

Phytochemical and Antioxidant Investigations of *Artemisia vulgaris*: HPLC isolation and Identification of p-Coumaric Acid, structural characterization and GC/MS Analysis of Essential oil compositions

Amani Amer Tawfeeq

Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Mustansiriya University, Baghdad, Iraq.

Corresponding Author email: Dr.amanitawfeeq@uomustansiriyah.edu.iq

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Abstract

Background: *Artemisia vulgaris* (Mugwort) is a traditional medicinal plant rich in volatile constituents and phenolic compounds that contribute to its varied biological activities.

Objectives: Currently, this work investigates the phytochemical isolation, structural identifications, and investigations of essential oil composition and antioxidant potential of Iraqi *Artemisia vulgaris*.

Methods: The aerial parts of *A. vulgaris* were macerated using an Acetone: Water mixture (80:20, v/v). The isolated phenolic acid compound was identified by liquid chromatography and spectroscopy analyses. The essential oils obtained through hydrodistillation were analyzed using gas chromatography. The evaluation and investigation for antioxidant activity were achieved by assaying the radical scavenging corresponding to ascorbic acid as a reference standard.

Results: The chromatographic analysis revealed that the isolated compound matched that of p-coumaric acid. Spectral characteristics, UV and FTIR, further confirmed its structural characteristics as a hydroxycinnamic acid derivative. The GC/MS analysis of extracted volatile oil identified several major constituents, including α -thujene (21.25%), p-cymene (10.72%), γ -terpinene (8.66%), and D-limonene (5.30%). The crude extract revealed concentration-dependent antioxidant activity, reaching 70.7% radical scavenging at 200 μ g/mL. Iraqi *Artemisia vulgaris* represents a valuable source of phenolic and terpenes compositions.

Conclusion: The successful isolation and identification of p-coumaric acid, together with the characterization of its essential oil constituents, contribute to a significant antioxidant activity, supporting the potential pharmaceutical and nutraceutical applications.

Keywords: P-coumaric acid, essential oil, *Artemisia*, GC/MS, HPLC, scavenging activity

1. Introduction

Mugwort, *Artemisia vulgaris*, is a flowering plant within the Asteraceae family. It belongs to the genus *Artemisia*, which includes several mugwort species. However, *Artemisia vulgaris* is the most widely recognized species under this name. Common name: Mugwort, Family: Asteraceae [1]. Mugwort contains the toxic compound santonin, which was once employed as an antihelminthic (anti-parasitic) drug, replaced by newer drugs of a similar kind, with less harmful compounds. Depending on the source of the plant, all significant components may be 1, 8-cineole, camphor, linalool, and thujone. *Artemisia vulgaris*, or mugwort, is a perennial herb applied for medicinal and culinary purposes [2].

Recent scientific research has examined its potential use in contemporary communication areas of medicine, especially in cancer prevention, neuroprotection, and metabolic health. They have broad-spectrum therapeutic capacities, including anti-inflammation, antimicrobial, and antioxidant activities [3]. *Artemisia vulgaris* is a fragrant, herbaceous, perennial plant that reaches a height of up to 1.5 meters (4 ft 11 in). The incredible and sturdy aroma of the *Artemisia* genus is primarily attributed to the high ranges of volatile terpenes that are key components of their essential oils. When growing this plant, it is essential not to forget its precise cultivation wishes. These species are found worldwide, particularly in the temperate regions of Europe, East Asia, the Americas, North Africa, and Australia [4]

1.1 Traditional use

The mugwort plant is thought to have warming and calming properties, which caused its recommendation for treating urological conditions, including dysuria and nephrolithiasis. Additionally, it became used to alleviate symptoms related to digestive problems and to sell essential well-being [5]. In medieval medicine, *Artemisia vulgaris*, regularly known as "mater herbarium" (the mother of herbs), was commonly applied to treat wounds, deal with gout, relieve leg fatigue, and function as a treatment for fevers. It was additionally valued for its potential to improve movement and ease digestive soreness, making it a versatile plant in traditional restoration practices. The plant also became widely used to treat gastrointestinal disorders believed to result from colds [6].

1.2 Phytochemical Characteristics

Artemisia vulgaris consists of many compounds, including sesquiterpenoid lactones, polyphenolics, phenyl propene derivatives, Fraxidin, Scopoletin, hydroxycinnamic acid, caffeoylquinic acid derivatives, sterols, carotenoids, and some glycosides. An incredible characteristic of this species is the presence of sesquiterpenoid lactones, such as Argabin, Santamarin, and Artemisinin. Additionally, the plant is rich in flavonoids, including derivatives of kaempferol and quercetin, which contribute to its medicinal and aromatic properties [7].

The essential oil of *Artemisia vulgaris* is characterized predominantly with a high abundance of monoterpenoids, sesquiterpenes constituents, moterpenoids and sesquiterpenoids and other terpenes [8]. Major volatilized compounds were reported and identified in the aerial parts include epoxide monoterpens, β -thujone, sabinene, and β -caryophyllene oxide, etc. whereas these constituents are largely absent from the alternative parts. The noticeable variation in the chemical profiles of aerial - extracted oils suggests and reflects the metabolic complexity of the species and biosynthetic pathways. Such compositional diversity may contribute to the broad range of biological and pharmacological activities associated with *A. vulgaris* [9].

1.3 Pharmacological activity

The radical molecules, particularly reactive oxygen species (ROS), also formed during normal cell processes, contributing to cellular damage that can result in pathological conditions, including cancer and neurodegenerative diseases. Antioxidants neutralize ROS and prevent cellular damage through donation of electrons, metal ions chelation, and the stimulation of metal-dependent enzymes such as superoxide dismutase (SOD) [10]. Many previous studies have reported significant antioxidant activity of *Artemisia* species due to their richness in polyphenolic, and phenolic constituents [11]. Furthermore, the plant is reported as having a strong biological activity. The antibacterial and

antioxidant properties of *Artemisia vulgaris* methanol extracts were evaluated due to their content of polyphenols and flavonoids.

The essential oil demonstrated significant activity against bacteria, and *Artemisia vulgaris* exhibited antifungal activity [11]. The alcoholic extract of *Artemisia vulgaris* exhibited inhibitory effects on various human cancer cell lines, including MCF-7 (breast), A549 (lung), and HeLa (cervical). Although traditional Chinese medicine (TCM) has used this herb to treat SARS, no formal clinical evidence is currently available regarding its efficacy in the treatment of COVID-19 [12], [13]. This study aimed to isolate phenolic acid compounds with structural identifications and GC/MS analysis for extracted essential oils to identify the components and value of the antioxidant activity of Iraqi *Artemisia vulgaris*.

2. Methods

A. Collection of Plant Materials:

The aerial parts of the plant materials, as seen in Fig. 1, were collected from the Al-Musaib region in December 2024. The collected plants were shade-dried at room temperature for 10 days and stored in a sterile container. Moreover, they were used further for extract preparation and essential oil extraction.



Fig. 1 *Artemisia vulgaris*

B. Extraction of *Artemisia vulgaris*

The air-dried aerial parts of plant material were prepared and powdered. About 150 gm was extracted by maceration using 1200 mL acetone: water (80:20, v/v) for all extraction processes (repeated three times until materials exhausted). The achieved extract was filtered, and the solvent was removed under reduced pressure [14]. The collected extract was dissolved in methanol, filtered through a 0.45 μm membrane filter, and analyzed by HPLC.

C. Hydro Distillation Method: An extraction method

The plant material is censored into small pieces and subjected to steam distillation using a Clevenger apparatus to extract the essential oils. As seen in Fig. 2, fifty grams of the crude material were extracted sequentially with 500 mL of distilled water, ensuring the entire sample was immersed using a Clevenger apparatus for 6 hours. The solvent then evaporated to dryness, which was repeated for the remaining four samples. The essential oil is collected in a tightly sealed, opaque glass bottle. The extracted oil was stored at 4°C until further analysis [15].



Fig. 2 Clevenger apparatus to extract essential oil of *Artemisia vulgaris*

D. GC-MS analysis

The hexane extract and essential oil were obtained from Iraqi *Artemisia vulgaris* and were investigated by gas chromatography-mass spectrometry (GC-MS). The column was Rxi-35Sil MS (diameter, 0.25 mm, length 3.0 m) at a temperature of 320°C. The optimum chromatographic condition of carrier gas was helium, and the column flow was controlled mode with a flow of 1.09 ml/min, 9.6 ml/min, and a split ratio of 5.0. The sampling time was 1.00 min; the column temperature was from 60.0 c to 300.0 c

E. HPLC analysis

The analysis was conducted on a Shimadzu LC-20A system fitted with a reverse-phase C18 column (250 × 4.6 mm, 5 μm). Separation was achieved using an isocratic mobile phase consisting of formic acid (1.5 ml): water (65 ml) and methanol (33.5 ml) . However, the elution is done at a flow rate of 1.0 mL/min. The analysis of extract and isolated compound examined the corresponding reference p-coumaric acid which prepared by dissolving 0.25 mg in 5 ml methanol [16].

F. Spectroscopy analysis

The characteristic features of expected isolated p-coumaric acid in the UV–Vis spectrum for this compound were achieved using a spectrophotometer over the wavelength range of 200–800 nm. The sample was prepared by dissolving it in methanol, and the characteristic absorption maxima were recorded for structural characterization. Moreover, the spectroscopic analysis, FTIR spectroscopy, was controlled in the range of 4000–400 cm⁻¹ using the KBr pellet method to characterize the major functions.

G. Antioxidant Activity

Certainly, the hydro-acetone extract was evaluated for its activity, a DPPH free radical scavenging investigation, following a modified form of the method reported by Kumar antioxidant assay. Concisely, dried extract diluted to 0.5 mL of serially or ascorbic acid standard (12.5–100 μg/mL) was mixed with 3 mL of methanol–DMSO and 0.3 mL of DPPH solution. The reaction samples were incubated at 37°C for 60 min, after which the extinction was measured at 517 nm using a plate reader. The measuring of neutralizing capacity was expressed as percentage inhibition and described by Baliyan % [17]:

$$\% \text{ Inhibition} = 100 \times [(A_0) - A_s / A_0]$$

The negative control: methanol–DMSO and DPPH; Positive control: ascorbic acid

3. Results and Discussion

3.1 Reversed-Phase HPLC

Chromatogram findings proposed that trans-4-hydroxycinnamic acid was detected at 315 nm. The separated compounds on the C18 column were represented by different peaks at a specific retention time for each one. The mobile phase is 100 ml of a mixture: water (65%): formic acid (1.5%): methanol (33.5%) under isocratic conditions. For all the separations were achieved and conducted at a flow rate of 1.0 ml/min, the isolation of the proposed isolated p- coumaric acid was achieved using a fraction collector system integrated with a compact analytical HPLC system. Moreover, the isolated compound was re-analyzed for identification with the same mobile phase. Coincidence of reference trans-4-hydroxycinnamic acid (p- coumaric acid). The analyzed compound revealed a retention time around 20.8 min under analytical HPLC conditions, which, according to that of the standard p- coumaric acid (Rt = 20.00 min), resulted in peaks as seen in Fig. 3. This work was followed by chromatographic re-analyses of the collected fraction, comparable with that of p-coumaric acid reference at RT \approx 9.0 min to confirm the identity of the isolated compound, as seen in Fig. 4.

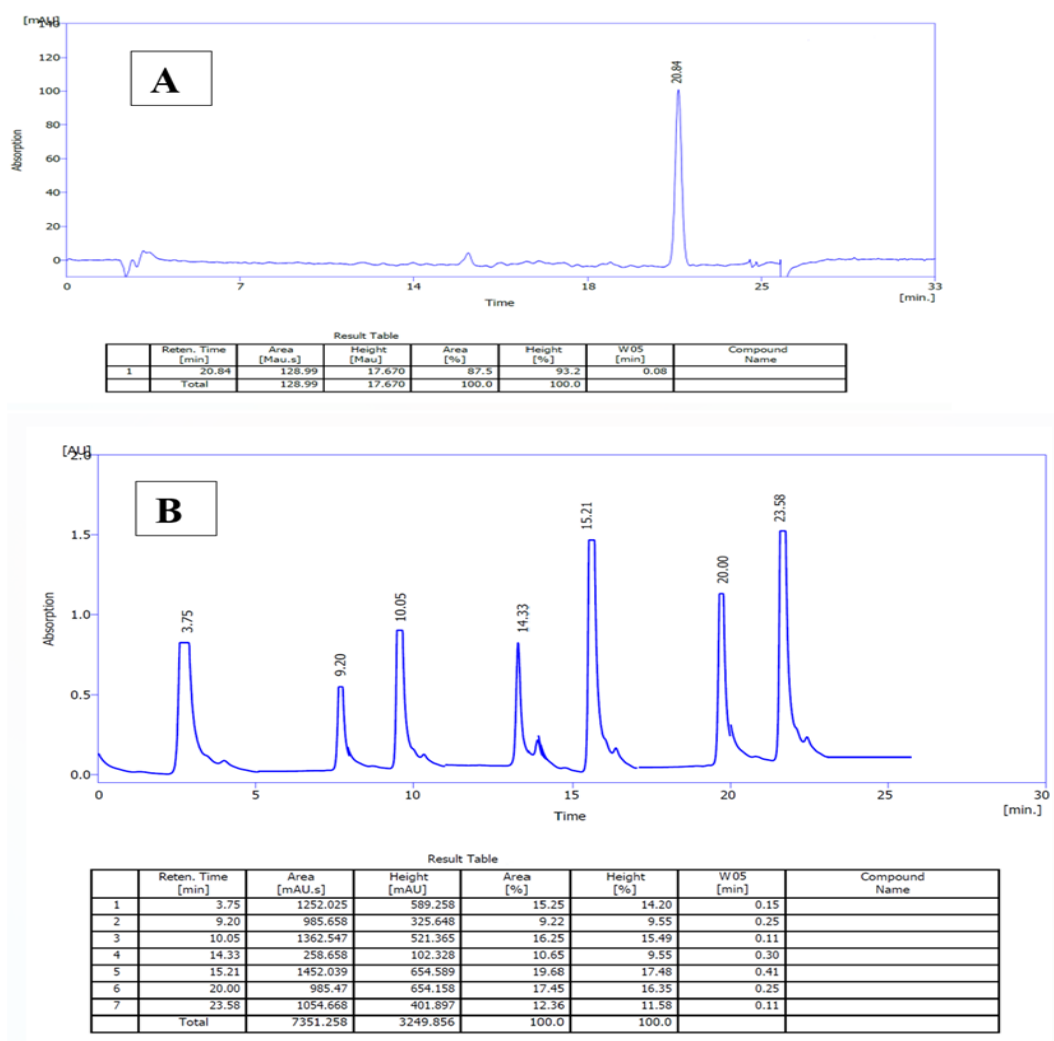


Fig. 3 HPLC chromatogram): A, P-coumaric standard, B; acetone-water cude extract.

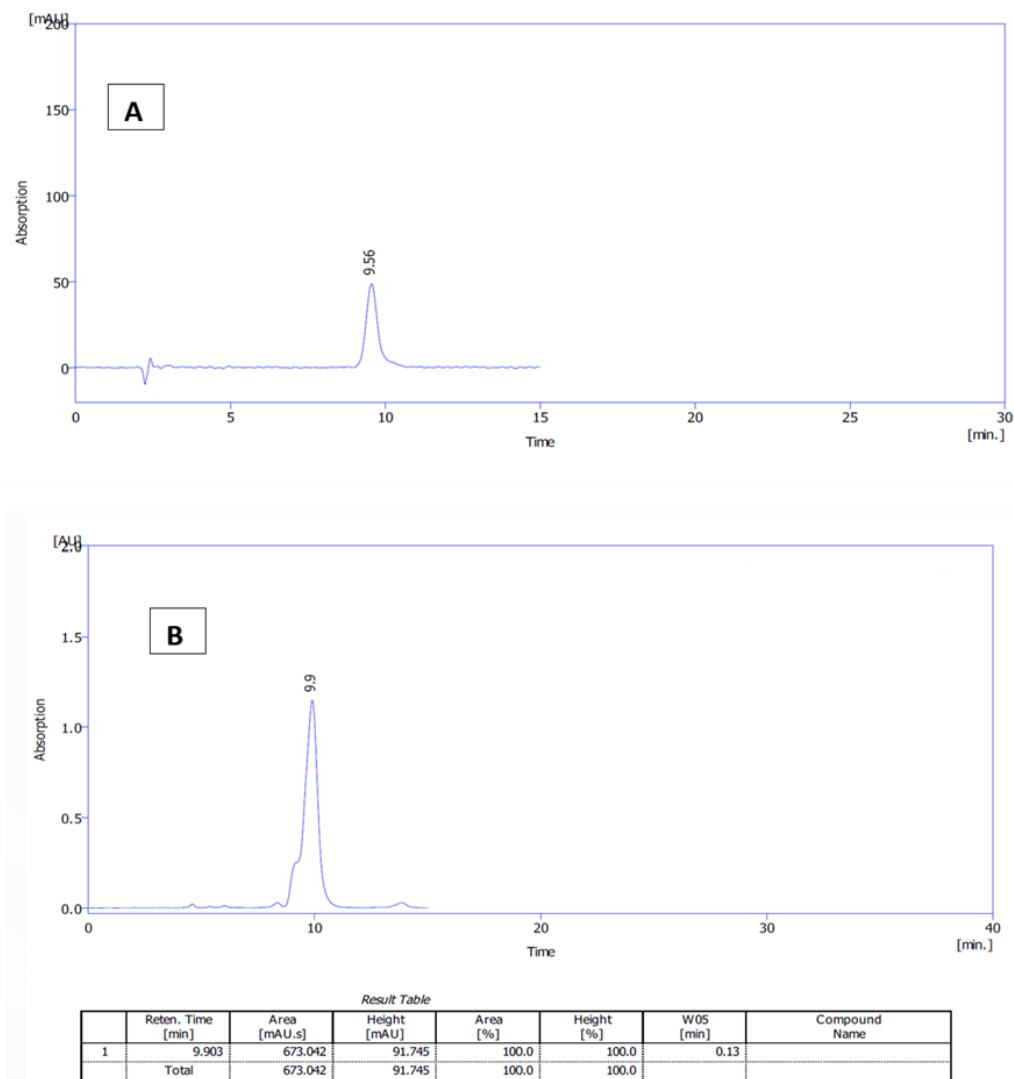


Fig. 4 HPLC Chromatogram: A; Isolated compound, B; P-coumaric standard.

3.2 Spectral Analysis:

Ultraviolet spectrophotometric investigation of the isolated compound exhibits strong peaks around 286 nm to 320 nm related to extended conjugation (aromatic ring), carboxylic acid, and unsaturated bond. The smallest peak was revealed between 220 nm and 233. The isolated compound matched that of standard coumaric acid, as seen in Fig. 5.

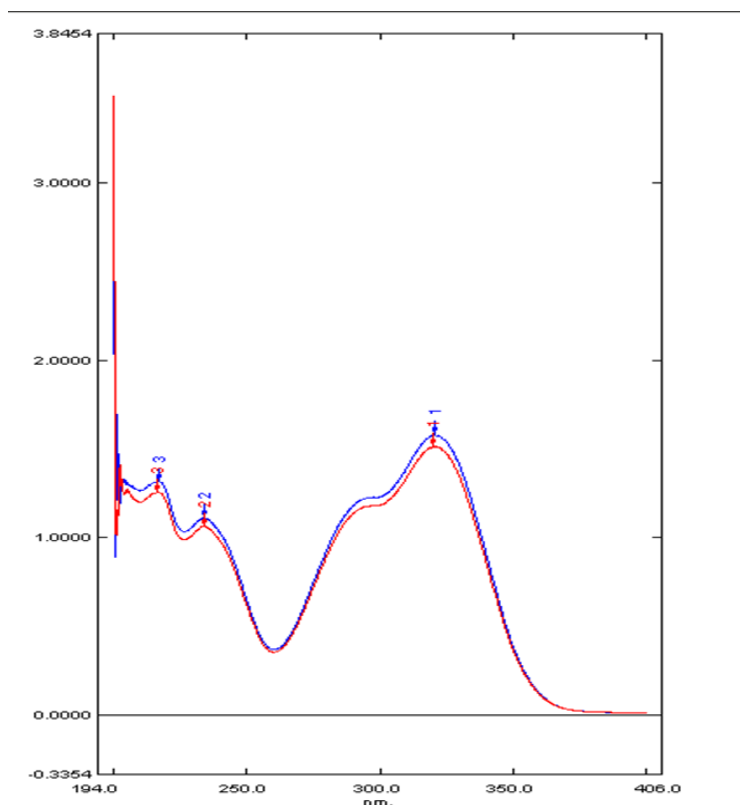


Fig. 5 UV spectrum of isolated P-coumaric acid

Moreover, the FTIR spectrum revealed a broad band at 3263–3649 cm^{-1} , in agreement with O–H stretching vibrations as characteristic of phenolic and carboxylic acid groups. A prominent absorption peak gave the carbonyl (C=O) at 1693 cm^{-1} , which represents the stretching vibration of the carboxylic acid. While the bands assigned at 1608 and 1531 cm^{-1} are attributed to aromatic and unsaturated C=C stretching. Peaks at 1303, 1234, and 1195 cm^{-1} indicate C–O stretching vibrations characteristic of phenolic and carboxylic functionalities. Furthermore, the bands between 890 and 690 cm^{-1} referred to bending vibrations of aromatic C–H out-of-plane, confirming the substituted aromatic ring, as seen in Table 1 and Fig. 6. Overall, the observed features of FTIR spectrums and bands matched with those reported for previous studies of p-coumaric acid, supporting the presence of hydroxy cinnamic acid [18], [19].

Table 1: FTIR spectrum Interpretation of P-coumaric acid

Observed bands (cm^{-1})	Interpretations
3200-3500 cm^{-1}	O-H stretching vibration of phenol and carboxylic acid
2986	aromatic C-H stretching
1693	C=O stretching of conjugated carboxylic acid
1608	C=C stretching of conjugated alkene
1531	C=C stretching of aromatic
1303	C–O stretching of carboxylic acid groups
1268 & 1195	C-O stretching of the phenolic group
1202	C-O stretching of phenol
891,860 and 783	C-H aromatic out of plane bending of the substituted benzene ring

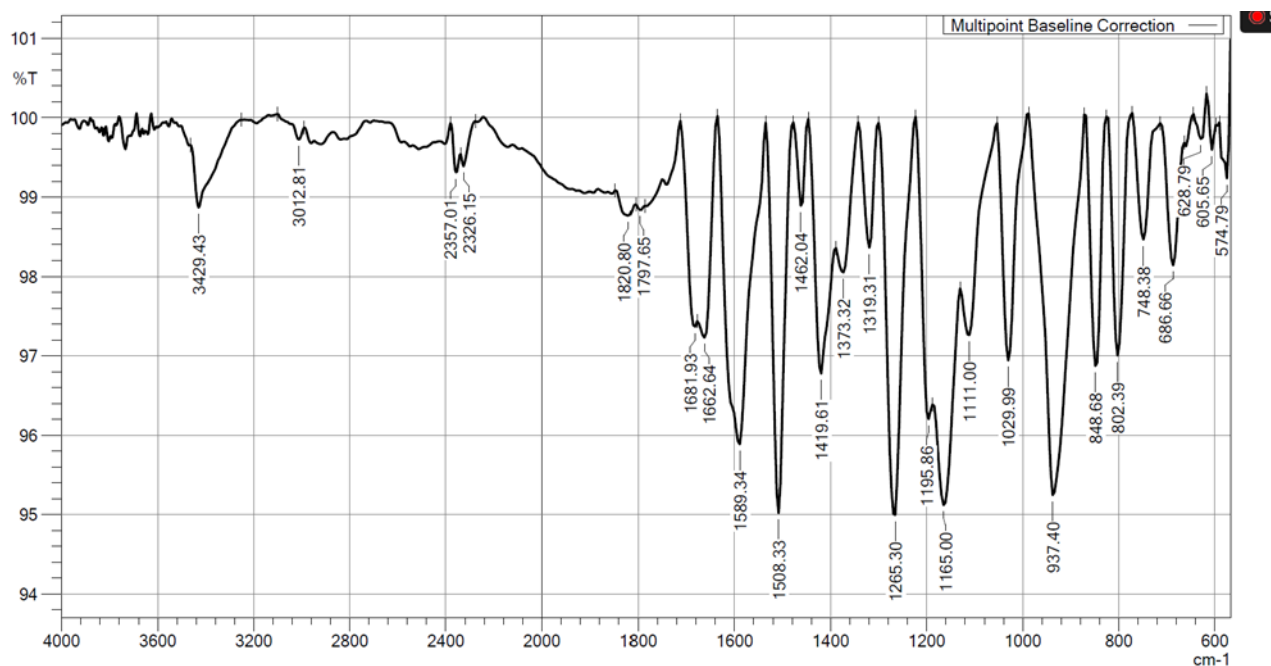


Fig. 6 FTIR spectrum of isolated p-coumaric acid from *Artemisia vulgaris* extract.

3.3. GC-MS chromatogram Analysis:

Chromatogram of GC-MS confirmed the analysis results in our work, and the determination of essential oil in Iraqi *A.vulgaris* active constituent extracted by the hydro-distillation method showed the presence of different groups of constituents. The analysis included different peaks, and Compounds were determined according to the NIST library of GC-MS, which showed a higher peak with R. time (retention time) of 31.789 min, 29.165 min, and 27.258 min. However, D-Limonene showed a peak with R. Time of 6.168 min, while alpha-Thujene observed the highest peak at R.Time of 5.778 min, followed by gamma-Terpinen at R. Time 6.346 min. p-Cimene appeared with a peak at R. Time 7.820 min. The lowest peak was observed at R. Time 8.768 min. Hence, the relative percentages of the predominant constituents were alpha-Thujene (21%), 4-p-Cimene (10%), and Dill apiol (10%), alpha-Limonene diepoxide (4%). The other compounds were unknown to the NIST library; the most common compounds were matched with a similarity index not less than 89%, shows Fig. 7 and Table 2.

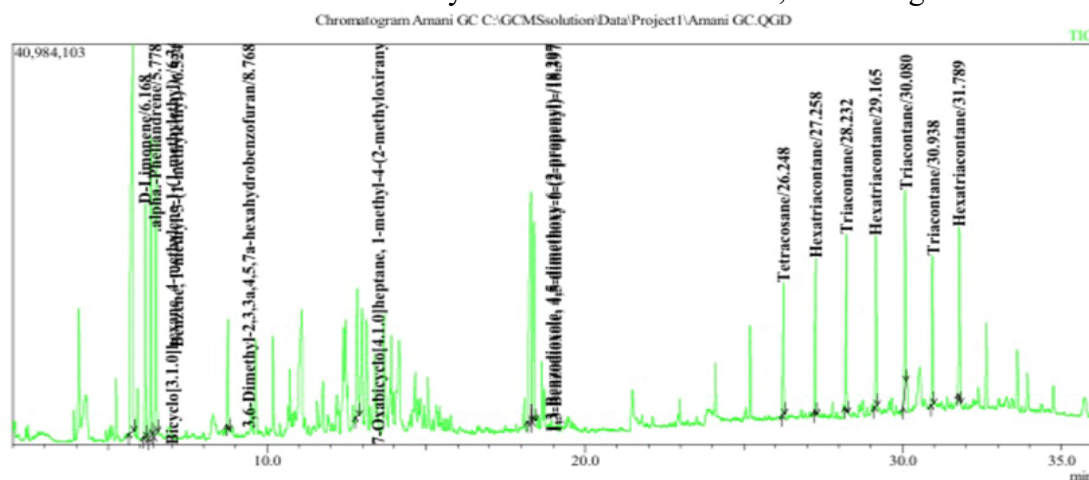


Fig. 7 Chromatogram peaks of Iraqi *A.vulgaris* extract.

Table 2: GC/ MS analysis of constituents of Iraqi *A. vulgaris*

Peak NO.	Name	R.Time	Area %
1	alpha.-Thujene	5.778	21.25
2	D-Limonene	6.168	5.30
3	gamma.-Terpinen	6.346	8.66
4	p-Cimene	6.524	10.72
5	3,9-Epoxy-1-p-menthene	8.768	2.97
6	.alpha.-Limonene diepoxide	12.832	4.29
7	Dill apiol	18.297	10.06
8	1,3-Benzodioxole,4,5-dimethyl	18.397	8.14
9	Tetracosane	26.248	3.01
10	Hexatriacontane	27.258	3.79
15	Hexatriacontane	31.789	4.23

From reported studies, the GC-Mass analysis of *Artemisia* from different geographical regions shows different compounds and percentages. In Iran showed that Alpha-pinene (5.8%), Menthol (9.71%), trans-pinocarveol (10.2%), furanone E (17.1%), and pinocarvone (8.5%)[20]. Were found to be the major constituents of the oil. In Turkey, the compositions with percentage ranges (60.8-5.06) were thujone, caryophyllene oxide, α -thujone, 8-cineole, and trans-caryophyllene [21]. In Syria, the findings parties trans- anethole 6.54%, camphor 8,65%, elemene 2.70 in addition to carene 5.69%, β -caryophyllene. In our study, the significant compounds have different yields in Iraqi *A. vulgaris*, as mentioned above. This reveals a marked difference in the composition and yields of aerial part compositions due to different climatic and geographical conditions.

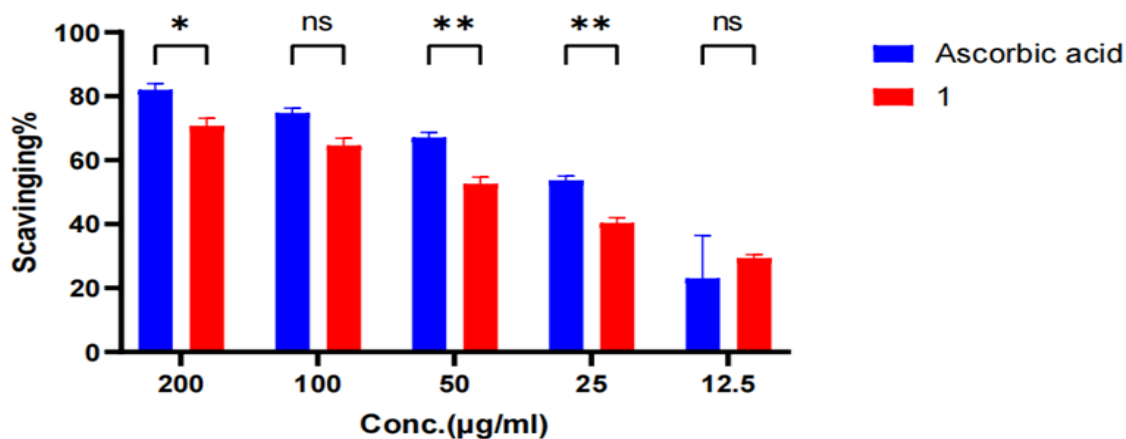
3.4 Anti-oxidant activity

Artemisia vulgaris possess a high antioxidant potential, which is attributed to terpenes and phenolic acids, and poly phenolic compounds as reported in previous anti-oxidant studies. The antioxidant prospective activity of medicinal plants is primarily attributed to various phytochemicals, including different vitamins, and various secondary metabolites. Previous researches have demonstrated significant antioxidant activity in essential oils and herbal extracts [22]. Phenolic constituents, mainly, compounds such as p-coumaric acid, revealed remarkable antioxidant effects owing to their hydroxyl-containing structures, which readily donate hydrogen atoms or electrons to neutralize reactive oxygen species and lipid peroxy radicals. The radical-scavenging competence improves cellular antioxidant defense systems by enhancing the activity of enzymes such as catalase, also glucose-6-phosphate dehydrogenase, superoxide dismutase, glutathione S-transferase, and peroxidase [23].

In this work, the results suggest that the antioxidant activity of *A. vulgaris* increases with increasing concentration up to a point, but then begins to decrease at the highest concentration tested (200 $\mu\text{g/mL}$). At 100 $\mu\text{g/mL}$, the antioxidant activity of sample is somewhat lower than at 200 $\mu\text{g/mL}$, but still higher than at lower concentrations. At 50 $\mu\text{g/mL}$ and 25 $\mu\text{g/mL}$, the antioxidant activity is lower than at 100 $\mu\text{g/mL}$. At 12.5 $\mu\text{g/mL}$, the antioxidant activity of plant is lower than at all other concentrations tested.as shown in Table 3 and Fig. 8.

Table 3: Scavenging activity of methanol extract

Concentration($\mu\text{g mL}^{-1}$)	Scavenging % (Mean \pm SD)	
	ascorbic acid	1
200	82.06 \pm 1.91	70.7 \pm 2.3
100	74.80 \pm 1.50	64.6 \pm 2.17
50	67.09 \pm 1.65	52.6 \pm 2.05
25	53.74 \pm 1.2	40.35 \pm 1.6
12.5	23.03 \pm 13.4	29.35 \pm 1.09

**Fig. 8** Scavenging % graph

Overall, this table provides information about the relationship between the concentration of extract and its antioxidant activity. The data can be used to determine the optimal concentration of *A. vulgaris* for a particular application, or to compare the antioxidant activity of this plant to other substances, listed in Table 4.

Table 4: Šídák's multiple comparisons test table

Number of families	1					
Number of comparisons per family	5					
Alpha	0.05					
Šídák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
Ascorbic acid - 1						
200	11.30	0.7070 to 21.90	Yes	*	0.0330	
100	10.15	-0.4504 to 20.74	No	ns	0.0648	
50	14.47	3.871 to 25.06	Yes	**	0.0047	
25	13.39	2.791 to 23.98	Yes	**	0.0093	
12.5	-6.327	-16.92 to 4.270	No	ns	0.4287	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2

Ascorbic acid - 1						
200	82.06	70.76	11.30	3.736	3	3
100	74.81	64.66	10.15	3.736	3	3
50	67.09	52.62	14.47	3.736	3	3
25	53.74	40.36	13.39	3.736	3	3
12.5	23.03	29.36	-6.327	3.736	3	3

From the above results of Šídák's multiple comparisons test for comparing the mean difference between each cell mean and the other cell mean in that row. The analysis was conducted for the Ascorbic acid - 1 treatment. The table shows the mean difference between the cell means, the 95% confidence interval of the difference, whether the mean difference is below the threshold, a summary of the result, and the adjusted p-value for each comparison. The adjusted p-values indicate the statistical significance of the mean difference after controlling for the multiple comparisons. The threshold alpha level used for the analysis was 0.05. The test details section provides the mean value, standard error of the difference, and the number of samples for each comparison. The anti-oxidant results support those of previous work [24]. In summary, Šídák's multiple comparisons test was used to compare the means of different concentrations of ascorbic acid with each other. The results show that the mean difference between the 200 µg/mL and 50 µg/mL concentrations, and the 25 µg/mL and 50 µg/mL concentrations, was both statistically significant ($p < 0.05$). However, the mean difference between the 100 µg/mL and 200 µg/mL concentrations, and the 12.5 µg/mL and 29.36 µg/mL concentrations, was not statistically significant ($p > 0.05$).

4. Conclusions

Essential oil was identified, and p-Coumaric acid was effectively isolated from *Artemisia vulgaris* and identified using UV–Vis, FTIR, HPLC, and MS analyses. The resulting chromatographic, spectral, and antioxidant data were reliable with the structural features of p-coumaric acid and other compositions. The results highlight the phytochemical importance of *A. vulgaris* as a source of bioactive phenolic constituents. Despite its traditional use, further clinical studies are needed to establish its safety and efficacy in modern medical practices.

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