

Original article

Phytochemical Screening, Biochemical Composition, and Antibacterial Activity of Leaves and Stems of Lavender from Al-Jabal Al-Akhder Region, Libya

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Abstract

Lavender is a common and popular aromatic Mediterranean herb that belongs to the *Lamiaceae* family. It is a species largely used in traditional medicine for its biological properties allotted primarily to polyphenols. The leaves and stems of the Lavender plant were collected from Ain-Mara and Ras-Elhelal in the Al-Gabel Al –Kadar region during the spring season of 2024. The Phytochemical screening and antibacterial activity were applied to the extracts of the leaves and stems of *Lavandula* plants. The results showed that the phytochemical screening of aqueous and methanolic extracts of the studied samples showed that some phytochemicals are present, while others are absent, like anthraquinone, which is absent in aqueous and methanolic extracts in leaves and stems of the lavender plant. The total carbohydrate, total amino acids, total proteins, and total phenols contents (ppm) were higher in leaves than in stems of lavender plant extracts. The anti-bacterial activity was carried out on two different species (*Staphylococcus* and *E. coli*). There are small variations in the inhibition zones of the studied lavender plant. A high effect was observed for high concentrations (100 and 75 %) of *E. coli* and *Staphylococcus* of lavender leaf extract only.

Keywords. Phytochemical, Antibacterial, Lavender, Plant, Libya.

Introduction

Most of the herbal products are categorized under GRAS (Generally Recognized as Safe) for human consumption and are efficient and rarely have side effects. So extensive research on plants and their essential oils has attracted the attention of many scientists and encouraged them to screen plants to study their chemical constituents and pharmacological effects that may lead to providing new alternative treatments and drugs for industrialized purposes [1]. Since ancient times, plants have been used by the world's populations in traditional medicine and have become an essential source of treatment, especially in developing countries. The WHO has estimated that 80 % of the world's population depends on plants in primary treatment [2]. It is well recognized that medicinal plants include essential components such as enzymes, vitamins, carbohydrates, lipids, and proteins that are used in meals as well as in the treatment of ailments and act as nutritional supplements. These active chemicals are necessary for life and are involved in several physiological and metabolic processes [3].

The genus *Lavandula* includes about 50 species cultivated around the world as an ornamental and medicinal plant [4]. This plant comes from the Mediterranean area, the Middle East, North Africa, and the Republic of Cape Verde. It is also successfully cultivated in Asia, Australia, and the Americas. In Europe, the main production of lavender is concentrated in the regions of France, Bulgaria, Spain, and Ukraine. French lavender, and in fact, the raw material obtained from it, is an important item in the economic balance of this country [5]. Although there are 39 different species in this genus, *Lavandula*, only three species have high commercial value, including Lavender (*Lavandula angustifolia* Mill.), *lavandin* *Lavandula intermedia* Emeric ex Loisel., and spike lavender (*Lavandula latifolia* Medik.) [6]. Therefore, the cultivation of these species as therapeutic and aromatic herbs has increased significantly over the past few years [7]. Currently, the most famous countries for cultivating Lavender are Bulgaria, France, Italy, Spain, Turkey, Croatia, Greece, Kashmir, South Africa, and regions in Northern Africa [8].

Even today, medicinal plants are still attracting scientists for their minimal side effects and their positive effects on human health. Indeed, research has focused on the study of natural sources of bioactive compounds. Several extracts, purified fractions, and components were found to exhibit antioxidant, antimicrobial, and anti-inflammatory activity with little or no toxic effects. These include flavonoids, phenolic acids, glycosides, and tannins. In Libya, many studies were carried out to estimate the chemical constituents as minerals, metals, phytochemicals, and antibacterial activities [9-105]. This study aims to investigate the chemical compounds of Lavender extract and their antibacterial activity against some bacterial species.

Methods

In this study, the plant was collected from Ain-Mara and Ras-El-Helal in the Al-Gabel Al –Kadar region during the spring season of (2024) year. The collected samples were identified in Seliphium herbarium, Botany Department, Faculty of Science, Omar Al- Mukhtar University. The plant taxonomy was given in (Table 1).

Table 1. The taxonomy of the Lavender plant

Kingdom	Plant Lavender
Family	Lamiaceac
Genus	Lavandula
Species	Lavandula spica l

The leaves and stems of the Lavender plant were separated and washed several times with distilled water. The samples were then dried in a dark and dry place for two days at 37°C. Then the samples were ground by mortar and stored in polyethylene bottles until analysis.

Extract preparation

The Extraction was carried out according to the method described by previous studies. 10 grams of leaves and stems of the Lavender plant were weighed and mixed with 100 ml of two different solvents (distilled water and methanol) in a conical flask and kept in a rotatory shaker at 150 rpm for 4 hours. Then, the extract was evaporated under reduced pressure using a rotary evaporator apparatus and allowed to dry in the incubator until complete dryness [9-12].

Phytochemical screening

The following tests were performed to detect various phytochemical constituents, which may be present in the studied plant extracts. All the phytochemical screening tests were carried out according to standard methods [9-15]. The methods are described as follows:

Screening for Carbohydrate Test

To 1ml of extract, 1ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes solution appeared green, showing the presence of reducing sugar.

Screening for Glycosides (Keller Kilianin Test)

5ml of each extract was added with 2 ml of glacial acetic acid, followed by the addition of a few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. The formation of the brown ring at the interface confirms the presence of glycosides.

Screening for Flavonoids: (Alkaline Reagent Test)

2 ml of the extracts were treated with a few drops of 20% sodium hydroxide solution, forming an intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, indicating the presence of flavonoids.

Screening for Anthraquinones

a) Bornträger's test

One ml of each extract of the successive aqueous ammonia or caustic soda is added and shaken. Rose-red color in the aqueous layer develops in the presence of anthraquinone glycosides.

b) Modified Bornträger's test

One ml of each extract of the successive extracts of the tested herbal preparations is hydrolyzed with alcoholic potassium hydroxide, the acidified and continues as Bornträger's test. Rose-Red develops in the aqueous layer in cases of the presence of anthraquinones.

Screening for Saponins: (Foam Test)

2ml of the extract was taken in a test tube, and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Determination of Carbohydrate

It was carried out as follows: One gram of each powdered sample was defatted with petroleum ether, and then extracted with hot 80% ethanol twice. The combined extracts were evaporated until dryness; the dried residues were dissolved in 10 ml of 10% aqueous isopropanol in a volumetric flask. One ml of sample containing the equivalent of 20-100 µg glucose was pipetted into thick-walled test tubes of 16 mm-

20 mm diameter. A reagent blank containing 1 mL of water and a set of glucose standards (e.g., 25, 50, and 75 µg glucose, in a volume of 1 ml) were prepared at the same time. One ml of 5.0% (W/ V) phenol was added to all tubes and mixed. Then, from a fast-flowing stream, 5 ml of concentrated sulphuric acid was added, directing the stream of acid on the surface of the liquid and shaking the tube simultaneously, to effect fast and complete mixing. The tubes were allowed to stand 10 min, shaken, and placed in a water bath at 25°C to 30°C for 20 min. before readings were taken. The color was stable for several hours. The absorbance of the characteristic yellow color was measured at 490 nm, and followed Beer's law of a standard calibration curve of glucose.

Estimation of total soluble protein

Crude protein was determined by converting total nitrogen to total protein (Total N × 6.25), and protein was expressed as mg protein/g.

Estimation of amino acids

A dry defatted sample of 0.1 g was hydrolyzed with 10 ml of 6 N HCl in sealed tubes for 24 hrs. at 110°C. After hydrolysis, the excess HCL was removed by evaporation under vacuum with occasional addition of water. The residue was dissolved in sodium citrate buffer PH 2.2, and any insoluble matter was filtered off. The optically clear solution (30 µl) is chromatographed in an amino acid analyzer, at the unit of analysis and scientific services, faculty of agriculture, Alexandria University.

Determination of total phenols by Folin Ciocalteu Method

The phenolic content in aqueous and ethanol extracts was quantified using the Folin-Ciocalteu method, following the procedure described by previous studies with gallic acid as the standard. Briefly, 10 mL of each extract was mixed with 3 mL of distilled water and Folin-Ciocalteu reagent. Samples (barley leaves and seeds) were placed in test cuvettes, followed by the addition of 0.5 mL Folin-Ciocalteu reagent and 2 mL of 20% sodium carbonate (Na₂CO₃). After incubation for 1 minute and cooling for 15 minutes, the absorbance was measured at 650 nm using a UV-Vis spectrophotometer. The results were calculated as milligrams of gallic acid equivalent per gram of fresh weight.

Determination of antioxidant activity by the Prussian blue method

One gram of the powdered sample was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml of methanol twice, and then extracted again with 10 ml 1% hydrochloric acid: methanol (v/v). The three combined extracts were evaporated under vacuum, and the residue was dissolved in 10 ml methanol. Half a milliliter of the solution was diluted with 3 ml of distilled water, 3 ml (0.008 M) of K₃Fe (CN)₆ was added, 3 ml of 0.1M HCl, and 1 ml 1% FeCl₃. The blue color is allowed to develop for 5 minutes, and the absorbance is measured at 720 nm.

Antibacterial activity

The agar well diffusion method was followed to determine the antibacterial activity on Mueller-Hinton agar (MH). Plates were swabbed (sterile cotton swabs) with pathogenic bacteria, wells 4 mm in diameter were made in each of these plates using a sterile cork borer, and about 100 µl of different organic solvents. Were added by sterile syringe into wells. The plates were incubated at 37 °C for 18 – 24 h. The zone of inhibition was measured using a meter rule as described by previous studies.

Results

Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing various diseases. The results of phytochemical screening depend on the color observation (qualitative tests). In this study, each extract of the studied plants was screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone. The obtained results were recorded in Table 2. The qualitative phytochemical analysis examined several important phytochemicals such as carbohydrates and/or glycosides, tannins, flavonoids, alkaloids, and/or triterpenes, saponins, and anthraquinone.

The results revealed that some phytochemicals are present while others are absent, like anthraquinone, which is absent in leaves and stems of the lavender plant. The phytochemical screening of aqueous extracts of the studied plants showed that flavonoids in the stem of Lavender were found in high amounts. While tannins, alkaloids in the stem, and flavonoids in leaves and anthraquinones in leaves and stems of lavender were absent, as shown in Table 2.

Table 2. The phytochemical screening of aqueous extracts of Lavender leaves and stems.

Phytochemical screening test	Parts of Lavender plant	
	Lavender leaves	Lavender stems
Carbohydrates and/or glycosides	+	+
Tannis	+	-
Alkaloid	+	-
Flavonoids	-	+++
Anthraquinenes	-	-
Saponins	+	+

+++ = high amount, ++ = moderate amount, + = low amount, - = no amount

Table 3 showed the results of the phytochemical screening of methanolic extracts of the studied plants showed that no constituent of the phytochemical was found in high amounts. While anthraquinones were absent in the leaves and stems of the lavender plant.

Table 3. The phytochemical screening of a methanolic extract of Lavender leaves and stems.

Phytochemical screening test	Parts of Lavender plant	
	Lavender leaves	Lavender stems
Carbohydrates and/or glycosides	++	++
Tannis	++	++
Alkaloid	+	+
Flavonoids	++	++
Anthraquinenes	-	-
Saponins	+	++

+++ = high amount, ++ = moderate amount, + = low amount, - = no amount

Table 4 illustrates the total carbohydrate, total amino acids, total proteins, and total phenols contents (ppm) of leaves and stems of Lavender plant extracts. In all the previously mentioned parameters, they noticed that all values were higher in leaves than stems of lavender plant extracts; in addition to that, all the values of all parameters were lower than 1 ppm.

Table 4. The total carbohydrate and total amino acid contents (ppm) of Lavender leaf and stem extracts.

Content Sample	Total carbohydrate	Amino acids	Total protein
Lavender leaves	0.601	0.796	0.71
Lavender Stems	0.139	0.452	0.273

Table 5 presents the contents of the total phenols antioxidant activity (ppm) of the Lavender plant, which revealed that there is no good relation between total phenolic content and antioxidant activity, where total phenol in Lavender leaves was 87.81 (ppm), and the antioxidant activity was 90.50 (ppm). In contrast to total phenol in Lavender stems, which recorded 10.71 (ppm), the antioxidant activity recorded 213.44 (ppm).

Table 5. The total phenols anti-oxidant activity contents (ppm) of Lavender leaves and stems extracts.

Content Sample	Total phenols	Anti-oxidant activity
Lavender leaves	87.81	90.50
Lavender Stems	10.71	213.44

The anti-bacterial activity (mm) of aqueous extracts of Lavender leaves and stems on the selective bacteria species presented in (Table 6). The anti-bacterial activity studies were carried out on two different species (*E.coli* and *Staphylococcus*). In this study, two different extracts were applied (aqueous and methanolic extracts). The results of the anti-bacterial activity (mm) of aqueous extracts of Lavender leaves and stems showed different effects of plant extracts. There are small variations in the inhibition zones of the studied plants; high effect was observed for high concentrations (100 and 75 %) for *E. coli* and *Staphylococcus* of Lavender leaf extract only. Generally, the values of inhibition zones were fluctuated between (5 -10 mm) for Lavender leaves and (5-7) mm for the stems of Lavender, respectively.

Table 6. The anti-bacterial activity by inhibition zone (mm) of aqueous extracts of Lavender leaves and stems on the selective bacterial species.

Plant Extract Type of bacteria	Lavender Leaves				Lavender Stems			
	%100	%75	%50	%25	%100	%75	%50	%25
Staphylococcus	5	8	5	7	5	3	5	7
E-coli	8	10	7	5	5	3	5	0

The anti-bacterial activity (mm) of aqueous extracts of Lavender leaves and stems on the selective bacteria species presented in (Table 7). The results revealed that most of the inhibition zones were higher than the inhibition zones of aqueous extracts. Lavender leaves at 75 % recorded the highest inhibition (15 and 12 mm) for Staphylococcus and E-coli respectively.

Table 7. The anti-bacterial activity by inhibition zone (mm) of methanolic extracts of Lavender leaves and stems on the selective bacterial species.

Plant Extract Type of bacteria	Lavender Leaves				Lavender Stems			
	%100	%75	%50	%25	%100	%75	%50	%25
Staphylococcus	7	15	9	8	14	8	7	9
E-coli	9	12	8	6	11	7	6	5

Discussion

Some studies used the phytochemical screening of hydroalcoholic extract of *Lavandula angustifolia* shown the presence of pharmacologically active substances such as tannins, flavonoids, saponins, terpenoids, carbohydrates, cardiac glucosides, alkaloids, coumarines, steroids, and proteins [106]. Medicinal plants contain some organic compounds that provide definite physiological action on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids [107]. These compounds are synthesized by primary or, rather, secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions. They are widely used in human therapy, veterinary, agriculture, scientific research, and countless other areas [108]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, and seeds [109].

It was reported that preliminary phytochemical screening of the crude extract of aerial parts, leaves, and flowers of lavender revealed the presence of different phytochemical classes. Flowers contained a markedly higher amount of terpenoids, flavonoids, and cardiac glycosides in aerial parts than in leaves. While aerial parts were richer in steroids than flowers and leaves. Proximate and nutrient analysis determine the nutritional significance of medicinal plants [110]. As various medicinal plant species are also used in food, assessment of their nutritional significance can help analyze the significance of these plant species [111]. Carbohydrates are the primary source of dietary energy and are essential for various physiological and metabolic functions. In subsistence communities with limited access to market foods, energy requirements are predominantly met through plant-based diets [112 & 113]. Several studies have demonstrated that many wild edible plants have carbohydrate concentrations that rival or even surpass those of cultivated crops. This is particularly evident in storage organs like rhizomes and tubers, as well as photosynthetic parts like leaves and flowers.

Proteins are large organic molecules made of amino acids and are vital for high-quality nutrition. They are essential for maintaining muscle mass, supporting immune responses, and aiding cell repair. Protein can be obtained from two main sources: animal and plant sources [114]. Animal protein, although higher in demand, is generally considered less environmentally sustainable. Therefore, a gradual transition from animal- to plant-based protein food may be desirable to maintain environmental stability, ethical reasons, food affordability, and greater food safety, fulfilling higher consumer demand, and combating protein-energy malnutrition. Due to these reasons, plant-based proteins are steadily gaining popularity, and this upward trend is expected to continue for the next few decades. Plant proteins are a good source of many essential amino acids, vital macronutrients, and are sufficient to achieve complete protein nutrition [110]. Polyphenols of plant origin comprise a wide variety of molecules that have a polyphenol structure (i.e., several hydroxyl groups on aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another [111]. Some studies reported that the phytochemical screening of hydroalcoholic extract of *Lavandula angustifolia* showed the presence of pharmacologically active substances such as tannins, flavonoids, saponins, terpenoids, carbohydrates, cardiac glucosides, alkaloids, coumarines, steroids, and proteins [112].

Positive high correlation between phenolic acid contents and the ability to reduce DPPH indicates that the phenolic compounds are the main co-authors of the antioxidant activity of *Lavandula angustifolia*. Previous research has investigated the relationship between total phenolic content and antioxidant capabilities of a variety of plants. The antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was evaluated by the agar diffusion method, finding inhibition halos of 10 mm and 9 mm, respectively. This demonstrates that the bioactive compounds contained in *Lavandula officinalis* are an alternative to the synthetic compounds currently used in the cosmetic, food, and pharmaceutical industries due to the properties they possess [113].

The antibacterial action of *Lavandula officinalis* is attributed to polyphenols, which produce an enzymatic inhibition of oxidized compounds, by reactions of sulfhydryl groups and by nonspecific interactions with proteins. It has been proven that polyphenols damage the integrity of the bacterial cell membrane, with a greater effect on Gram-positive bacteria, which are more susceptible to the antimicrobial action of bioactive compounds [114]. The bioactive compounds present in the extracts directly affect the bacterial cell membrane, and the cytotoxic activity directly affects the mitotic phase of gram-positive and gram-negative bacteria. Microorganisms such as *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* are susceptible to the bioactive compounds of *Lavandula officinalis* extract, where rosmarinic acid, carnosol, flavonoids, caffeic acid, and carnosic acid prevail [115]. Some studies found good positive linear relationships; others found poor linear correlations or could not explain the relationship between total antioxidant activity and Phenolic concentration as reported [115].

Conclusion

The leaves and stems of lavender plant are a good raw material for many industries, including cosmetics and pharmaceuticals due to moderate content of phytochemical constituents, in addition leaves and stems of lavender plant had high nutritional value for their total carbohydrate, amino acid, protein phenols contents, for the antibacterial activity, The leaves and stems of lavender plant showed different effects for E-coli and *Staphylococcus* where lavender leaves at 75 ppm recorded the highest inhibition zone (15 and 12 mm) for *Staphylococcus* and E-coli respectively.

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Conflict of Interest

The authors declare that there are no financial, personal, or professional relationships that could be perceived as influencing the results or interpretations presented in this work.

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