Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2026; 39(3)

A Comparative Phytochemical Profiling of Phenolics, Flavonoids and Alkaloids in Alfalfa Cultivars Grown in Iraq

Izdehar Yahia Obaid[1*], **and Bushra M. J. Alwash** [1]
[1,2]Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding Author

Received: 26/October/2025
Accepted: 08/March/2026
Published: 20/July/2026
doi.org/10.30526/39.3.4313

Abstract

Natural substances obtained from medicinal plants have long served as valuable sources of therapeutic agents because they contain important secondary metabolites. This study aimed to identify the qualitative and quantitative content of some active secondary metabolites (SMs) in the aerial parts of three alfalfa (*Medicago sativa* L.) cultivars (local cultivar LC, Green Gold cultivar GC, and Victoria cultivar VC) grown in Iraq. This was achieved by detecting phenols, flavonoids, and alkaloids in the crude extract and determining their qualitative and quantitative levels using colorimetric methods and HPLC analysis. The results showed a clear difference among the three studied cultivars in both the quantity and quality of the studied SMs. The VC cultivar stood out with the highest content of phenolic compounds (295.8) and alkaloids (4.11), surpassing the other two cultivars. In comparison, GC cultivar recorded the highest flavonoid content (219.09), and its phenolic concentration (285.61) exceeded that of the LC. Meanwhile, LC surpassed GC in alkaloid content, registering a value of (3.70). These differences may be influenced by soil conditions, environmental factors, and genetic diversity among the cultivars in regulating the biosynthetic pathways of secondary metabolites.

Keywords: *Medicago.sativa*, phenolic compounds, HPLC, cultivar.

1. Introduction

Natural substances obtained from medicinal plants have long served as valuable sources of therapeutic agents because they contain organic compounds that are produced in small amounts by plants, known as secondary metabolites (SMs). These natural products are not essential for plant growth and development but contribute to diverse biological processes and play an essential function in mediating plant association with the surrounding environment, especially under biotic and abiotic stress conditions[1, 2]. These biologically active molecules represent the foundation for numerous therapeutic drugs used today[3]. Many herbal treatments are considered relatively safe when used appropriately, easy to store, and often more effective, with minimal side effects compared to chemical-based treatments, and they have been used to treat various illnesses since ancient times [4, 5]. The *Medicago* genus, a member of the *Fabaceae* family, is widely distributed around the world, consisting of approximately 83 species; about two-thirds of them are annuals, while the others are perennials [3, 6]. Among these, *Medicago sativa* (commonly known as alfalfa, or *jett* in Iraq) is one of the most important perennial herbaceous plants belonging to the Fabaceae family[7, 8]. Studies have shown that alfalfa has antimicrobial, anti-inflammatory, anticancer, and antioxidant activities. Additionally, it may help lower cholesterol levels and contribute to neuroprotection, antiulcer effects, estrogenic activity, and the treatment of atherosclerosis. In recent years, alfalfa has found a place in human diets beyond medicinal use [9]. And it is used in the treatment of various health disorders due to its rich content of secondary metabolites, in addition to containing many vitamins and minerals [3, 10]. *Medicago sativa* (*M. sativa*) is considered an important source of alkaloids, phenols, and flavonoids [11]. Several types of phenolic

compounds have been found in *M. sativa*, especially phenolics and flavonoids, which are among the most important secondary metabolites[12] playing a key role in disease prevention owing to their potent anti-inflammatory and antioxidant activities [13, 14]. Alkaloids are one of the secondary metabolites that possess a nitrogen atom within their molecular structure, primarily located in plants. They represent one of the largest groups of plant-derived active compounds [15, 16]. Because of the presence of these important SMs in *M. sativa*, it has come to possess numerous therapeutic properties. This plant has many cultivars, including cultivated, domesticated, and hybrid ones (cultivars). Although many studies focused on the chemical composition of *M. sativa*, there is still a lack of local research comparing the quantity and quality of secondary metabolites, especially alkaloids, between different *M. sativa* cultivars grown in Iraq. This raises the question of whether secondary metabolite production is affected by cultivar variation within the same species. For this reason, this study aimed to extract and isolate flavonoids, phenolic compounds, and alkaloids, as well as to determine and compare their concentrations in the aerial parts of three *M. sativa* cultivars cultivated in Iraq.

1. Materials and Methods

2.1 Collection and identification of plant material

The seeds of three *Medicago sativa* cultivars, including the local cultivar (LC), Green gold cultivar (GC), and Victoria cultivar (VC), were approved by the seed certification department at the Iraqi ministry of agriculture. They were cultivated in October in Baghdad, and the plants were collected at the flowering stage in April. The plant materials were washed and allowed to air-dry under shade, and then ground with an electric blender.

2.2 Extraction and fractionation of some bioactive compounds

Shade-dried ground material of *M. sativa* cultivars was extracted according to the scheme shown in **Figure 1**, [17, 18].

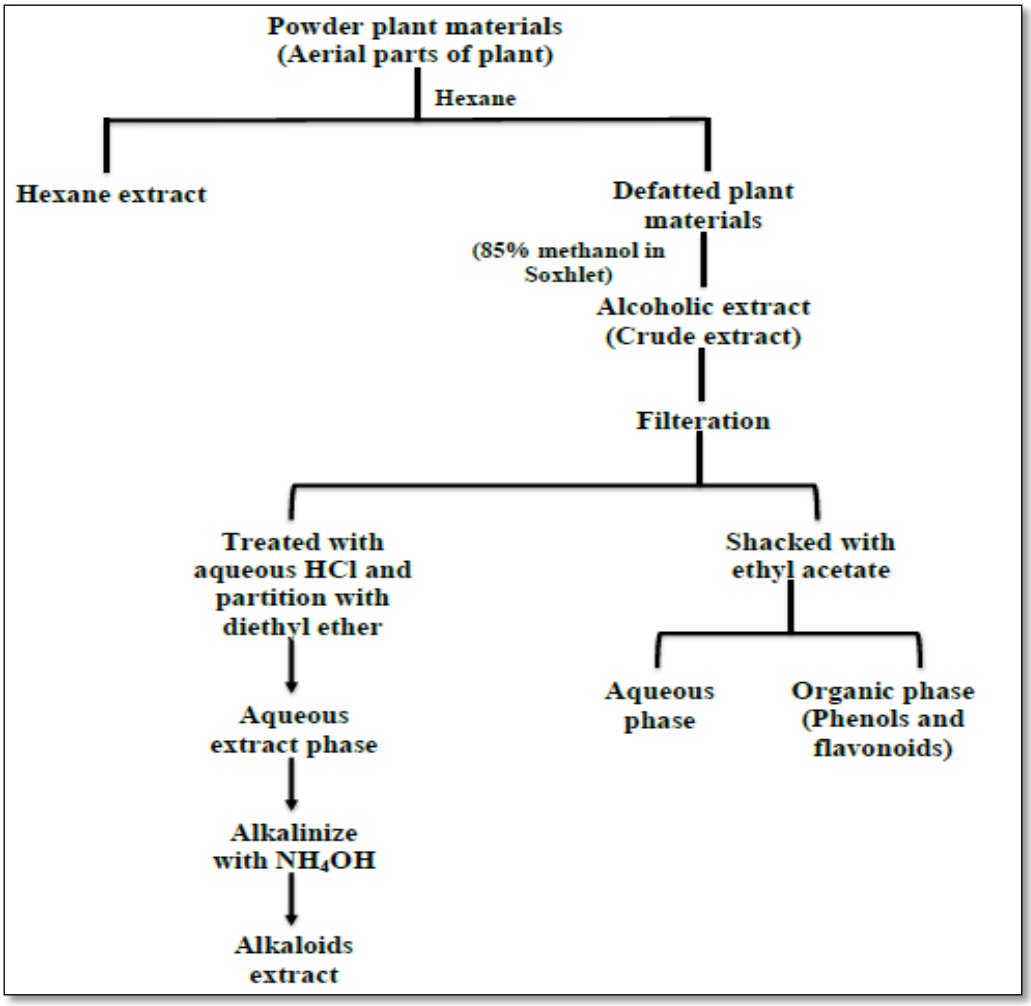

Figure1. Extraction and separation of alkaloids and phenolic compounds

1.3 Phytochemical screening

The extracts obtained from the above procedures were subjected to chemical tests to detect bioactive constituents following well-recognized procedures for detecting bioactive constituents [19, 20].

1.4 Test for Phenolic Compounds

Ten milligrams of each extract were dissolved in 10 ml of distilled water and subsequently filtered, then 1 ml of 5% w/v $FeCl_3$ solution was added to the filtrate solution. A deep green or deep blue coloration indicates the presence of phenolic compounds.

1.5 Test for Flavonoid compounds

1ml of 10% lead acetate solution was carefully mixed with 5 ml of the methanolic extract. The appearance of a yellow color indicates a positive reaction for flavonoids, showing the existence of flavonoid compounds in the sample.

2.6 Test for Alkaloid compounds

About 10 ml of each methanolic extract were blended with 5 ml of 1% HCL and heated in a steam bath with continuous stirring. Mayer's reagent (which was prepared by dissolving 1.35 g of mercuric chloride ($HgCl_2$) in 60 ml of water and 5 g of potassium iodide (KI) in 10 ml of water) and Wagner's reagent (containing 1.27 g of I_2 and 2 g of KI dissolved in 100 ml of distilled water) were then introduced into the reaction mixture. A white precipitate was formed upon treatment with Mayer's reagent and a reddish-brown precipitate after the addition of Wagner's reagent was considered indicative of the presence of alkaloids in the extract.

2.7 Estimation of total phenolic compounds, flavonoids, and alkaloids

2.7.1 Phenolic content

Total phenolic concentration of the methanolic extract was quantified using the Folin-Ciocalteu colorimetric method. In this method, 100 µl of the extract was mixed with 500 µl of the Folin-Ciocalteu reagent (Merck, Germany) and 1.5 ml of 20% sodium carbonate solution. The mixture was vortexed thoroughly, and the volume was adjusted to 10 ml distilled water. After a 2 h reaction, the absorbance was measured at 765 nm, and the phenolic concentrations were calculated by a gallic acid (Sigma-Aldrich, Germany) calibration curve. Results were expressed as mg GAE per gram of dry weight [21].

2.7.2 Flavonoid content

Total flavonoid concentration in the crude extract was quantified by using the aluminum chloride colorimetric assay. Briefly, 50 µL of the crude extract (1 mg/mL ethanol) was diluted to 1 mL with methanol, followed by the addition of 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution, After 5 min of incubation, 0.3 mL of 10% $AlCl_3$ solution was introduced, and the mixture was kept for 6 min. Subsequently, 2 mL of 1 mol/L NaOH solution was added, and the final volume was adjusted to 10 mL with double-distilled water. The reaction mixture was allowed to stand for 15 min, and the absorbance was recorded at 510 nm. The flavonoid concentration was determined using a rutin calibration curve, and results were expressed as milligrams of rutin equivalent (mg RE) per gram of dry weight [22].

2.7.3 Alkaloid content

Accurately measured aliquots of standard solution of atropine (0.4, 0.6, 0.8, and 1.0) were put into separatory funnels. To each funnel 5 ml of phosphate buffer (pH 4.7) and 5 ml of bromocresol green (BCG) solution were added. The resulting mixtures were extracted with 1, 2, 3, and 4 ml of chloroform, respectively, through vigorous shaking. The chloroform layers were collected in a 10 ml volumetric flask and diluted to adjust the solution with chloroform. The absorbance of the resulting chloroform complexes was measured at 470 nm using a UV spectrophotometer (SHIMADZU UV-1800) against a reagent blank prepared in the same manner without the addition of atropine [23].

2.8 Identification of bioactive constituents

HPLC was utilized for the qualitative and quantitative determination of phenols, flavonoids, and alkaloids isolated from the three cultivars of M. sativa. The analysis was performed using a reverse-phase C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase

consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). A gradient elution program was applied as follows: 0–5 min, 10% B; 5–20 min, 10–60% B; 20–30 min, 60–90% B; followed by re-equilibration to initial conditions.
The flow rate was maintained at 1.0 mL/min, the column temperature was set at 30°C, and the injection volume was 20 µL. Detection was carried out using a UV–Vis detector at 280 nm for phenolic compounds and alkaloids and 360 nm for flavonoids.
Identification of compounds was achieved by comparing retention times with authentic standards, while quantification was based on calibration curves constructed from standard solutions at different concentrations.
The percentage of the compounds found in the plant material was calculated using the following equation:
Percentage of compound in the plant =
(AUC of plant sample / AUC of the standard) × Conc. St × DF × 100 / Weight of dry plant material used for extraction.
Where:
DF= Dilution factor.
AUC = Area Under Curve.
Conc. St. = Concentration of standard utilized in HPLC.

3. Results

The qualitative chemical screening of the crude extract is shown in **Table 1**. The results indicate the presence of phenols, flavonoids, and alkaloids in all M. sativa cultivars. The LC cultivar exhibited a stronger positive reaction in the flavonoid test compared to the other cultivars.

Table 1. Phytochemical screening of some active compounds in different cultivars of *Medicago sativa*.

Compound / Cultivar	Phenolic compounds	Flavonoids	Alkaloids
LC	+	+	++
GC	++	+++	+
VC	+++	++	+++

+ represent presence of phytochemical, ++ moderate presence, +++ strongly presence.

3.1 Estimation of total phenolic compounds, flavonoids, and alkaloids

The quantitative contents of total phenols, flavonoids, and alkaloids in *M. sativa* cultivars are presented in Table 2. The VC cultivar recorded the highest phenolic content (295.8 mg/100 g); while the LC cultivar recorded the lowest (269.08 mg/100 g), the highest flavonoids content was in the GC cultivar (219.09 mg/100 g), while the LC cultivar recorded the lowest (197.0 mg/100 g), and the last total alkaloids content was highest in the VC cultivar (4.11%), while the GC cultivar recorded the lowest (3.02%) **(Table 2**).

Table 2. Total phenols , flavonoids and alkaloids.

No	Active compound name	LC	GC	VC
1	Total phenolics content (mg / 100 gm)	269.08	285.61	295.8
2	Total flavonoids content (mg / 100 gm)	197.00	219.09	205.9
3	Total alkaloids content %	3.70	3.02	4.11

3.2 Identification of bioactive constituents

According to the HPLC analysis, the VC cultivar contained the highest levels of phenolic acids compared with the GC and LC cultivars. The differences in phenolic acid profiles were observed among the cultivars **Table 3**.

Table 3. Quantitative analysis of phenolic acids in *M.sativa* cultivars.

Active compound name	LC (ppm)	GC (ppm)	VC (ppm)
p-coumaric acid	74.6	80.6	84.7
Ferulic acid	77.4	81.6	84.1
Gallic acid	145.0	152.6	152.0
Caffeic acid	52.3	58.9	59.7
Chlorogenic acid	41.6	48.7	54.9

As shown in **Table 3**, the VC cultivar exhibited the highest concentrations of most phenolic acids. Gallic acid was the most abundant phenolic acid in the GC cultivar. In contrast, the LC cultivar recorded the lowest levels of all detected phenolic acids. The HPLC analysis confirmed the presence of several flavonoids, whose concentrations varied among cultivars, as shown in **Table 4** and **Figures 2-4.**

Table 4. Quantitative Analysis of flavonoids in *M.sativa* Cultivars.

Active compound name	LC (ppm)	GC (ppm)	VC (ppm)
Catechine (ppm)	120.6	130.6	141.6
Rutin (ppm)	98.0	114.5	112.6
Epicatechin (ppm)	62.5	77.4	77.9
Qurcetine (ppm)	63.9	70.6	71.1
Apigenin (ppm)	71.6	80.6	82.4

As noted in the table above, the GC cultivar recorded the highest level of rutin, whereas the VC cultivar exhibited the highest concentrations of the remaining flavonoid.

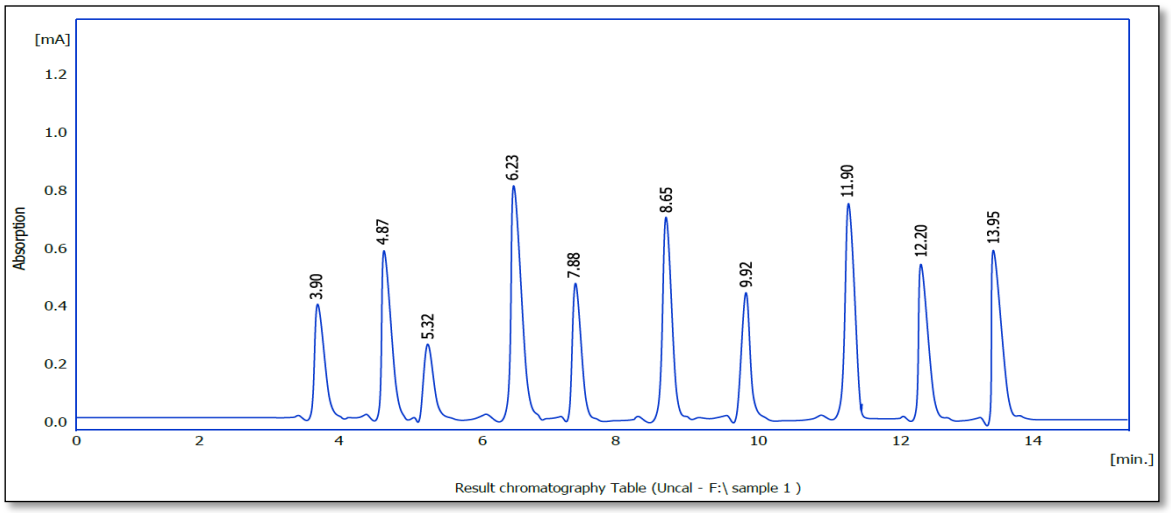

Figure 2. HPLC chromatogram of Phenolics and Flavonoids in LC sample. Peaks correspond to: epicatechin (3.90 min), ferulic acid (4.87 min), chlorogenic acid (5.32 min), gallic acid (6.23 min), caffeic acid (7.88 min), catechin (8.65 min), quercetin (9.92 min), rutin (11.90 min), apigenin (12.20 min), and *p*-coumaric acid (13.95 min).

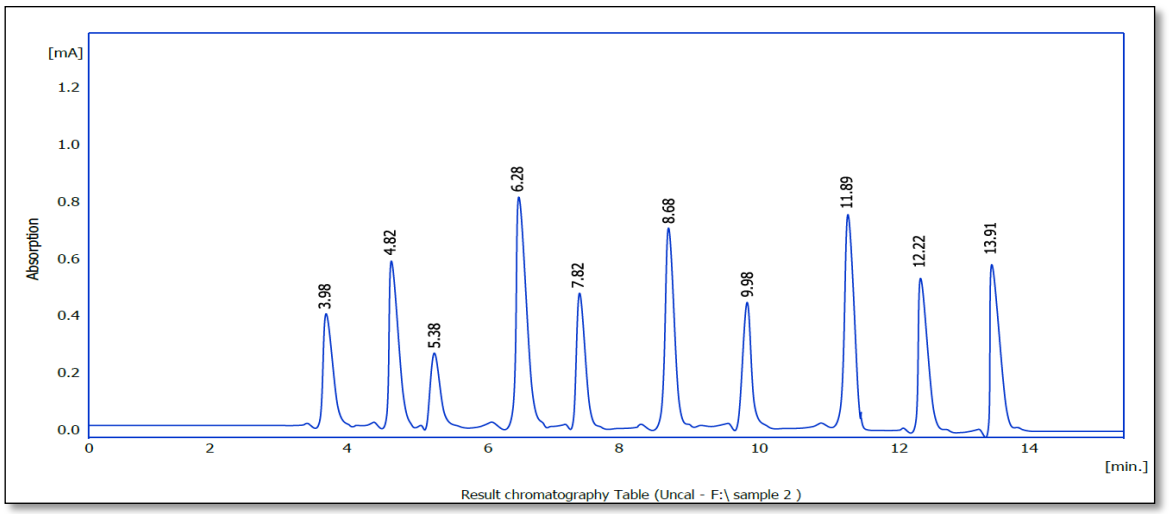

Figure 3. HPLC Chromatogram of Phenolic and Flavonoid Compounds in GC sample. Peaks correspond to: epicatechin (3.98 min), ferulic acid (4.82 min), chlorogenic acid (5.38 min), gallic acid (6.28 min), caffeic acid (7.82 min), catechin (8.68 min), quercetin (9.98 min), rutin (11.89 min), apigenin (12.22 min), and *p*-coumaric acid (13.91 min).

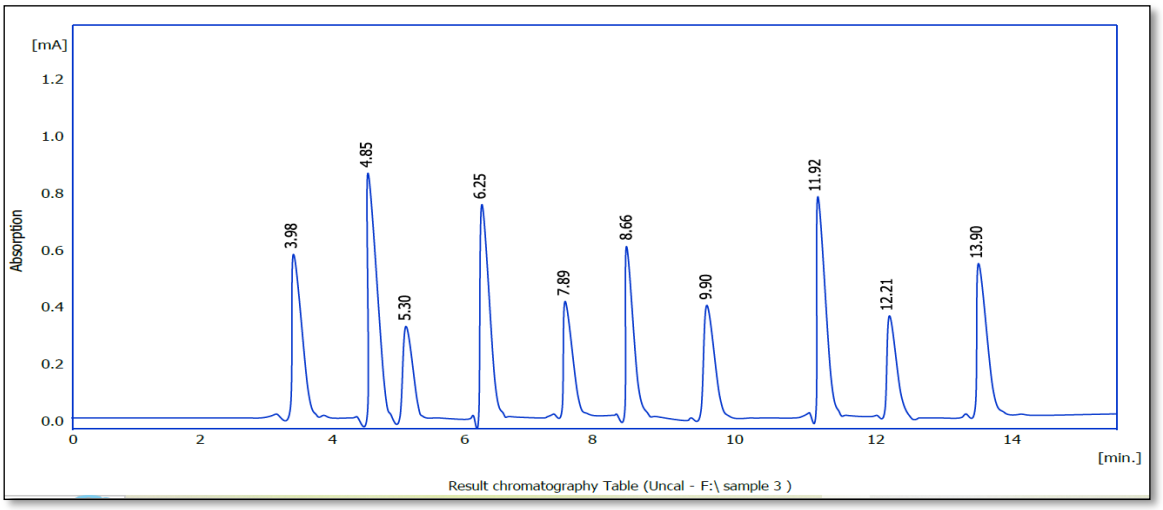

Figure 4. HPLC Chromatogram of Phenolics and Flavonoids in VC sample. Peaks correspond to: epicatechin (3.98 min), ferulic acid (4.85 min), chlorogenic acid (5.30 min), gallic acid (6.25 min), caffeic acid (7.89 min), catechin (8.66 min), quercetin (9.90 min), rutin (11.92 min), apigenin (12.21 min), and p-coumaric acid (13.90 min).

Regarding alkaloids, the HPLC analysis identified the presence of several compounds, including scopolamine, isoquinoline, lupanine, stachydrine, sparteine, and homostachydrine, as shown in **Table 5** and **Figures 5-7.**

Table 5. Quantitative Analysis of Alkaloid Compounds in *M.sativa* Cultivars.

Active compound name	**LC (PPM)**	**GC (PPM)**	**VC (PPM)**
Scopolamine	74.08	66.80	92.8
Isoquinoline	32.62	27.44	42.2
Lupanine	25.90	29.08	33.52
Stachydrine	40.25	44.08	51.42
Sparteine	18.98	22.65	26.98
homoStachydrine	16.69	18.98	21.09

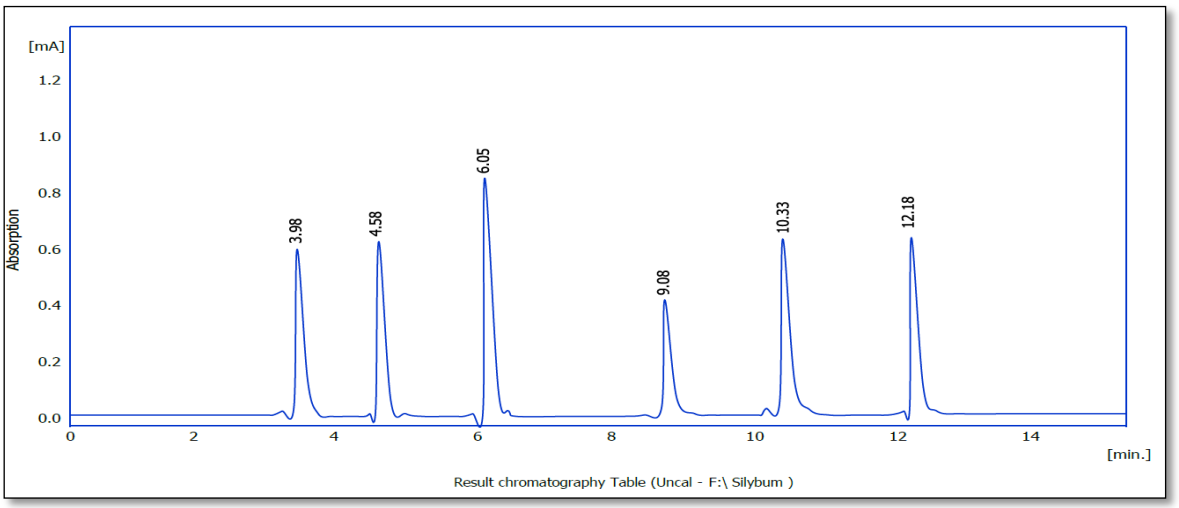

Figure 5. HPLC Chromatogram of alkaloid Compounds in LC sample. The peaks correspond to the following compounds and retention times: Lupanine (3.98 min), Stachydrine (4.58 min), Sparteine (6.05 min), Homostachydrine (9.08 min), Isoquinoline (10.33 min), and Scopolamine (12.18 min).

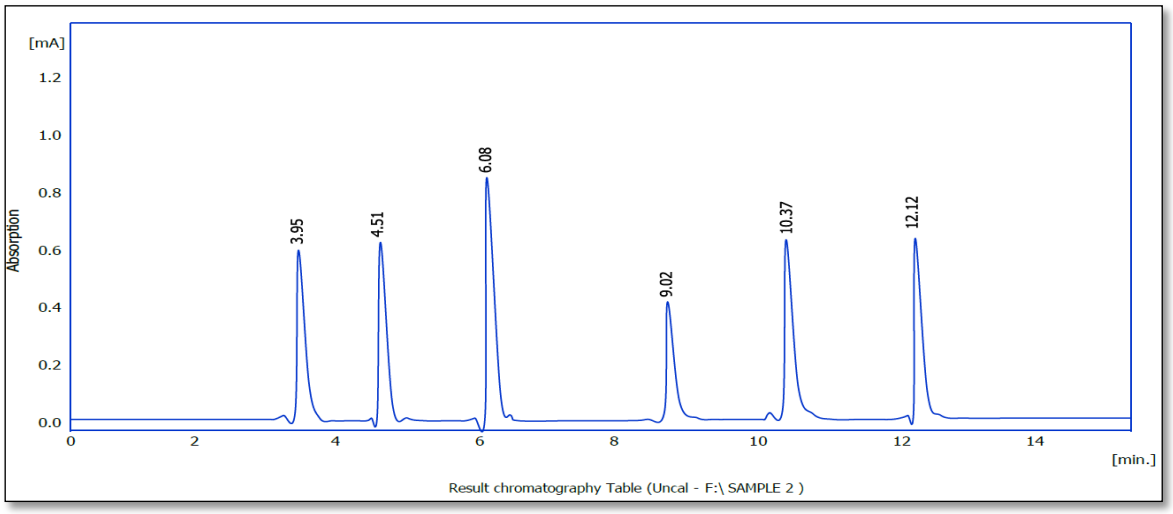

Figure 6. HPLC Chromatogram of alkaloids in GC sample. The peaks correspond to the following compounds and retention times: Lupanine (3.95 min), Stachydrine (4.51 min), Sparteine (6.08 min), Homostachydrine (9.02 min), Isoquinoline (10.37 min), and Scopolamine (12.12 min).

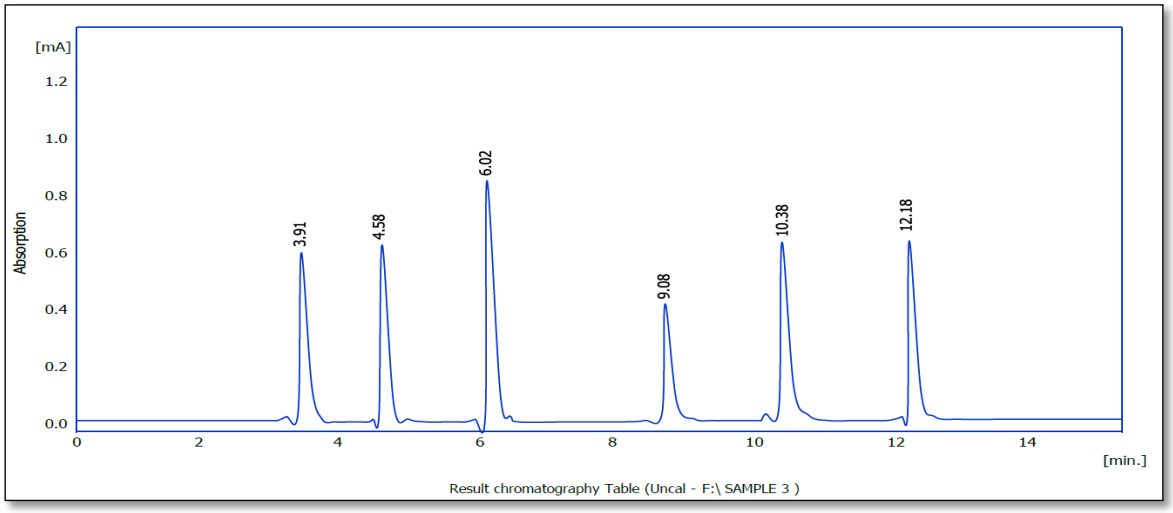

Figure 7. HPLC Chromatogram of alkaloid Compounds in VC sample. The peaks correspond to the following compounds and retention times: Lupanine (3.91 min), Stachydrine (4.58 min), Sparteine (6.02 min), Homostachydrine (9.08 min), Isoquinoline (10.38 min), and Scopolamine (12.18 min).

4. Discussion

Plant secondary metabolites include phenolics, flavonoids, and alkaloids, which are synthesized through internal biosynthetic pathways in plants [24, 25].

In this study, quantitative analysis using HPLC, as previously revealed, showed notable differences in the total content of phenolics, flavonoids, alkaloids, and individual secondary metabolites among three *Medicago sativa* cultivars grown in Iraq. The obtained contents align with previous research indicating that *M. sativa* Cultivars differ genetically in their production of secondary metabolites. 26. The biosynthesis pathways of phenolic and flavonoid compounds are primarily governed by the phenylpropanoid pathway, where key enzymes such as phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), and flavonol synthase essential [27]. Genetic variation affecting the expression or activity of these enzymes likely contributes to the differential accumulation of these compounds, even when plants are grown under similar environmental conditions [28].

In addition to differences in total content, notable ones were also observed in specific phenolic acids (e.g., p-coumaric acid, ferulic acid, gallic acid, caffeic acid, and chlorogenic acid) and flavonoids (e.g., rutin, catechin, epicatechin, quercetin, and apigenin). These differences may reflect how each cultivar regulates metabolic flux through the shikimate and phenylpropanoid pathways [29]. Also, the bioactive compounds vary in quantities and qualities depending on the cultivar and conditions of culture, soil, and environmental factors [6].

Similarly, the levels of alkaloids, including Scopolamine, Isoquinoline lupanine, stachydrine, sparteine, and homostachydrine also varied among cultivars. Scopolamine and Isoquinoline were detected in all three *M. sativa* cultivars, suggesting their consistent detection under the applied analytical conditions. However, as these compounds are not commonly reported in *M. sativa*, further verification using more selective techniques such as LC–MS is recommended. The slight variations among cultivars may be attributed to differences in gene regulation or how each cultivar responds to internal and external stress signals. Although the environmental conditions were generally controlled, subtle factors such as light intensity, soil nutrient availability, and interactions with soil microbes can still influence the production of secondary metabolites. Ultimately, the interplay between genetics and the environment even under uniform growing conditions contributes to the observed differences. Minor gene–environment interactions can influence gene expression and secondary metabolite biosynthesis.

5. Conclusion

This study demonstrated significant differences among *M. sativa* cultivars in their phenolic, flavonoid, and alkaloid contents, indicating that each cultivar possesses a distinct phytochemical profile. The differences could possibly be related to genetic background or environmental conditions, although further studies are needed to confirm these assumptions. To the best of our knowledge, this work represents the first comparative investigation in Iraq involving these cultivars, particularly VC and GC in comparison with LC.

Acknowledgment

The authors express their gratitude to the heads of the Chemistry and Physics Department at the University of Baghdad's College of Sciences for Women for providing labs for conducting scientific research and for helping them finish this work.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

Not applicable.

Ethical Clearance

The authors declare that neither humans nor animals have been used in any of the investigations described in this work.

References

1. Ramaroson ML, Koutouan C, Helesbeux JJ, Le Clerc V, Hamama L, Geoffriau E, Briard M. Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. Molecules. 2022;27(23):8371. https://doi.org/10.3390/molecules27238371
2. Al-Rawi A, Hassan FM, Alwash BM. In vitro stimulation of ergosterol from *Coelastrella terrestris* using squalene and antioxidant effect. Syst Rev Pharm. 2020;11(11):364–369.
3. Al-Dabbagh F. Five isolated phytochemicals from Iraqi *Medicago sativa* with comparative RP-HPLC study. Egypt J Health Care. 2024;15(2):1029–1037. https://doi.org/10.21608/ejhc.2024.359308
4. Salm S, Rutz J, Blaheta RA, Bachmeier BE. Clinical benefits of herbal medicines for non-life-threatening ailments. Front Pharmacol. 2023;14:1234701. https://doi.org/10.3389/fphar.2023.1234701
5. Bhutia KN, Basnett DK, Bhattarai A, Bhutia S. Herbal products sold in Sikkim Himalaya region, India: a mini survey. Glob J Med Pharm Biomed Update. 2023;18:14. https://doi.org/10.25259/GJMPBU_43_2022
6. Djedid IK, Terzaghi M, Brundu G, Cicatelli A, Laouar M, Guarino F, Castiglione S. Genetic diversity and differentiation of eleven Medicago species from Campania region revealed by nuclear and chloroplast microsatellites markers. Genes (Basel). 2022;13(1):97. https://doi.org/10.3390/genes13010097

7. Rouki V, Boskabady MH, Marefati N, Sotoudeh R, Gholamnezhad Z. Therapeutic effects of *Medicago sativa* against cyclophosphamide toxicity in rats. Avicenna J Phytomed. 2024;14(1):112–125. https://doi.org/10.22038/AJP.2023.22911
8. Bakhtiar Z, Hassandokht M, Fozi V, Mirjalili MH. Agro-morphological traits, essential oils, phytochemicals and bioactivity of *Medicago* species. Chem Biol Technol Agric. 2025;12(1):25. https://doi.org/10.1186/s40538-025-00740-y
9. Al-Snafi AE, Khadem HS, Al-Saedy HA, Alqahtani AM, El-Saber G. A review on *Medicago sativa*: a potential medicinal plant. Int J Biol Pharm Sci Arch. 2021;1(2):22–33. https://doi.org/10.30574/ijbpsa.2021.1.2.0302
10. Raeeszadeh M, Moradi M, Ayar P, Akbari A. The antioxidant properties of *Medicago sativa* L. (alfalfa) and its potential therapeutic effects in preventing nicotine-induced liver damage. Oxid Med Cell Longev. 2022;2022:2691577. https://doi.org/10.1155/2022/2691577
11. Vahidipour HR, Cheniany M, Lahouti M, Ganjeali A, Matin MM. Correlation of secondary metabolites with organ and harvest time in alfalfa. *Int J Adv Biol Biomed Res*. 2024;12:28–43.
12. Horvat D, Viljevac Vuletić M, Andrić L, Baličević R, Kovačević Babić M, Tucak M. Forage quality, phenolics and antioxidant activity in alfalfa. Plants (Basel). 2022;11(20):2735. https://doi.org/10.3390/plants11202735
13. Xie J, Gao Y, Zhang Y, Wang L, Li H. Phenolic acids from medicinal and edible plants. *Food Funct*. 2024;15(4):1967–1985. https://doi.org/10.1039/D4FO00044F
14. Iqbal Y, Ponnampalam EN, Suleria HAR, Cottrell JJ, Dunshea FR. LC-ESI/QTOF-MS profiling of chicory and lucerne polyphenols. Antioxidants (Basel). 2021;10(6):932. https://doi.org/10.3390/antiox10060932
15. Ferrante C, Menghini L. *Alkaloids: Botanical and Pharmacological Overview*. Singapore: Bentham Science Publishers; 2023. https://doi.org/10.2174/9789815123678123030004
16. Wilson DN. Use of alkaloids in plant protection. In: Ramawat KG, Mérillon JM, editors. Alkaloids: Chemistry and Biology. Berlin: De Gruyter; 2022. p. 337–352. https://doi.org/10.1515/9783110771558-013
17. Lafta AA, Hamad MN. Isolation of alkaloids from *Papaver rhoeas* in Iraq. *Iraq J Pharm Sci*. 2021;30(2):78–85. https://doi.org/10.31351/vol30iss2pp78-85
18. Salman ZO, Alwash BMJ, Kadhim EJ. Isolation and identification of flavonoid compounds from *Euphorbia milii* cultivated in Iraq and evaluation of its genetic effects on two cancer cell lines. Baghdad Sci J. 2024;21(9):2805–2819. https://doi.org/10.21123/bsj.2024.9276
19. Harborne JB. Phytochemical Methods. 1st ed. London: Chapman and Hall; 1973. p. 278.
20. Kokate CK, Gokhale SB, Purohit AP. Pharmacognosy. 29th ed. Pune: Nirali Prakashan; 2009. p. 635.
21. Kingne F, Tonfack DF, Tsafack DH, Karuna MSL, Womeni HM. Phenolic content and antioxidant activity of mango and avocado leaves. Int J Phytomed. 2018;10(4):181–190. https://doi.org/10.5138/097501852289
22. Habibatti S, Zohra AF, Khalid H, Anwar S, Mansi I, Awadh Ali NA. Antioxidant and anti-inflammatory activity of Onopordum acanthium. Int J Phytomed. 2017;9:92–100.
23. Ajanal M, Gundkalle MB, Nayak SU. Total alkaloid estimation by UV spectroscopy. *Anc Sci Life*. 2012;31(4):198–201. https://doi.org/10.4103/0257-7941.107361
24. Ibragić S, Dahija S, Karalija E. Stress-induced metabolite regulation and epigenetic effects. *Epigenomes*. 2025;9(2):10. https://doi.org/10.3390/epigenomes9020010
25. Ramakrishna A, Ravishankar GA. Abiotic stress and secondary metabolites. Plant Signal Behav. 2011;6(11):1720–1731. https://doi.org/10.4161/psb.6.11.17613
26. Cen H, Wang T, Liu H, Wang H, Tian D, Li X, Zhang Y. Overexpression of *MsASMT1* in alfalfa enhances secondary metabolite accumulation. Front Plant Sci. 2020;11:489. https://doi.org/10.3389/fpls.2020.00489
27. Vogt T. Phenylpropanoid biosynthesis. Mol Plant. 2010;3(1):2–20. https://doi.org/10.1093/mp/ssp106
28. Reshi ZA, Mir SR, Dar GH. Secondary metabolite pathways and regulation in plants. Plants (Basel). 2023;12(8):1615.
29. Fraser CM, Chapple C. The phenylpropanoid pathway in Arabidopsis. Arabidopsis Book. 2011;9:e0152. https://doi.org/10.1199/tab.0152
30. Zhao Y, Liu G, Yang F, Liang Y, Gao Q, Xiang C, Li X, Yang R, Zhang G, Jiang H, Yu L, Yang S. Multilayered regulation of secondary metabolism in medicinal plants. Mol Horticult. 2023;3(2):11. https://doi.org/10.1186/s43897-023-00059-y