



Separating and Identifying of Some Alkaloids and Phenols Compounds from the Sage Plant and Studying their Antioxidant and Biological Activity

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Abstract

This study included the separation of some active compounds from the leaves of sage plant using soxhlet device and then the acid hydrolysis process of the methanolic extract to separate the phenolic compounds. The alkaloid components were isolated from the methanolic extract and analysed using high-performance liquid chromatography (HPLC) method. The results showed that the extract contain five phenolic compounds: Caffeic acid, Gallic acid, Kaempferol, Rutin, Quercetin, in addition to the alkaloids *Quinine* and *Scopolamine*. The biological activity was also tested on two types of bacteria that infect the plant (*Acrobacterium tumfaciens*, *Pseudomonas savastanoi*), where the phenols showed high inhibitory effects at a concentration of 400 mg/cm³ with a diameter of 22 mm against *Pseudomonas savastanoi* bacteria, while the highest inhibitory amount of phenols against *Acrobacterium tumfaciens* bacteria was 13 mm at a concentration of 400 mg /cm³, and the alkaloids showed high inhibitory effects against *Acrobacterium tumfaciens* bacteria with the highest inhibitory diameter of 13 mm at a concentration of 100 mg/cm³, while its highest effect against *Pseudomonas savastanoi* bacteria was at a concentration of 100 mg/cm³ at 12 mm. The antioxidant activity and effectiveness of the methanolic extract showed antioxidant activity of 69.680 µg/ml at a concentration of 15.6 µg/ml, while the lowest effect was at a concentration of 250 µg/ml at 4.728 µg/ml compared to ascorbic acid.

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1. Introduction

Sage is an aromatic medicinal plant belonging to the *Lamiaceae* family. Its original habitat is the Middle East and the Mediterranean region. ^[1] Sage is extensively utilised in traditional medicine for its antiseptic, anti-scabies, antibacterial, anti-syphilitic, and anti-inflammatory properties. It is said that it has been used to treat fever and some digestive disorders in several locations in the Middle East. ^[2] Many researches and studies indicate that sage has a strong antioxidant effect. ^[3] Sage leaves are a rich source of many active compounds, including polyphenols and phenolic acids. The acids comprise caffeic acid, rosmarinic acid, salvinolic acids, saged coumarin, sagenic acid, and ferulic acid. The primary flavonoids include luteolin, apigenin, hispidolin, kaempferol, and quercetin. The predominant phenolic acids in sage species are derivatives of caffeic acid, primarily existing in a double-bonded configuration, exemplified by rosmarinic acid ^[5] Sage leaves also contain many active compounds such as alkaloids ^[19].

2. Materials and Methods

Collection Plants

After planting the plant, the leaves of the sage plant were collected from the city of Mosul, cleaned, washed well and dried.

Preparation of the Plant Extract

The extraction process was carried out using a continuous extraction device (Soxhlet), where the sample was ground after drying for the purpose of extraction based on the polar scale, starting from petroleum ether and ending with methanol. If you took 50 grams of the powdered leaf sage were taken and then placed in the device with the addition of 500 ml of solvent and the device was operated for 8 hours daily until the solvent used became colorless and the process was repeated according to the polarity and the raw methanol extract was taken and the solvent was evaporated using a rotary evaporator and then the extract was placed in dark, sterile glass bottles in a cool place to protect it from damage [4, 5].

Separation of Phenols from Crude Methanolic Extract:

The main goal of acid hydrolysis process is to break the glycosidic bond and release phenols from sugar molecules because phenols are usually not found free but are bound in the form of glycosides inside the plant. Take 1 gram of methanolic extract and add 50 ml of hydrochloric acid (1N) Hcl and heat it at 100°C for an hour, then put the solution in a separating funnel and add 25 x 2 ml of ethyl acetate with good shaking, then take the upper layer and ignore the lower layer, then evaporate the lower layer of ethyl acetate using a rotary evaporator and keep the phenols in sterile glass bottles tightly closed and store them until they are used and diagnosed using an HPLC device [10, 11].

Separation of Alkaloids from the Crude Methanolic Extract

Combine 1 g of the methanolic extract with 5% sulphuric acid and ether in a 50/50 v/v ratio, then transfer the solution to a separating funnel and shake vigorously for 10 minutes. The highest two layers will consist of ether, whereas the lower levels contain the alkaloids that are extracted, leaving the upper layer disregarded. Introduce 10-molar sodium hydroxide, followed by the addition of 30 ml of chloroform, and thereafter position the mixture in a separating funnel, stirring for 10 minutes. Consequently, the first two layers will be disregarded, leaving the lowest layer containing chloroform and alkaloids. They are placed in sterile, opaque glass bottles until they are diagnosed using HPLC technology and the inhibitory and antioxidant activity tests are performed [12].

Diagnosis of Active Compounds using HPLC

The samples were analyzed using HPLC model (SYKAM) Germany. The mobile phase = (methanol: D.W: formic acid) (70 : 25 : 5) The column is C18-ODS 25 cm * 4.6 mm) and the UV detector - 280 nm with a flow rate of 1.0 ml / min [13, 14].

Biological Effectiveness

The bacteria used in the study are *Pseudomonas savastion*, *Acrobacterium tumfaciens*

Agricultural media used

The agar nutritional medium was utilised in accordance with the manufacturer's guidelines.

Antibiotics

The sensitivity of bacteria to antibiotics was tested as shown in the table 1:

Table 1: Inhibitory effect of standard antibiotics against bacteria used under study

Antibiotic	<i>Acrobacterium tumfaciens</i>	<i>Pseudomonas savastanoi</i>
Amoxicillin (AMC10)	-	-
Azithromycin (AZM15)	15	12
Trim Methbrin (TMP10)	-	-
Nalidixic acid (NA30)	10	-
Ciproder (CIP10)	20	15

(-) no effect.

Biological Efficacy Test

The study assessed the inhibitory activity against the bacteria through the diffusion method, creating holes of 6 mm in diameter at three replicates for each bacterium. A prepared bacterial suspension was applied and compared with the standard McFarland solution, which was subsequently incubated. Following a 30-minute incubation at 37 °C to facilitate absorption, the extract was prepared to evaluate its inhibitory activity against the bacteria utilised in the study. Concentrations of 400, 200, and 100 mg/cm³ were achieved by dissolving 1 gramme of the extract in 2.5 cm³ of dimethyl sulfoxide (DMSO) to yield a 400 mg/cm³ concentration. After that, the rest of the concentrations were made by dilution, then the mixture was sterilized by pasteurization at a temperature of 62 for 10-15 minutes, then 0.5 microliters of each concentration were taken and injected using a micropipette into the agar holes at a rate of three replicates for each isolate. And incubate at 37°C in the incubator for 16 hours [14].

Antioxidant Activity Test

DPPH test (1,1-Diphenyl-2-picrylhydrazyl) was used to determine the antioxidant activity of methanolic extract of sage leaves [6]

DPPH (is an organic solvent with a purple color that turns yellow when in contact with a reducing agent. The method of work was as follows:

1. A series of dilutions of the extract (15.6, 31.2, 62.5, 125, 250, 500) micrograms/ml were prepared and 10 ml of Methanol:DMSO solution was added to each of them at a ratio of 9:1.
2. Dissolve 0.02 g of DPPH in 100 ml of Methanol:DMSO at a ratio of 9:1.
3. Add 0.3 ml of DHHP to each tube.
4. Blank negative control tubes were prepared containing DPPH solution and Methanol:DMSO solution Only.
5. Sample tubes containing DPPH solution and Methanol:DMSO solution were prepared
6. Then the concentrations prepared from the plant extracts

were added at a rate of 3 replicates for each concentration.

7. The positive control tube containing DPPH solution, Methanol:DMSO solution and ascorbic solution was prepared.
8. The results were read on the spectrophotometer at a wavelength of 517 nm^[7]

The results were read according to the following equation:

$$\text{Antioxidant activity \%} = \frac{[(\text{OD blank} - \text{OD sample}) / \text{OD blank}] \times 100}{1}$$

OD blank: negative control tube

OD sample: positive sample tube^[15]

3. Results and Discussion

Diagnosis of phenolic compounds using HPLC

Phenolic chemicals were extracted from the methanolic extract of sage leaves utilising high-performance liquid chromatography (HPLC). The findings indicated that the extract comprised five phenolic compounds: Caffeic acid, Gallic acid, Kaempferol, Rutin, and Quercetin, the highest concentration of which was Gallic acid at a retention time of 10.08, while the lowest concentration was Kaempferol at a retention time of 6.25, and also Quercetin at a retention time of 3.90, as shown in Table (2) and Figures (1-6). These results were similar to the study^[20] on the presence of phenolic compounds, where 14 phenolic compounds were detected, The primary chemicals identified were methyl rosmarinate (24.86 mg/100 g) and ferulic acid (6.06 mg/100 g), accompanied by five flavonoids, with Rutin, Naringin, and Quercetin being the principal constituents^[24].

Table 2: Phenols compound isolated from sage leaves using HPLC

No.	Name	Ret.time	Con.%
1	Caffeic acid	12.8	19
2	Gallic acid	10.08	29
3	Kaempferol	6.25	14
4	Rutin	5.97	24
5	Quercetin	3.90	14

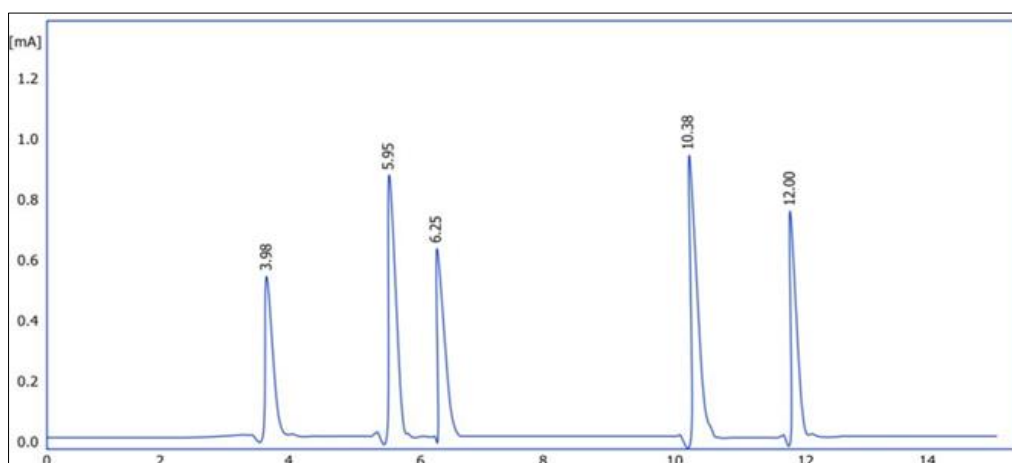


Fig 1: Curve of phenolic compounds isolated from sage leaves using HPLC technique.

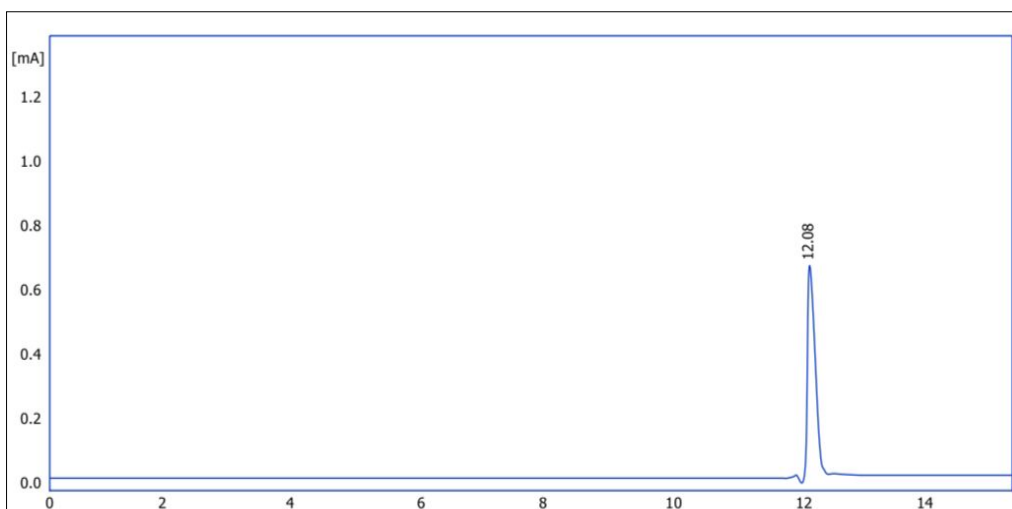


Fig 2: Standard calibration curve for caffeic acid using HPLC technique

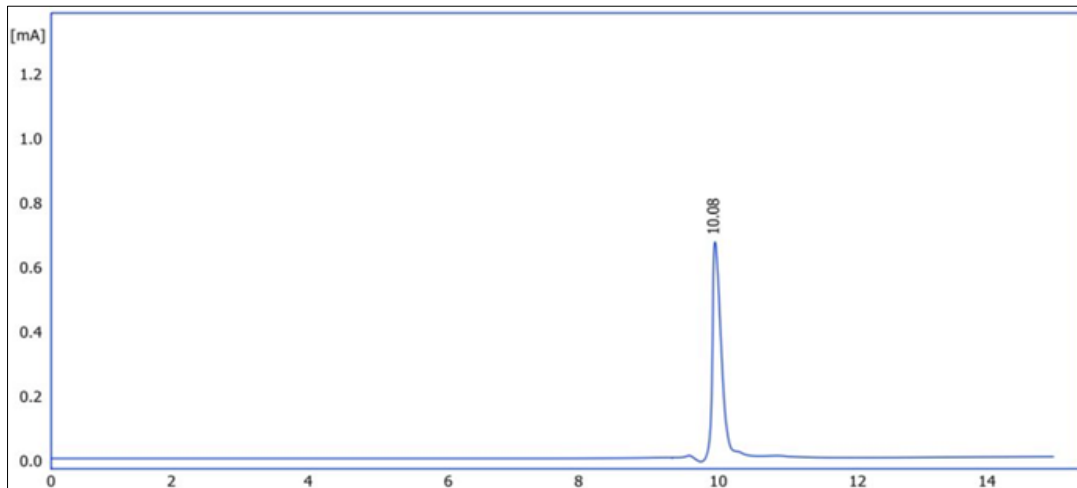


Fig 3: Standard calibration curve for calcic acid using HPLC technique

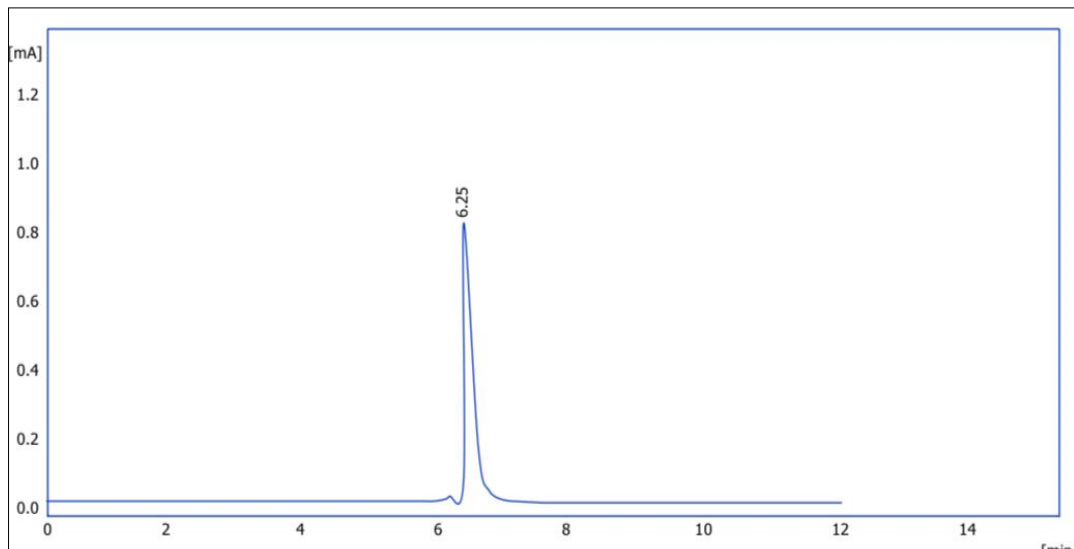


Fig 4: Standard calibration curve for camphor using HPLC technique

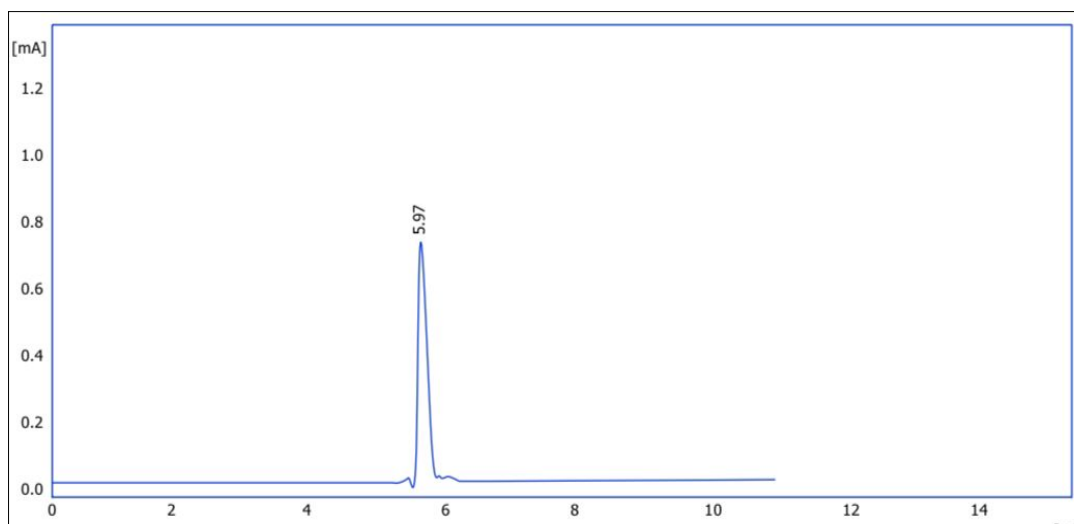


Fig 5: Standard calibration curve for rutin using HPLC technique

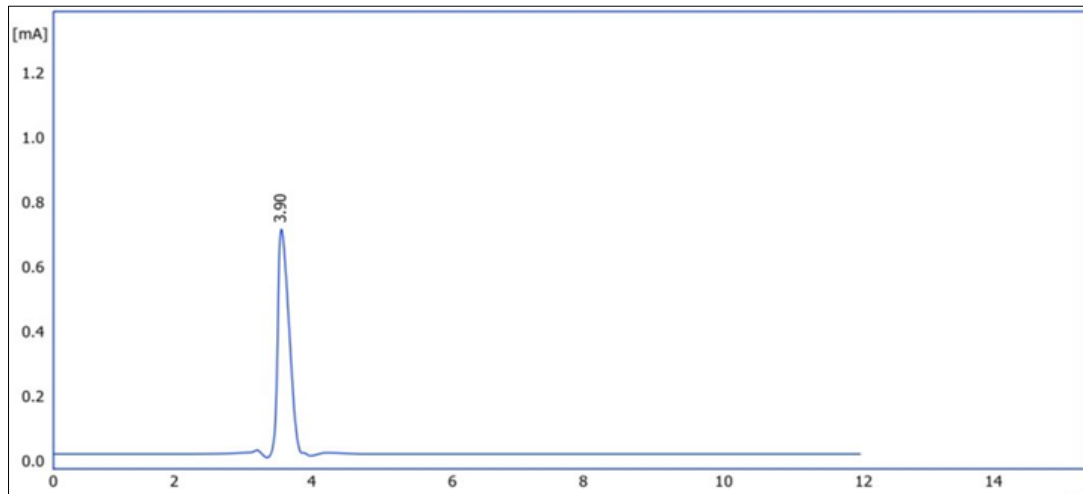


Fig 6: Standard calibration curve for quercetin using HPLC technique

Alkaloids diagnosis by HPLC

Two alkaloids were identified in sage leaves, scopolamine and *quinine*. The results showed that the scopolamine alkaloid appeared at a retention time of 10.08/min at a concentration of 30% and the *quinine* alkaloid appeared at a retention time of 4.10/min at a concentration of 35% as

shown in Table (3) and Figures (7-9). The findings of this study were analogous to those of the study of [18], which indicated that the methanolic extract of sage leaves contains many active compounds including alkaloids, which parallels the findings of our present investigation [22].

Table 3: Alkaloids isolated from sage plant using HPLC technique

No	Name	Ret.time	Con%
1	Scopolamine	10.08	30
2	<i>Quinine</i>	4.10	35

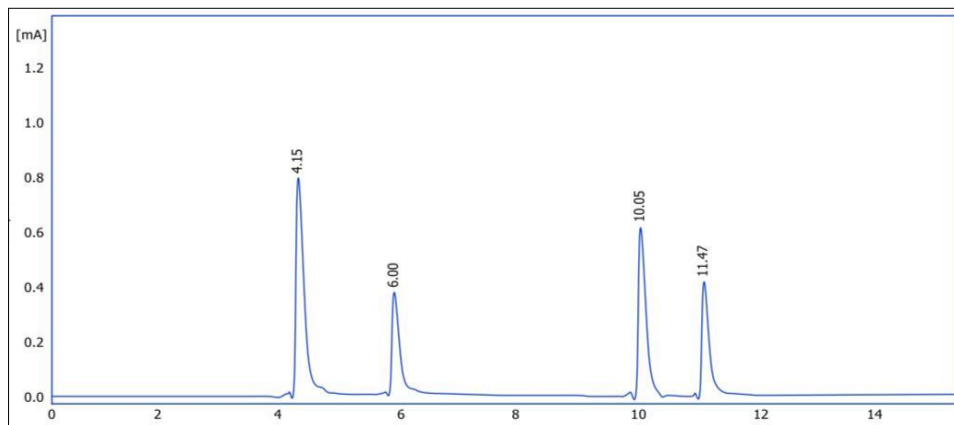


Fig 7: Curve of Alkaloids compounds isolated from sage leaves using HPLC technique.

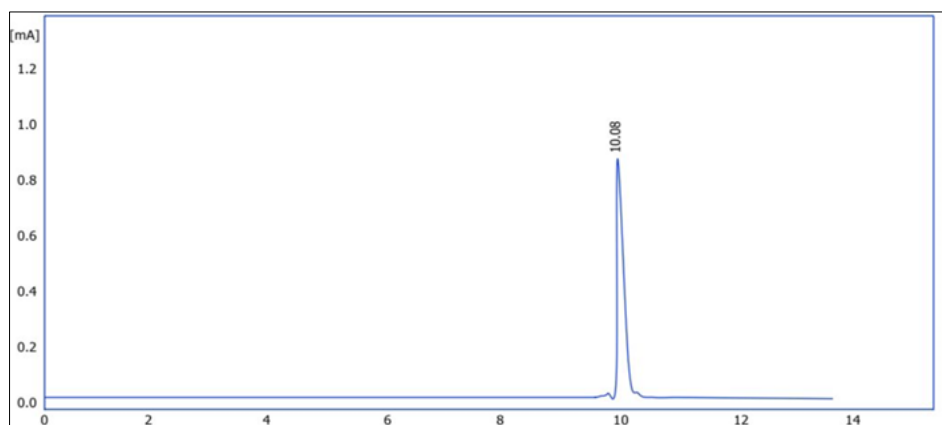


Fig 8: Standard calibration curve for Scopolamine using HPLC technique

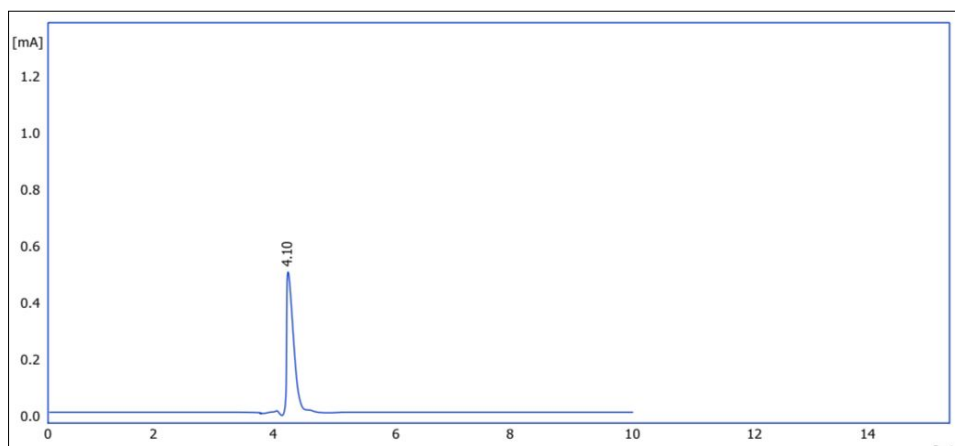


Fig 9: Standard calibration curve for quinine using HPLC technique

Antioxidant activity of methanolic extract of sage leaves

The results of the antioxidant activity showed that the methanolic extract of sage leaves has a high antioxidant activity at low concentrations, and the result was the opposite at high concentrations, i.e. the effect was less than the standard compound ascorbic acid, as the concentration of 15.6 showed the highest effect of 69.680, while the lowest effect was at the concentration of 250, of 4.728, as shown in Table (4) and Figure (10). The reason for this effect is due to the presence of active compounds in the methanolic extract, which include phenols and alkaloids, as the study [19] showed

their antioxidant effects with good activity exceeding 80% inhibition at a concentration of 0.01 mg/ml. These results were similar to the study [8], which indicated the presence of a good amount of phenols and flavonoids, which may be responsible for the antioxidant activity due to the ability to remove free radicals of the hydroxyl group present in the chemical structure, antimicrobial against living organisms Minute. These results were also similar to the study [9]. The methanolic extract showed great antioxidant activity and there is a correlation between the polyphenol content and antioxidant activity.

Table 4: Antioxidant activity of methanolic extract of sage leaves

Concentrations	Ascorbic acid		M.S	
15.6	13.469	0.13469	69.680	0.69680
31.2	14.421	0.14421	67.176	0.67176
62.5	26.258	0.26258	58.831	0.58831
125	26.938	0.26938	42.837	0.42837
250	54.693	0.54693	4.728	0.0472
500	70.748	0.70748	52.712	0.52712

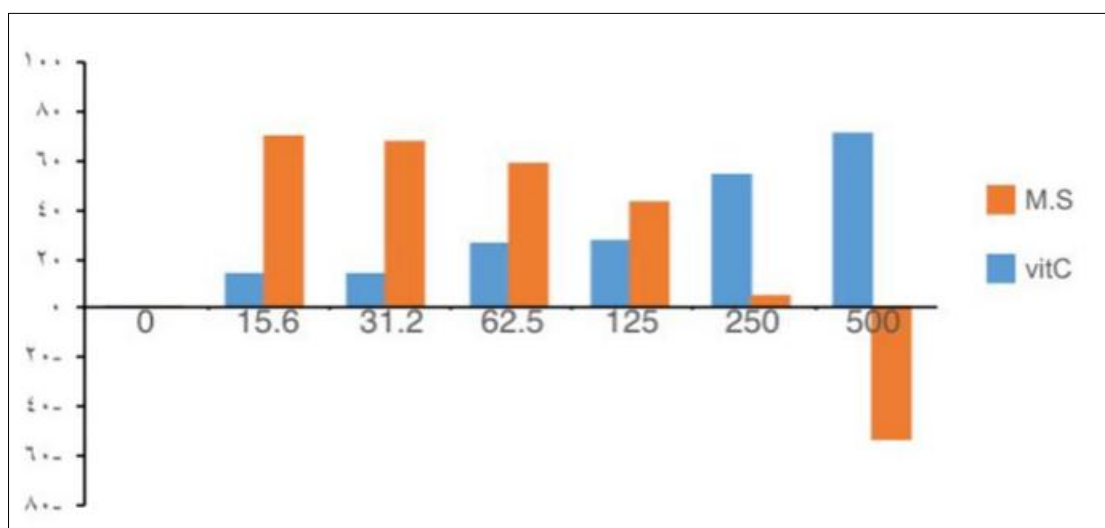


Fig 10: A diagram showing the antioxidants of the methanolic extract of sage leaves.

Testing the inhibitory activity of phenolic compounds isolated from sage leaves

The phenolic compounds isolated from sage leaves showed the highest effect rate at the concentration of 400 mg/cm³ and an inhibition amount of 22 mm against *Pseudomonas*

savastanoi bacteria, followed by concentrations of 200, 100 mg/cm³ with an effect amount of 21.19 mm. The concentration of 400 mg/cm³ gave the highest inhibition amount of 13 mm against *Acrobacterium tumfaciens* bacteria, followed by concentrations of 200, 100 respectively

with an inhibition amount of 9.8 mm as shown in Table (5) and Figure (11). The outcomes of our research were comparable to the study [16], Phenolic compounds, abundant in sage, exhibited inhibitory effects against bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*,

and *Salmonella typhimurium*), indicating sage's potential application in the pharmaceutical and food industries as a natural antibacterial and antioxidant agent. The study [21] also demonstrated the effect of phenolic compounds on the antimicrobial activity against *B. subtilis*.

Table 5: shows the inhibitory effect of phenolic compounds isolated from sage leaves.

Transactions	Types of Bacteria		Extract Effect
	<i>Acrobacterium</i>	<i>Pseudomonas</i>	
400	13c±0.7	22a±0.4	18a
200	9c±1	21a±0.6	15b
100	8c±0.5	19a±0.5	13b
Effect of bacteria	10b	21a	

- Mean diameter of the circle calculated using three isolates for each bacterium.
- Distinct letters, both horizontally and vertically, denote significant differences at a significance level of 0.01≥P.
- The diameter of the inhibition circle is measured in millimetres.
- The figures following (±) denote experimental error.

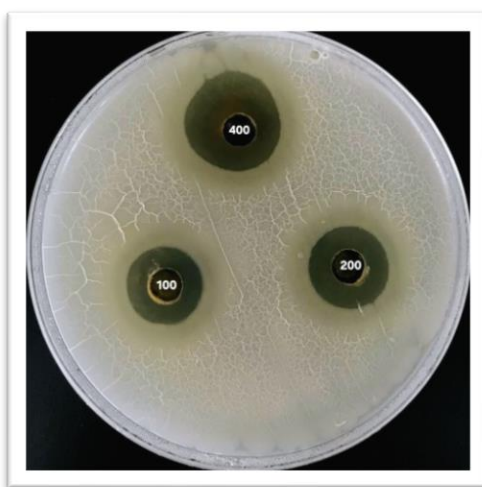


Fig A: Shows the inhibitory effect of phenols against *Pseudomonas savastanoi*.

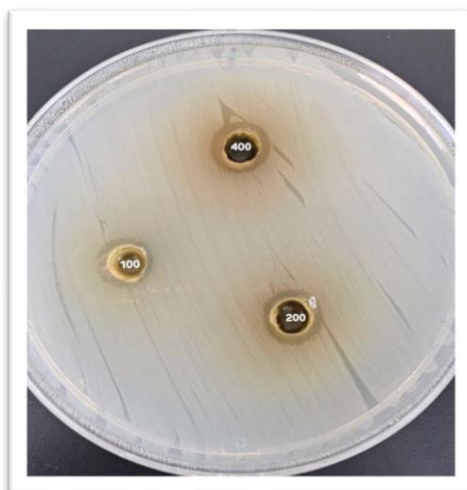


Fig B: Shows the inhibitory effect of phenols against *Acrobacterium tumfaciens*.

Fig 11: Shows the Inhibitory Effect of Phenolic Compounds in Sage Leaves.

Inhibitory Activity Test of Alkaloid Compounds Isolated from Sage Leaves

The alkaloid compounds isolated from sage leaves showed the highest effect rate at the concentration of 100 mg/cm³ and an inhibition amount of 13 mm against *Acrobacterium tumfaciens* bacteria, followed by concentrations of 400, 200

mg/cm³ respectively with an inhibition amount of 12, 10 mm. The concentration of 100 mg/cm³ gave an inhibition amount of 12 mm against *Pseudomonas savastanoi* bacteria, followed by concentrations of 400, 200 mg/cm³ respectively with an amount of 9, 9 mm as shown in Table (6) and Figure (12). This study was similar to the research demonstrating

that sage extract exhibited antibacterial efficacy against *Salmonella typhi*, *Listeria monocytogenes*, and

Staphylococcus aureus. Nonetheless, no substantial antibacterial action was detected against *E. coli* [17, 23]

Table 6: shows the inhibitory effect of alkaloid compounds isolated from sage leaves.

Transactions	Types of Bacteria		Extract Effect
	<i>Acrobacterium</i>	<i>Pseudomonas</i>	
400	12b±0.5	9c±0.5	11b
200	10c±0.6	9c±0.2	9c
100	13a±0.5	12b±0.2	13a
Effect of bacteria	12a	10b	

- Mean diameter of the circle calculated using three isolates for each bacterium.
- Distinct letters, both horizontally and vertically, signify substantial differences at a significance level of $0.01 \geq P$.
- The diameter of the inhibition circle is measured in millimetres.
- The figures following (\pm) denote experimental error.



Fig A: Shows the inhibitory effect of alkaloids against bacteria *Acrobacterium tumefaciens*

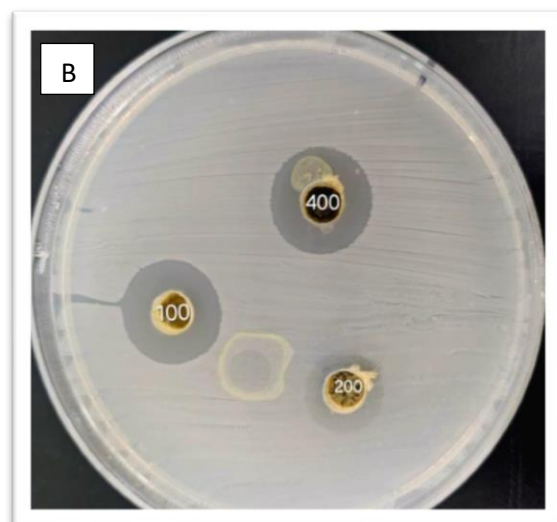


Fig B: Shows the inhibitory effect of alkaloids against bacteria *Pseudomonas savastanoi*

Fig 12: Shows the inhibitory effect of alkaloids in sage leaves

References

1. Ghorbani A, Esmailizadeh M. Pharmacological properties of *Salvia officinalis* and its components. *J Tradit Complement Med.* 2017;7(4):433-40.
2. Al-Mijalli SH, Assagaf H, Qasem A, El-Shemi AG, Abdallah EM, Mrabti HN, *et al.* Antioxidant, antidiabetic, and antibacterial potentials and chemical composition of *Salvia officinalis* and *Mentha suaveolens* grown wild in Morocco. *Adv Pharmacol Pharm Sci.* 2022;2022:2844880.
3. Francik S, Francik R, Sadowska U, Bystrowska B, Zawiślak A, Knapczyk A, *et al.* Identification of

- phenolic compounds and determination of antioxidant activity in extracts and infusions of salvia leaves. *Materials* (Basel). 2020;13(24):5811.
4. Jakovljević M, Jokić S, Molnar M, Jašić M, Babić J, Jukić H, *et al.* Bioactive profile of various *Salvia officinalis* L. preparations. *Plants* (Basel). 2019;8(3):55.
 5. Sharma Y, Velamuri R, Fagan J, Schaefer J, Streicher C, Stimson J. Identification and characterization of polyphenols and volatile terpenoid compounds in different extracts of garden sage (*Salvia officinalis* L.). *Pharmacogn Res.* 2020;12(2): [page numbers if available].
 6. Khiya Z, Oualcadi Y, Gamar A, Berrekhis F, Zair T, Hilali FE. Correlation of total polyphenolic content with antioxidant activity of hydromethanolic extract and their fractions of the *Salvia officinalis* leaves from different regions of Morocco. *J Chem.* 2021;2021:8585313.
 7. Sotiropoulou NS, Megremi SF, Tarantilis P. Evaluation of antioxidant activity, toxicity, and phenolic profile of aqueous extracts of chamomile (*Matricaria chamomilla* L.) and sage (*Salvia officinalis* L.) prepared at different temperatures. *Appl Sci* (Basel). 2020;10(7):2270.
 8. Wali AF, Hamad EA, Khazandar AA, Al-Azzawi AM, Sarheed OA, Menezes GA, *et al.* Antimicrobial and *in vitro* antioxidant activity of *Salvia officinalis* L. against various re-emergent multidrug resistance microbial pathogens. *Ann Phytomed.* 2019;8(2):115-20.
 9. Pop AV, Tofană M, Socaci SA, Nagy M, Borş MD, Salanță L, *et al.* Studies on total polyphenols content and antioxidant activity of methanolic extracts from selected *Salvia* species. *Bull UASVM Food Sci Technol.* 2015;72(1).
 10. Najm MR, Sultan FI. Separation and identification of some natural products from cumin seeds and the study of biological activity. *AIP Conf Proc.* 2023;2862(1).
 11. Harborne JB. *Phytochemical methods.* 3rd ed. London: Chapman & Hall; 1998.
 12. Ataei-Azimi AB, Delnavaz-Hashemloian H, Ebrahimzadeh A, Majd A. High *in vitro* production of anticanceric indole alkaloids from periwinkle (*Catharanthus roseus*) tissue culture. *Afr J Biotechnol.* 2008;7:2834-9.
 13. Mradu G, Saumyakanti S, Sohini M, Arup M. HPLC profiles of standard phenolic compounds present in medicinal plants. *Int J Pharmacogn Phytochem Res.* 2012;4(3):162-7.
 14. Rajeshkumar S, Malarkodi C. *In vitro* antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. *Bioinorg Chem Appl.* 2014;2014:581890.
 15. Chaves N, Santiago A, Alías JC. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used. *Antioxidants* (Basel). 2020;9(1):76.
 16. Mokhtari R, Kazemi Fard M, Rezaei M, Moftakharzadeh SA, Mohseni A. Antioxidant, antimicrobial activities, and characterization of phenolic compounds of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and thyme-sage mixture extracts. *J Food Qual.* 2023;2023:2602454.
 17. Karagözlü N, Özeşer T. Green synthesis, characterisation, and antibacterial activity of silver nanoparticles obtained from *Salvia officinalis* extract. *Czech J Food Sci.* 2024.
 18. Bouteldja R, Doucene R, Aggad H, Abdi FZ, Belkhdja H, Belal A, *et al.* Phytochemical screening, acute toxicity and antidiabetic activity of ethanolic extract of *Salvia officinalis* L. in Wistar rat. *Agric Conspec Sci.* 2023;88(4):351-7.
 19. Maliki I, Moussaoui AE, Ramdani M, ElBadaoui K. Phytochemical screening and the antioxidant, antibacterial and antifungal activities of aqueous extracts from the leaves of *Salvia officinalis* planted in Morocco. *Moroc J Chem.* 2021;9(2):902.
 20. Sabry MM, Abdel-Rahman RF, El-Shenawy SM, Hassan AM, El-Gayed SH. Estrogenic activity of sage (*Salvia officinalis* L.) aerial parts and its isolated ferulic acid in immature ovariectomized female rats. *J Ethnopharmacol.* 2022;282:114579.
 21. Tohma H, Köksal E, Kılıç Ö, Alan Y, Yılmaz MA, Gülçin İ, *et al.* RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of *Salvia* L. species. *Antioxidants* (Basel). 2016;5(4):38.
 22. Najm MR, Sultan FI. Evaluation of phytochemical constituents by GC-MS, HPLC and biological activity of *Peganum harmala* L. seeds extract. *IOP Conf Ser Earth Environ Sci.* 2022;1060(1):012097.
 23. Saadallah HA, Sultan FI, Al-Tarjuman JK. Phytochemical analysis of terpenes from *Eucalyptus camaldulensis* and study of its biological effect against *Macrophomina* spp. *IOP Conf Ser Earth Environ Sci.* 2023;1262(5):052021.
 24. Sultan F, Al-Farha AAB, Shaaban I. Separation and identification of some fatty acids and phenolic compounds from *Portulaca oleracea* L. and study their biological effect on two types of pathogenic bacteria.

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