Biochemical Characterization of *Laurus nobilis* L. Leaf Extracts and Evaluation of Their Inhibitory Activity Against Selected Pathogenic Bacterial Isolates

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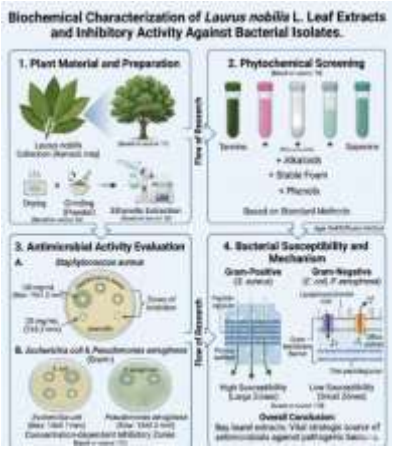
Abstract

The bay laurel (*Laurus nobilis*) belongs to the laurel family and is considered one of the most important medicinal and aromatic plants with significant economic and therapeutic value. This study aims to achieve two main objectives: to evaluate the phytochemical composition of bay leaves and to determine the natural bioavailability of compounds found in these leaves as potential alternatives to synthetic compounds. Reviews of scientific literature and previous studies have confirmed that the leaves contain several classes of secondary metabolites, such as flavonoids (e.g., apigenin, quercetin, and luteolin), alkaloids, tannins, and volatile essential oils. It was concluded by research that those components of bay extract had significant antibacterial/antimicrobial activity against a wide variety of pathogenic microorganisms including Gram positive & Gram Negative bacteria .The results show a clear positive correlation; as the concentration of bay leaf extract increased from 25 mg/ml to 100 mg/ml, the diameters of the inhibition zones increased for all tested strains. The highest inhibition value was reached at a concentration of 100 mg/ml for *S. aureus*, at 19/1.2 mm**.**

So, this indicates the potential use of bay laurel oil & extracts in food safety as a natural antimicrobial agent or preservatives. The research explored the uses of bay in traditional medicine to ease neurological conditions such as seizures, migraines and headaches etc. There is still much to do in terms of extracting the bioactive compounds from bay so that we can maintain their bioactivity with the use of modern technologies, therefore it is important to invest in bay & its use in the medical/pharmaceutical fields as a safe, renewable source of natural antioxidants.

Keywords: Biochemical, *Laurus nobilis* L., Inhibitory Activity, Pathogenic Bacterial



Graphic abstract

Introduction

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The noble bay (*Laurus nobilis* L.), The bay tree, an evergreen member of the laurel family (Lauraceae), comprises some of the most well-known species of evergreen trees found in the Mediterranean basin where it is native, although it has a large distribution into the tropics and many subtropical areas, primarily in East Asia and the Americas. The bay tree has a conical crown and can grow to heights of about 12-18 m. The leaves are lanceolate, thick and leathery, with a shiny green surface and have a strong and aromatic aroma from the large number of volatile oils contained in them. The genetic resources of the bay trees in the Levant, particularly in Syria, are strategically important because of their biodiversity; they form a strong base to confront climate change. However, local populations of these bay trees suffer from human and environmental stresses, making accurate documentation of local genetic types necessary to sustain the populations and genetic diversity of the species. Research has demonstrated that the variation in bay laurel group morphology and phenology is directly impacted by ecological conditions as well as by heredity; bay laurel grows in areas that experience very different climatic environments (e.g., from wet coastal to arid mountain sites). In terms of geological altitude from sea level; these trees can be found at an approximate elevation of one thousand three hundred fifty meters [7,8].

Bay leaves and their respective fruits are secondary metabolite-rich food sources. Some of the flavonoids found within bay trees are apigenin, quercetin and luteolin; other important compounds include alkaloids, glycosides and tannins. In addition to these components, bay leaves have large amounts of tocopherols (vitamin E), vitamins A, C, and B9, as well as important minerals like copper, iron, and potassium. A nutritional analysis has shown that 100 grams of dry bay leaves provide an energy content of around 313 calories, along with a high content of both fiber and carbohydrates. Bay leaves and fruits have long been used as traditional remedies in relation to nervous disorders such as epilepsy and migraine headaches. Bay leaves have also been used as diuretic and alleviating pain resulting from any type of gastrointestinal problem or rheumatism. A recent scientific study indicated that aqueous extracts of bay leaves were effective treatments for experimentally induced diarrhea, suggesting that plants can safely be an alternative to chemical medications. Additionally, The bay has a very wide range of biological activity against bacteria, yeast and mold [16]. Bay has long been associated with various traditional industries, such as the production of "Bay Soap," which has been manufactured in Aleppo for thousands of years, and as an aromatic flavouring in the food industry [17,18]. Due to these diverse applications of bay and differences in its morphological and chemical characteristics due to environmental factors [19,20], a thorough and comprehensive analytical profile of this plant is needed; therefore this study will analyze the qualitative properties (value) of the active constituents of the bay laurel plant and determine the effect of an ethanolic extract of bay leaves on some bacterial pathogens.

Materials and methods

sample collection

plant *Laurus nobilis* (leaves) obtained from the local markets of Ramadi city and confirmed the diagnoses and classifications at the Museum of Natural History / Baghdad. The theme of drying the leaves and grinding them well to a fine powder in preparation for the extraction process [21], agricultural environments, dyes, solutions and solvents: the theme of preparations as needed and according to standard standards, the bacteria that cause the disease, the samples are obtained from the isolates of the bacteria characteristic of the disease.

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Preparation of plant extract

plant leaves *(L. nobilis*) From selected sites the samples were air dried at room temp. and ground in an electrical grinder to form a very fine powder. The active ingredients were extracted with distilled water (1/10 vol.) which was Stirred Continuously for Twenty-four Hours. The extract was filtered through filter paper (Whatman No. 1) to prepare the raw extract which was concentrated using a rotary evaporator[22].

Chemical Analysis

Perform specific chemical tests to detect the active groups in the leaf extract according to standard approved methods[23], as follows:

Detection of tannins: Add 1 ml of 1% ferric chloride (FeCl3) solution to 2 ml of the extract; the appearance of a dark green or blue color indicates the presence of tannins.

Detection of carbohydrates: Using the Mollich test by adding α-naphthol reagent followed by concentrated sulfuric acid; the appearance of a purple ring at the interface between the two samples indicates a positive result.

Detection of alkaloids: Using Meyer's reagent; the appearance of a creamy white precipitate indicates the presence of alkaloids.

Detection of flavonoids: By adding a small amount of concentrated hydrochloric acid and magnesium sulfate (Shinuda test); the appearance of a pink or red color indicates the presence of flavonoids.

Detection of saponins: Add 5 ml of the concentrated extract to a test tube; the appearance of a dark, stable foam for 15 minutes indicates the presence of saponins.

Detection of phenols: By adding a solution of alferlic acid, potassium cyanide, and ferric chloride. The appearance of a greenish-blue color indicates the presence of phenolic compounds.

Antimicrobial activity

To assess the antibacterial activity of prepared noble bay (*Laurus nobilis*) leaf extracts, the susceptibility of targeted pathogenic bacterial isolates was quantitatively evaluated using the standard disc diffusion method on agar medium. The pathogenic bacterial strains used in this study were clinically obtained from local hospital laboratories in Anbar Governorate, confirmed using the automated VITEK® 2 system, and maintained on slanted nutrient agar media at 4°C for subsequent experimental steps. Sterile filter paper discs (6mm in diameter) were thoroughly impregnated with specific concentrations of the plant extracts and carefully placed on the surface of Mueller-Hinton agar plates pre-inoculated with bacterial suspensions modified according to the McFarland turbidity standard of 0.5. After a 24-hour incubation period at 37°C, the antibacterial activity was determined by measuring the apparent inhibition zones (in millimeters) around each disc using a digital vernier caliperometer. All biological tests were performed in triplicate to ensure statistical reliability[24].

Statistical Analysis

The experiments were conducted using a completely randomized design (CRD). Results were reported as mean ± standard deviation (SD), based on three replicates per treatment. Comparisons of extract concentration (25, 50, and 100 mg/ml) and positive control (ampicillin) were made using appropriate statistical tests. There were statistically significant differences between concentrations and the diameter of the inhibition zone for all bacterial strains studied. A clear positive correlation was demonstrated between increasing concentration and the size of the inhibition zone for all bacterial strains studied**.**

Results and Discussion

Chemical Content

The findings of this inquiry, and the supported references found in the literature, indicate that *L.*

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nobilis represents an excellent resource due to its dual nutritional and medicinal properties. The variety of flavonoid constituent compounds − such as apigenin, quercetin and luteolin − along with alkaloidal plant constituents in its leaves and fruits contribute to the physiological properties of many antioxidant and anti-microbial benefits associated with noble bay. Additionally, the current study demonstrates there is agreement with the past works of researchers [24], documenting that noble bay's phytochemicals possess an extensive range of inhibiting activities against multiple strains of bacteria, yeast and fungus, therefore representing significant potential for either food preservation or in the pharmaceutical industry.

The study identified through morphological analysis that variation in characteristics of leaf size and growth across various geographical areas of Syria and the Levant was due to an interaction between genetics and the environment (climate, temperature, relative humidity). The wide range of genetic variation reported for cave populations in Syria, Lebanon, and Turkey by [25,26] also shows that the species exhibits a remarkable ability to adapt to its environment from coastal areas through to the mountainous highlands [27]. The plant's ability to tolerate extreme cold weather as recorded in 1985 [28] is also an indicator of the genetic resilience of the species, which makes it to be an extremely important species to be used in agricultural improvement programs to reduce the impact of climate change [29].

The results of the medicinal extract of laurel leaves for the treatment of digestive ailments and the control of diarrhea confirm the traditional use of this herbal remedy as a carminative, a digestive aid, and an analgesic [7,30]. The therapeutic action of the three major essential vitamins contained in laurel (vitamin A, vitamin C and vitamin B9) along with the major mineral content of potassium and iron has been shown to increase the overall value of laurel as both a pharmacological agent and as a calorie and cholesterol-free dietary supplement.[32] The economic history of Maryland's production of "laurel" soaps demonstrates the investment potential of extracting essential oils from laurel plants. The significant differences in nutritional components (protein, fat and carbohydrate) as well as active ingredient concentration in a variety of extracts (ethylacetate, hexane and oleic acid) from laurel bind the extracted target compounds to their specific intended industrial or medical use[33].

Table 1: Qualitative Analysis of Active Compounds

No.	Test type	qualitative result
1	Tannins	+
2	Carbohydrates	+
3	Glycosides	+
4	Phenols	+
5	Resins	-
6	Flavonoids	+
7	Saponins	+

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8	Alkaloids	+
9	Proteins	-

There is a current interest in the potential enhancement of the use of aqueous extracts containing beneficial bioactive materials. This analysis summarises how the chemical composition of extracts from the noble bay plant (*Laurus nobilis*) differs throughout its range. In one instance, of the many compounds reported, 1,8-cineole has been documented to be the most abundant with an average concentration of 81.89% in the northern Adriatic Sea; moreover previous studies found that 1,8-cineole to be present at high concentrations in bay leaves harvested from different regions of the planet [34]; however, alpha-pinene, e-caryophyllene, and methyleugenol were observed to be more frequently present in southern samples. The level of methyleugenol is important because it is a phenolic compound which has been demonstrated to demonstrate antimicrobial and antioxidant activities, and the expressions of phenolics are impacted by environmental factors including temperature and the amount of sunlight received [35]. The above observational data supports the conclusion that sources of origin greatly influence chemical profiles, probably through multiple interactions between genes and local environmental pressures/conditions. The results will differ in respect to the chemical activity depending on regional sources of origin, which reinforces the importance of optimizing regional differences for both harvesting and extraction methods. [36] **.**

Inhibitory Activity of Leaf Extracts

Table 2 shows that studying the inhibitory activity of plant extracts is a crucial step in the search for natural alternatives to traditional antibiotics, especially given the increasing phenomenon of bacterial resistance. The table below presents the results of the disk diffusion method to evaluate the effect of bay leaf extract (*Laurus nobilis*) at different concentrations on the growth of three pathogenic bacterial strains. The standard antibiotic ampicillin was used as a positive control to provide a comparative basis for evaluating the relative antibacterial efficacy of noble laurel extracts. All strains tested displayed a positive correlation between concentration of bay leaf extract and increase in diameter of inhibition zone, from 25 mg/ml to 100 mg/ml with the greatest increase in diameter being seen with S. aureus at a concentration of 100 mg/ml = 19/1.2 mm. Increased concentration of bay leaf extract allows for more phytochemicals to be deposited into the areas between cells and therefore provides a greater concentration available to penetrate the bacterial cell wall and interfere with its critical processes. The results also indicate that Gram positive bacteria are more sensitive to bay leaf extract than their Gram-negative counterparts (i.e., *Staphylococcus aureus* vs. *E. coli* or Pseudomonas aeruginosa) which can be attributed to the differences in the structure of their bacterial cells.

Peptidoglycan represents the primary structural element of the cell wall of gram-positive cells, being the element in which chemical constituents of the extract can easily pass through to enter into the cell due to the thickness of the peptidoglycan of gram-positive cells; Whereas gram-negative cells utilize an outer membrane made of lipopolysaccharides, which is the barrier that keeps large/hydrophilic chemicals from passing through to the inside of the cell; thus, this helps to account for their higher resistance and the smaller zone of inhibition therefore, of these two bacteria isolates.

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A study shows that bay leaf extracts have selective inhibition depending on both bacterial isolates and extract concentrations; the basis of this ability comes from phenolic compounds found in bay leaves disrupting plasma membrane permeability, inhibiting cellular enzymes, and interfering with bacterial genetic material.

Table 2: Effect of *L. nobilis* leaf extract on some bacterial species

Bacterial Species	antibiotic ampicillin	Concentration 100 mg/ml	50 mg/ml concentration	25mg/ml concentration
Staphylococcus aureus	22 ±0.4	19 ±1.2	15 ±0.8	12 ±0.5
Escherichia coli	18 ±0.6	14 ±0.7	11 ±0.5	9 ±0.3
Pseudomonas aeruginosa	16±0.3	13 ±0.5	10 ±0.4	7 ±0.2

. *aeruginosa* at the highest concentration tested (25 mg/ml), the extract had the least degree of inhibition (7 mm) against the S. aureus isolate. This relatively low level of inhibition can be attributed to the general resistance of the microorganism and several factors contributing to the overall amount of this resistance including efflux pumps capable of excreting foreign material from the bacterium at the time of entry or low permeability of the outer membrane of the organism. While the extract had a greater degree of inhibition than the standard antibiotic (positive control), the diameter produced with the 100 mg/ml extract (19mm) was similar to that produced by synthetically produced antibiotics suggesting that bay leaves could potentially be a source for the development of antimicrobial agents following purification of their active components.

The assessment of the presence of phenolic compounds in spice and herb substances that are found in different types of essential oil is receiving more attention for their many different functions such as antioxidants, antimicrobial properties, and flavoring abilities. Bay is part of the laurel plant family which originates from the Mediterranean area. The laurel plant family includes many different types of aromatic plants that are widely used in the Mediterranean region's cuisine as well as for their medicinal purposes in treating various types of diseases (including various forms of infections). The noble bay (Laurus nobilis) is another member of the laurel plant family and is an aromatic tree that can reach a height of 2–10 meters. The noble bay contains approximately 1.3% essential oils, mono- and triterpene polar flavonoids, alkaloids, glycosylated flavoring agents, megastigmans, and phenolic compounds. It has been established that the noble bay exhibits many different pharmacological effects to include antimicrobial, cytotoxic, and immunomodulatory effects. The essential oil of the bay contains eucalyptol, alpha-terpenyl acetate, linalool, methyl eugenol, sabinene, and carvacrol. The composition of each essential oil is influenced by where it is grown, how high up, how many hours sunlight, and conditions of growth [37,38]. The compounds found in bay laurel essential oils provide high levels of antibacterial activity against foodborne pathogens (such as Salmonella, Staphylococcus aureus, Escherichia

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coli, and Listeria monocytogenes), as well as spoilage bacteria (for instance *P. aeruginosa*), and antifungal activity as well (38). The high levels of antibacterial activity found in bay laurel essential oils are due to the combined effects of terpenes (linalool), lactones, oxides (1,8-cineole) and monoterpenes (camphene and alpha-pinene). All of these essential oils contain a variety of chemical structures, either alone or together with many other chemicals or at many different concentrations and have a unique way of inhibiting many pathogens by changing the way that the cells are made up, breaking down proteins and inhibiting the action of various enzymes. Although bay leaf oil has been studied for its antibacterial properties, it does not negatively affect beneficial bacteria in the gut. Prior studies show that bay leaf compounds from the Adriatic coast have demonstrated antibacterial activity against two different types of bacteria: Staphylococcus aureus and Streptococcus pyogenes. These two types of bacteria represent the Gram-positive family of bacteria and are typically associated with skin infections. The gram-negative bacteria used in the test were *E. coli* and *P. aeruginosa*. Additionally, *Candida albicans* was used in the test because it commonly occurs in the skin ,40,] [7 ,39.

Figure 1: Effect of the highest concentration(100mg/ml) of plant extract on *Staphylococcus* bacteria (right), *E. coli* (left)

extract and greater inhibitory activity against the isolated bacteria. Also, there was a variation in the sensitivity to the alcoholic extract within the species of bacteria tested; however, the Gram positive species exhibited greater sensitivity compared to the Gram-negative species. (*Staphylococcus aureu*s) showed a greater response to the extract compared to Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), attributed to structural differences in cell wall permeability.

Conclusions

This study concluded that bay laurel *L. nobilis* is an important strategic provider of secondary metabolites with excellent therapeutic and food value. Qualitative analysis of the plant's leaves demonstrated that they contain several different types of bioactive compounds, with flavonoids, tannins, alkaloids and volatile oils being the most abundant. Experimental results clearly show that the alcoholic extract of the plant exhibits bactericidal effects against the isolated bacteria tested. Results showed a strong correlation between higher concentrations of alcoholic

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