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Article

# Chromatographic Separation and Identification of Many Chemical Compositions and Antibacterial Activity of *Laurus nobilis*

**Fatimah I. Sultan[1], Mohammed Fadhil Haddad[2], Yassar Yahya AL-Tamer[3,] Mahmood Esmail Mohammed[4]**

1. Plant Production Techniques Dept., Technical Agricultural College, Northern Technical University, Mosul, Iraq
2. Northern Technical University, Mosul, Iraq
3. Department of Medical Laboratories Techniques, Al-Noor University College, Mosul, Iraq
4. Mosul University, Iraq

* Correspondence: [1]drfatimah@ntu.edu.iq, [2]Mohammed.75.haddad@ntu.edu.iq, [3]yasaar.yahya@alnoor.edu.iq, [4]mahjsbio74@uomosul.edu.iq

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**Abstract:** Phytochemical and antimicrobial screening leaves of Laurus nobilis , It was included the separation and identification of some active constituents from the leaves of Laurus nobilis and their activities were tested against some types of gram positive, gram negative bacteria. The active constituents were separated through Soxhlet and Column Chromatography (CC) and The column was eluted using petroleum - ether-ethyl acetate at 10:1 V/V intervals to separate various terpene compounds and fatty acids of L1F1 extract from Laurel leaves, As 20 active compounds were diagnosed by a device GCMS analysis technique, an of them (Terpinen-4-ol, . Linalool, gamma.-Terpinene, Eucalyptol, Eucalyptol, alpha.-Terpinyl acetate)and separating of some free phenolic compounds. As 6 Phenolic compounds (Luteolin, Kaempferol, Kaempferol, Qurcetine, Rutin, Apigenin, P. Coumaric acid) were identified by High Performance Liquid Chromatography (HPLC). The active separated compounds (terpene ,fatty acids, phenolics), each according to it's separation and purification, showed a different inhibitory effect against the micro-organisms (m.o) under study by using Disc Diffusion Method.

**Keywords:** GC-MS Analysis, Laurus Nobilis, Fatty Acids, HPLC Analysis, Phenolic Compounds, Antibacterial Activities.

## Introduction

**Many scientific studies have shown the antimicrobial and antioxidant activity of *L. nobilis* extracts [1]**

Bay leaves grow in the form of a tree or a bush and sometimes reach 10 meters in height, and they have leaves with a fragrant smell because they contain volatile oils. They are always used as ornamental trees, and they also have many important medicinal uses [2], [3].

Called commonly Bay leaf. The family It belongs to Lauraceae; it is endemic in the Mediterranean region. The Bay leaf is rather common in the Algerian Tell and in the region of Constantine [4].

The only plant found in the Mediterranean region is a *Laurus nobilis L* of the family Lauraceae [5]. In most areas where the bay leaf plant is found, it is used for many medical uses, as it is used to treat digestive disorders, and it is used against arterial hypertension and in the treatment of viral infections such as influenza [6].

The current study aims to separate and chemically characterize the active compounds using GC/MS and HPLC devices for petroleum ether extract and IMS extract of laurel leaves, study the biological activity, and evaluate the antibiotic activity against three bacterial strains: *Staph. aureus, Bacillus subtilis, E. coli, Pseudo. Aeruginosa.* To get better results, a number of antibiotics were tested on the pathogenic bacteria used in the study first, and as a comparison with the effect of the active compounds separated from bay leaves [7].

## Materials and Methods

The leaves of *Laurel* were collected from Mosul and classified and verified by taxonomists. and according to international classification sources. The leaves were dried according to the correct scientific methods.

Preparation of plant extracts:

An amount of 100 gm of crushed bay leaf was extracted for 8-10 hours per day using was Soxhlet extraction device, with 1 liter of two solvents (petroleum ether and IMS). The extracts were concentrated to 25 mL using a vacuum rotary evaporator at 50 °C**.** Thus, two crude extracts were obtained, one of which is the crude petroleum ether extract and the other the crude IMS extract

**Extraction of the Active Constituents**

**The study utilizes thin-layer chromatography (TLC) technology to detect active constituents and identify the mobile phase of a separating column (silica gel).**

Silica gel sheets prepared by Mercke were used, with a thickness of 0.25 mm and dimensions of 20 x 20 cm. The samples were loaded on one end of the board in the form of spots along the starting line with the help of a capillary tube. The bottom and not in contact with the solvent system, which is chosen with a certain polarity, depending on obtaining the best separation of the extract to be separated from its components

The container was covered with its lid and left at the laboratory temperature until the separation solution rose a distance before the end, after which the plate was lifted from the container and dried with nitrogen gas, then spots were shown by washing it with a Phosphomolybdic acid hydrate reagent, and the same method was adopted in determining the mobile phase of the separation column for all separated compounds (Kirchner, 1978) [8].

**Separation and purification of the active compounds from using a chromatographic separation column (silica gel)**

The crude extract was purified using column chromatography to obtain the active compounds in the form of effective aggregates (Fractionation). By taking 300 mg of each previously prepared crude extract and mixing it with an amount of 25 g of silica gel, the sample was placed in a chromatographic separation column filled with a prepared silica gel (60-120 mesh). The column was eluted with petroleum -ether-ethyl acetate at 10:1 V/V intervals, and 2 gm of petroleum ether extract was dissolved using Laurel leaves. The extract was separated using a column chromatographic filled with silica gel (60-120 mesh). A chromatography apparatus was filled with 30 gm of gel and a solvent (10:1 V/V) for a chromatography study. Two crude petroleum ether extract fractions, L1F1 and L1F2, were obtained based on the similarity of spots on the TLC plate. The IMS extract was filled, and the column was eluted using a solvent (petroleum-ether: methanol; 5:1 V/V). PE: M, L2F1 fractions were obtained based on spot similarity on the TLC plate, and the same solution system was used in the column. The parts were evaporated using a rotary evaporator under pressure [8], [9].

**Instruments Used to Diagnose Active Compounds**

**GC- MS Analysis:**

The University of Basra's Food Research and Consumer Protection Laboratory has identified various terpene compounds and fatty acids in the L1F1 extract from Laurel leaves, The Japanese-made GC MS QP210 Ultra was used for separation using an MS-GC thermal system at( 40)°C for one minute Increase the distance from( 150 – 280) m at a rate of 5 m/min, then perform an auto-injection process by injecting 1 microliter into the gas device, The study examined the esterification process of oil samples, specifically AOC-AHIMADZU, (20i+5), and their separation conditions using GC-MS solutions program and schematics [10], [11].

**High Performance Liquid Chromatography (HPLC)**

Identified Different phenolic compounds of L2F1 extract from Laurel leaves in the Ministry of Science and Technology in Baghdad by High-performance liquid chromatography (HPLC) analysis conducted on a SYKAMN HPLC system (Germany ) equipped with a C18-ODS column (250 × 4.6 mm, 5 μm). Samples (100 μL) were injected into the system. The mobile phase was composed of 95% acetonitrile + 0.01% Triflouroacetic acid (solvent A) and 5% acetonitrile + 0.01% Triflouroacetic acid (solvent B) at 1 mL/min. The gradient program was as follows: 10% A from 0–5 min; 25% A from 5-7 min; 40% A from 7–13 min; then returning to initial conditions. Detection of phenolic compounds was carried out with a UV-visible detector at 278 nm.

**Antibacterial activities of the prepared extract of *Laurus nobilis*:**

The antibacterial properties of these extracts were evaluated on all studied bacteria using a modified method [12]. The inoculums were prepared in nutrient broth and incubated at 37 °C for 18-24 hours, with the density adjusted to the 0.5 McFarland standard. The Muller-Hinton agar plate was inoculated using sterile cotton swabs with the inoculum. Excess inoculums were removed by pressing and rotating swabs against the side of the tubes above the liquid level, and the swabs were streaked all over the surface of the application. Finally, the inoculums were dried, and 50 ml of prepared extracts were re-dissolved in DMSO and placed in 6mm diameter walls on the inoculated plates, The plates were inoculated at (37)°C for 18-24 hours, and the diameters of each zone of inhibition, including wells and discs, were measured, the data was recorded and compared with the standard antibiotics [13].

(Ax (25μg/ml) , C(10μg/ml) , SXT(25μg/ml) Also, we are using various concentrations (400-200-100-50-25-12.5) mg/cm$^3$ of plant extracts under study. The extract that produced a zone of inhibition was further studied using different concentrations of this extract, ranging from (400-12.5) mg/cm$^3$ (14,15) mm.

**Statistical Analysis**

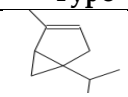
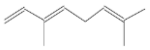
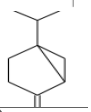
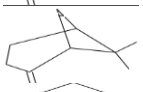
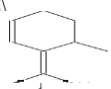
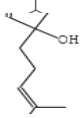
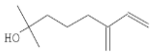
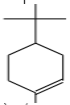
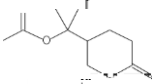
The data were statistically analyzed using the SAS statistical analysis program and a completely randomized design. Means were compared using Duncan's multiple range test at a significance level of $p \leq 0.01$.

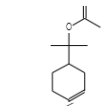
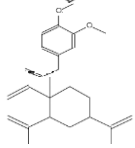
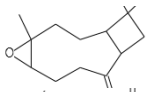
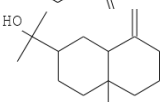
**Results and Discussion**

Through analysis and chemical examination, as indicated by previous scientific studies [15], [16]. it was found that bay leaf is rich in many effective compounds, such as terpenes and fatty acids, as well as phenolic compounds. Through the results of the current study, many effective terpene compounds and some fatty acids and other compounds were identified using a GC/MS Table (1). (Figure 1) presents the principal components identified in the L1F1 extract of Bay leaf through GC/MS analysis, with the compound concentrations expressed as Area values and Area percentages. The most abundant compound is Eucalyptol, which dominates the extract with a concentration of 97,577,880 and an Area% of 64.45%, indicating it as the major component by a significant margin. Following this, alpha.-Terpinyl acetate is the second most concentrated compound, comprising 10.67% of the extract and an Area of 16,152,295. Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- ranks third with 6.41% (Area: 9,712,108), and alpha.-Pinene follows with 4.67% (Area: 7,067,407), suggesting moderate abundance. beta.-Pinene is next, contributing 3.36% (Area: 5,093,174), while Terpinen-4-ol represents 2.29% (Area: 3,469,505). Less concentrated but still noteworthy are Benzene, 1-methyl-3-(1-

methylethyl)- with 1.60% (Area: 2,419,609) and 3-Cyclohexene-1-methanol, alpha.,alpha.,4-trimethyl-R- at 1.30% (Area: 1,963,695). Several minor constituents were also identified, such as gamma.-Terpinene (0.59%, Area: 899,553), delta.-Terpineol, acetate (0.57%, Area: 860,346), Linalool (0.55%, Area: 836,487), and Methyleugenol (0.66%, Area: 998,353). Other trace components include Cyclohexanemethanol, alpha., alpha.-dimethyl-4-methylene- (0.43%, Area: 646,488), 3-Allyl-6-methoxyphenol (0.41%, Area: 617,058), beta.-Myrcene (0.39%, Area: 594,678), Cyclohexene, 4-methyl-3-(1-methylethylidene)- (0.37%, Area: 561,977), Caryophyllene oxide (0.38%, Area: 570,059), and Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- with 0.32% (Area: 480,377). The least abundant compounds are 2-Naphthalenemethanol, decahydro-alpha.,alpha.,4a-trimethyl-8-methylene-, [2R-(2.a (0.30%, Area: 453,675) and Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (0.28%, Area: 422,886). The compositional profile highlights that the extract is heavily dominated by a few major constituents, particularly Eucalyptol, while the rest are present in much smaller quantities, suggesting a complex but uneven distribution of phytochemical components.

**Table 1.** Principal components of the L1F1 extract of Bay leaf **by** GC/MS analysis.

| Peak | R.time | Area | Area% | Name | Type |
|---|---|---|---|---|---|
| 1 | 6.389 | 480377 | 0.32 | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- |  |
| 2 | 6.553 | 7067407 | 4.67 | .alpha.-Pinene |  |
| 3 | 7.514 | 9712108 | 6.41 | Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- |  |
| 4 | 7.598 | 5093174 | 3.36 | .beta.-Pinene |  |
| 5 | 8.036 | 594678 | 0.39 | .beta.-Myrcene |  |
| 6 | 8.707 | 561977 | 0.37 | Cyclohexene, 4-methyl-3-(1-methylethylidene)- |  |
| 7 | 8.790 | 2419609 | 1.60 | . Benzene, 1-methyl-3-(1-methylethyl)- |  |
| 8 | 9.032 | 97577880 | 64.45 | Eucalyptol |  |
| 9 | 9.867 | 899553 | 0.59 | .gamma.-Terpinene |  |
| 10 | 10.927 | 836487 | 0.55 | . Linalool |  |
| 11 | 12.631 | 646488 | 0.43 | Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylene- |  |
| 12 | 13.048 | 3469505 | 2.29 | Terpinen-4-ol |  |
| 13 | 13.364 | 1963695 | 1.30 | 3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl- R- |  |
| 14 | 16.912 | 860346 | 0.57 | .delta.-Terpineol, acetate |  |
| 15 | 17.679 | 617058 | 0.41 | 3-Allyl-6-methoxyphenol |  |

| | | | | | |
|---|---|---|---|---|---|
| 16 | 17.826 | 16152295 | 10.67 | alpha.-Terpinyl acetate |  |
| 17 | 18.831 | 998353 | 0.66 | Methyleugenol |  |
| 18 | 19.201 | 422886 | 0.28 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- | |
| 19 | 23.483 | 570059 | 0.38 | Caryophyllene oxide |  |
| 20 | 24.186 | 453675 | 0.30 | 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.a |  |

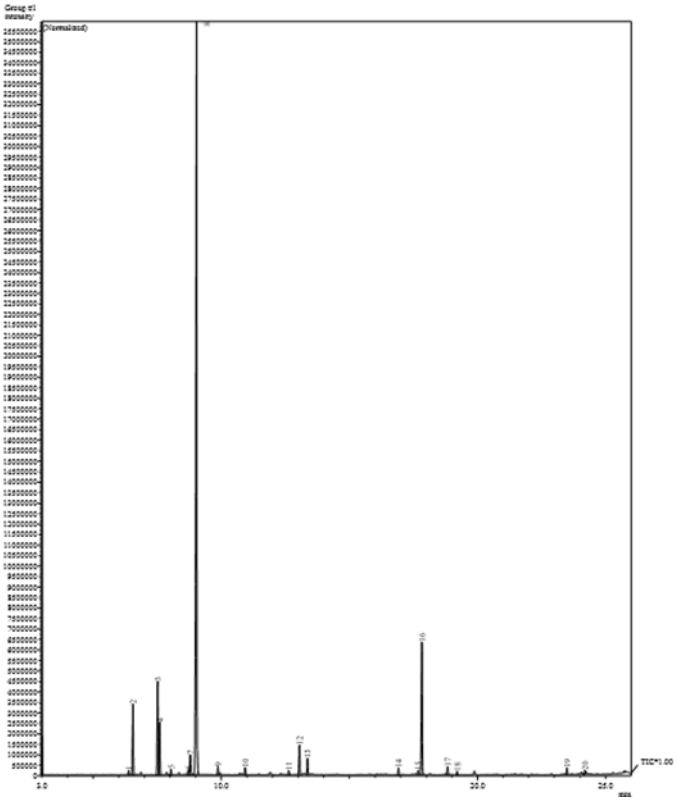


**Figure 1.** GC/MS chromatograms of Principal components of the L1F1 extract for bay leaves.

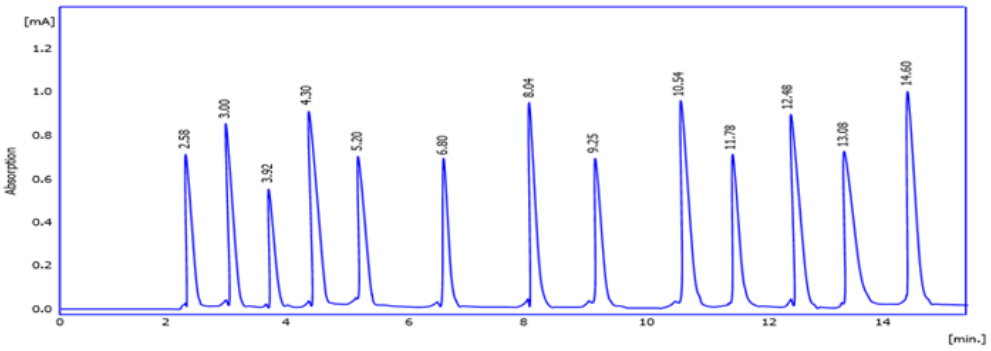
A number of phenolic compounds were also isolated and identified from the fraction (L2F1) produced from the IMS extract of bay leaves. The phenols whose names, Table (2) and Figures (2 and 3) present the results of the HPLC analysis of the phenolic compounds in the extract fraction (L2F1) from Laurus nobilis, comparing their retention times and concentrations with those of the corresponding standards. The standard phenolic compounds assessed were luteolin, kaempferol, quercetin, rutin, apigenin, and p-coumaric acid, with retention times (Rt) of 3.00, 4.30, 5.20, 6.80, 9.25, and 10.54 minutes, respectively. The L2F1 extract showed remarkably close retention times, confirming the presence of these compounds: luteolin at Rt 3.00 min with a concentration of 41.5 mg/g, kaempferol at Rt 4.32 min with 62.5 mg/g, quercetin at Rt 5.25 min with 36.9 mg/g, rutin at Rt 6.85 min with 62.5 mg/g, apigenin at Rt 9.25 min with 24.9 mg/g, and p-coumaric acid at Rt 10.85 min with a concentration of 33.6 mg/g. Among these, kaempferol and rutin exhibited the highest concentrations (62.5 mg/g each), followed by luteolin (41.5 mg/g) and quercetin (36.9 mg/g), while apigenin and p-coumaric acid were

present in relatively lower amounts. These results indicate a rich phenolic composition in the L2F1 fraction, with kaempferol and rutin being the dominant phenolic constituents.

The researcher [17] proved by means of chromatographic analysis GCMS *Laurus nobilis* leaf oil that it is rich in fatty acids and phenolic compounds, and The [18] proved the presence of 11 phenolic compounds from the *Laurus nobilis* that were identified by HPLC analysis Gallic Acid, Catechin, Coffeic Acid, Syringic Acid, Rutin, Coumaric Acid, Naringenin, Quercetin, Ferulic Acid, Vanillin, and Cinnamic Acid, and the results showed HPLC illustrated that syringic acid, Gallaic acid, and Rutin in highest concentrations respectively and phenolic compounds ranged from (0.21 - 21.42) µg/g the lowest concentration was Cinnamic, The results of the current study also agreed with the of the researcher in the diagnosis of phenolic compounds

**Table 2**. The HPLC analysis of the fraction was used to determine the Ret time of the standard and extract phenolic compounds (L2F1) from *Laurus nobilis.*

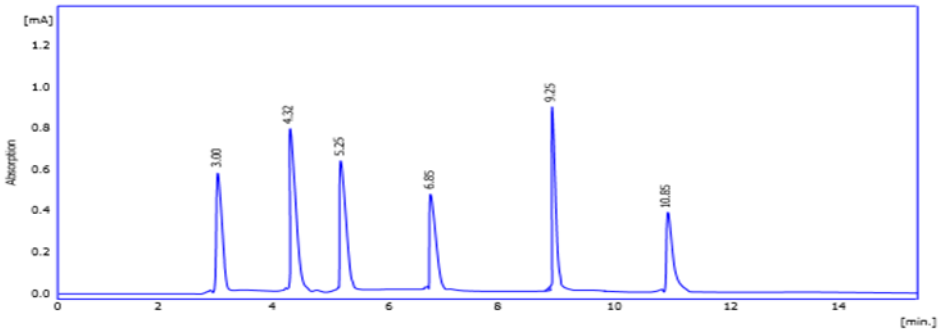
| Standard | Phenolic compounds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Luteolin** | | **Kaempferol** | | **Qurcetine** | | **Rutin** | | **Apigenin** | | **P.Coumaric acid** | |
| | $R_t$ min 3.00 | Conc. (mg/g) | $R_t$ min 4.30 | Conc. (mg/g) | $R_t$ min 5.20 | Conc. (mg/g) | $R_t$ min 6.80 | Conc. (mg/g) | $R_t$ min 9.25 | Conc. (mg/g) | $R_t$ min 10.54 | Con. (mg/g) |
| Extract fraction (L2F1) | 3.00 | 41.5 | 4.32 | 62.5 | 5.25 | 36.9 | 6.85 | 62.5 | 9.25 | 24.9 | 10.85 | 33.6 |




Result chromatography Table (Uncal - F:\ mix phenolic compound ( 10 PPM )

| No | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|---|---|---|---|---|---|---|---|
| 1 | 2.58 | 2156.24 | 730.25 | 6.25 | 6.30 | 0.15 | Gallic acid |
| 2 | 3.00 | 3214.56 | 766.98 | 8.22 | 8.15 | 0.20 | Luteolin |
| 3 | 3.92 | 5481.59 | 562.14 | 4.59 | 4.52 | 0.10 | Ferulic acid |
| 4 | 4.30 | 4120.35 | 784.55 | 8.25 | 8.22 | 0.20 | Kaempferol |
| 5 | 5.20 | 2005.98 | 730.22 | 7.25 | 7.25 | 0.15 | Qurcetine |
| 6 | 6.80 | 3201.57 | 731.25 | 8.23 | 8.41 | 0.15 | Rutin |
| 7 | 8.04 | 2212.65 | 784.58 | 9.25 | 9.33 | 0.20 | Chlorogenic acid |
| 8 | 9.25 | 3201.65 | 720.11 | 5.22 | 5.21 | 0.15 | Apigenin |
| 9 | 10.54 | 3369.58 | 784.65 | 9.32 | 9.35 | 0.20 | p-Coumaric acid |
| 10 | 11.78 | 5246.12 | 774.58 | 6.56 | 6.47 | 0.15 | Caffeic acid |
| 11 | 12.48 | 1854.55 | 732.65 | 9.25 | 9.22 | 0.20 | Curcumin |
| 12 | 13.08 | 1625.25 | 720.1 | 6.55 | 6.30 | 0.15 | Vanillic acid |
| 13 | 14.60 | 1245.98 | 785.65 | 9.25 | 9.20 | 0.20 | Hydrobenzoic acid |
| | Total | 83963.29 | 9607.14 | 100.00 | 100.00 | | |

**Figure 2.** HPLC analysis of standard phenolic compounds.




Result chromatography Table (Uncal - F:\ LA ))

| No | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|---|---|---|---|---|---|---|---|
| 1 | 3.00 | 8594.25 | 580.59 | 17.58 | 17.45 | 0.20 | |
| 2 | 4.32 | 10225.65 | 750.21 | 19.55 | 19.22 | 0.25 | |
| 3 | 5.25 | 7485.94 | 623.05 | 17.12 | 17.52 | 0.20 | |
| 4 | 6.85 | 3256.00 | 436.59 | 12.66 | 12.65 | 0.15 | |
| 5 | 9.25 | 18978.55 | 992.22 | 22.98 | 22.90 | 0.30 | |
| 6 | 10.85 | 2001.25 | 420.25 | 10.25 | 10.33 | 0.15 | |
| | Total | 90941.26 | 3762.59 | 100.00 | 100.00 | | |

**Figure 3.** HPLC analysis of phenolic compounds presented in fraction (L2F1) *Laurus nobilis.*

The table (3 and 4) and also images 1(A-H) showed the antibacterial activity of *Laurus nobilis* extracts against some pathogenic bacteria: (*Staph. aureus, Bacillus subtilis, E.coli, Psedo aeruginosa*). The crude extracts of *Laurus nobilis*, containing fatty acid and phenolic compounds, exhibited antibacterial activity compared to antibiotic compounds (Ax 25µg/ml, C 10µg/ml, SXT 25µg/m). The following concentrations of the separated active ingredients were used (400-200-100-50-25-12.5) mg/cm$^3$. This means(1, 2, 3, 4, 5, 6) consecutively in the images.

The effectiveness of the isolated fatty acids against the bacteria under study was very high, with a concentration of 400 mg/cm$^3$ against *Staph. aureus* at a concentration of 27 mm, followed by the other concentrations for all the bacterial species used, as shown in Table 3

Table 4 illustrates the results of the inhibitory activity of the isolated phenolic compounds against the bacteria under study. The activity was very high at a concentration of 400 mg/cm$^3$ against Staph. aureus (36 mm), followed by the other concentrations. For all the bacterial species used, the effect was moderate compared to the antibiotics, which had no effect on the bacteria.

The results of previous studies were similar to the results of our current study. The researcher's study [19], which determined the antimicrobial activity of the aqueous extract of Laurus nobilis leaves, showed a high effect at different concentrations against the isolated bacteria like *Staph aureus, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa* [20]. The ability of the effective compounds extracted from the leaves of *Laurus nobilis* to exhibit antibacterial activity utilizing various strategies for extraction (in vitro) [21].

The researcher N. Al-Ogaili (2020) also revealed that the ethanolic extract of the leaves of *Laurus nobilis* has a vital effect against several types of bacteria; among them [22], the results of K. A. Sakran (2021), who used the ethanolic extract of both cold and hot types, were more inhibitive than the hot aqueous extract of *Laurus nobilis* leaves against the *Pseudomonas aeruginosa* germ [23].

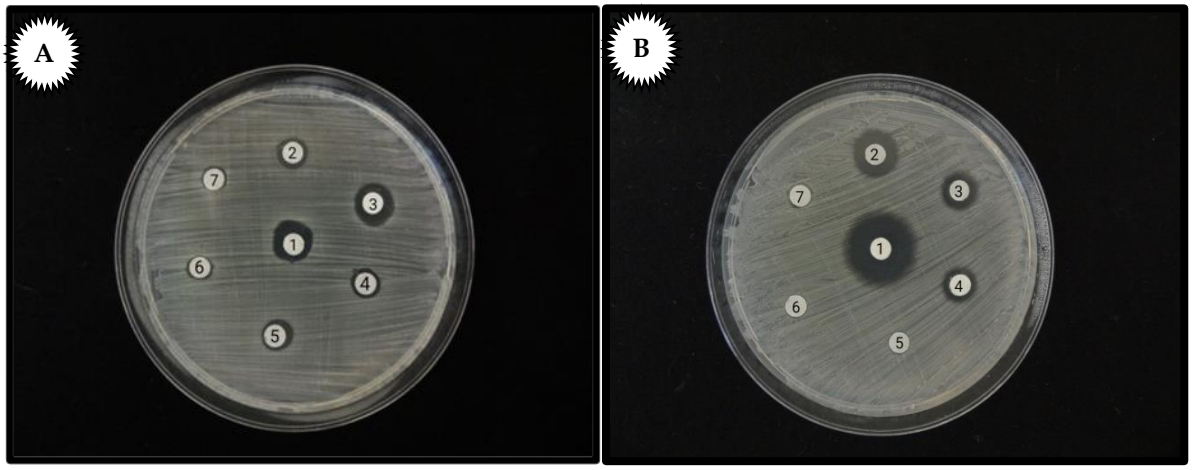
**Table 3.** Antimicrobial efficacy of (Fatty acid & terpene) of *Laurus nobilis* extract (L1F1).

| **Concentration (mg/c3) / Zone inhibition in (mm)** | **Microorganism** | | | | **Concentration Effect** |
|---|---|---|---|---|---|
| | ***Staph. aureus*** | ***Bacillus subtilis*** | ***E.coli*** | ***Pseudo. aeruginosa*** | |
| **400** | 27a ±0.5 | 18c ±0.5 | 25a ± 1.1 | 25a ± 1.5 | 23a |
| **200** | 16d ±0.4 | 12g ±1.1 | 14ef ± 0.5 | 20b ± 1 | 15b |
| **100** | 14f ±0.5 | 15de ±1 | 13fg ±1 | 15de ± 0.3 | 14c |
| **50** | 13g ±0.3 | 14ef ± 1 | 11h ± 0.5 | 10h ± 0 | 12d |

| | | | | | |
|---|---|---|---|---|---|
| **25** | 9h ± 0.6 | 12g ± 0.9 | 0i ± 0 | 0i ± 0 | 5e |
| **12.5** | 0i ± 0 | 10h ±1 | 0i ±0 | 0i ±0 | 2f |
| **Bacteria Effect** | 13a | 12b | 11c | 10d | |
| **Antibiotic compounds** | | | | | |
| **Ax (25µg/ml)** | - | - | - | - | - |
| **C (10µg/ml)** | - | - | - | - | - |
| **SXT (25µg/ml)** | - | - | - | - | - |

**Table 4.** Antimicrobial efficacy of (phenolic Compound) *Laurus nobilis* extract (L2F1).

| **Concentration (mg/c3) / Zone inhibition in (mm)** | **Microorganism** | | | | **Concentration Effect** |
|---|---|---|---|---|---|
| | ***Staph. aureus*** | ***Bacillus subtilis*** | ***E.coli*** | ***Pseudo. aeruginosa*** | |
| **400** | 36a ± 0.5 | 17e ± 0.6 | 16f ± 0.3 | 18e± 1 | 21a |
| **200** | 30b ± 1 | 10k ± 0.5 | 13hi± 0.5 | 15fg ± 0.5 | 17b |
| **100** | 25c ± 0.4 | 0L ± 0 | 12j ± 0.4 | 13ij ± 0.3 | 12c |
| **50** | 19d ± 0.5 | 0L± 0 | 13ji ± 0.5 | 0L ± 0 | 8d |
| **25** | 15fg ± 0.6 | 0L± 0 | 14gh ± 0.5 | 0L ± 0 | 7e |
| **12.5** | 0L± 0 | 0L ± 0 | 18k ± 0.3 | 0L ± 0 | 2f |
| **Bacteria Effect** | 21a | 5d | 13b | 7c | |
| **Antibiotic compounds** | | | | | |
| **Ax (25µg/ml)** | - | - | - | - | - |
| **C (10µg/ml)** | - | - | - | - | - |
| **SXT (25µg/ml)** | - | - | - | - | - |



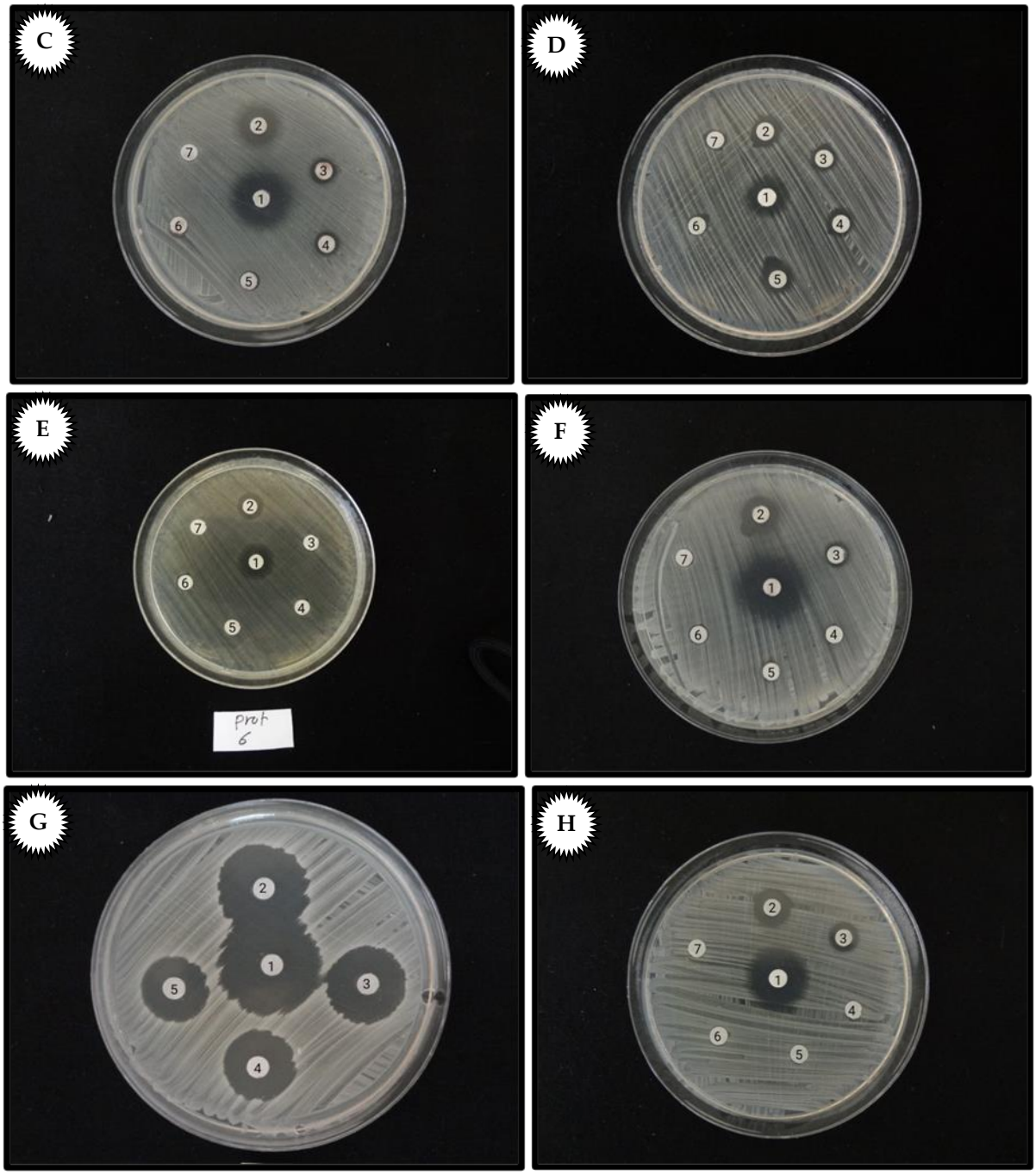



**Figure 4.** The inhibitory effect of the active compounds isolated from Laurus nobilis against the bacteria used in the study.

A. *Effect of fatty acids separated from Laurus nobilis plant on Bacillus at different concentrations.*
B. *Effect of fatty acids separated from Laurus nobilis on E.coli at different concentrations.*
C. *Effect of fatty acids separated from Laurus nobilis on Psedo. aeruginosa at different concentrations.*
D. *Effect of phenolic compounds isolated from Laurus nobilis on E.coli at different concentrations.*
E. *Effect of phenolic compounds isolated from Laurus nobilis on Bacillus at different concentrations.*
F. *Effect of fatty acids isolated from laurel staph aureus germ at different concentrations.*
G. *Effect of phenolic compounds isolated from Laurus nobilis staph aureus germ at different concentrations.*
H. *Effect of phenolic compounds isolated from Laurus nobilis Psedo. aeruginosa at different concentrations.*

## Conclusion

This study confirms that *Laurus nobilis* leaves are a rich source of bioactive terpenes, fatty acids, and phenolic compounds with notable antibacterial activity. GC–MS analysis identified eucalyptol and $\alpha$-terpinyl acetate as the dominant constituents of the petroleum ether fraction, while HPLC analysis revealed kaempferol and rutin as the major phenolic compounds in the IMS fraction. Both isolated fractions exhibited concentration-dependent inhibitory effects against Gram-positive and Gram-negative bacteria, with particularly strong activity against *Staphylococcus aureus*, in some cases exceeding the effect of standard antibiotics. These findings support the medicinal value of *Laurus nobilis* and indicate its potential as a natural antimicrobial source for pharmaceutical and related applications.

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