demonstrated that the phenological traits of nine *Medicago* species from Portugal and suggested that the differences in phenological indicators may be due to the variability in phenolic compounds, including isoflavones (18). High isoflavone species showed lengthy elongation and pre-flowering periods. They noted that temperature, light and dryness may affect *Medicago* specie's bioactive component content. Genetic factors were more important, they believed. The TPC and TFC in the aerial sections of the examined samples varied more due to plant genotype than environmental conditions.

Conclusion

This study found that nine plant samples from different locations of Iraq have identical phenolic and alkaloid contents, indicating that they belong to the same genus. Rutin is found in all plant species in high amounts relative to other phenolic and flavonoid chemicals, indicating that they are from the same genus. The current study showed the significant impact of these plants due to their antioxidant, anti-inflammatory and antibacterial properties, but it may take further research to determine where these compounds are concentrated in the plants (roots, stems, leaves, fruits).

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Authors' contributions

OAA and RHA contributed to the overall supervision of the work and provided critical revisions to the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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