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Antibacterial Activities of Bay Leaf (*Laurus nobilis*) On Some Bacterial Isolates

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ABSTRACT

Laurus nobilis is one of the most well known most, frequently used plants is from Lauraccaefamily which contains up to 2,500 species that grows in the subtopics and tropics of the Mediterranean region and Indonesia. This study was supposed to investigate the antimicrobial effect of L. nobilis leaves ethanol extract on Staphylococcus aureus, Salmonella typhi and Escherichia coli. This preliminary study examined the antimicrobial effect of L. nobilis leaves ethanol extract. The method used is Agar Well diffusion for determination of the zone of inhibition and the minimal bactericidal concentration to investigate the activity of L. nobilisleaves ethanol extract at 100% concentration. The results revealed that the extract of L.nobilisleaves had antibacterial activity against Staphylococcus aureus with a zone of inhibition (16.3 ± 1.5mm), Salmonella typhi with (14.5±0.5mm) and weak antimicrobial activity against Escherichia coli (11.3±1.1mm). Also, through the minimum bactericidal concentration experiment, the L. nobilis leaves ethanol extract had activity against Staphylococcus aureus and Salmonella typhi, it killed the bacteria in all concentration. Starting from 5 x10^{7} to 5 xl0^{4}. But the activity on Escherichia coli just weaken concentration 5x10^{7} and 10^{6}. This research has concluded that the L. nobilis leaves ethanol extract exhibited a significant antimicrobial effect against Staphylococcus aureus and Salmonella typhi than Escherichia coli that is considered a kind of multidrug resistant bacteria.

INTRODUCTION

Spices, herbs, and essential oils have drawn attention due to their many uses, including their capacity as antioxidants, antimicrobials, and flavoring agents, phenolic compounds found inthem. The bay leaf is unique to the Mediterranean region and a member of the Lauraceae family.A member of the Lauraceae family, the aromatic plant is commonly employed in Mediterranean cuisine as a spice and in traditional medicine to cure a number of infectious diseases. (Sirikene*t al*., 2018).

Pathogenic microorganisms that are resistant to multiple antibiotics (MDR) have rapidly grown in both humans and animals. Therefore, infections brought on by resistant microorganisms have

affected public health and presented a risk to humans. Researchers began looking for new methods of illness prevention or treatment as a result of the microorganisms' resistant characteristics. These days, antibiotics can be replaced by compounds derived from plants such as essential oil. Some Gram negative and positive microbes are resistant to the antimicrobial effects of bay laurel, cinnamon, oregano and clove-like plants (Yilmaz*et al*., 2019).

As a fragrant shrub and evergreen tree in the Lauraceae family, laurel (L.) *nobilis*, also known as bay leaf, is one of the most popular culinary spices in all Western and Asian nations. In addition to Mexico and other temperate and warm regions of the world, it is grown and is native to the Mediterranean nations of Turkey, Spain, Morocco, Greece, and Portugal. It ranges in height from two to ten meters (Da Sheila *et al*., 2020).

The plants are naturally grown between 600 and 800 meters 'above sea level in coastal regions. The leaves and berries of the plant are frequently used to flavor and improve meals, particularly meats, sauces, and soups (Yilmaz*et al*., 2019). In addition to its distinctive perfume, it is used to treat illnesses all over the world. Other than spoilage bacteria, this plant's components, including its essential oils and organic acids, have demonstrated potent antibacterial, activity against severalfoodborne pathogen microorganisms (Algabri*et al*., 2018).

Essential oils are hydrophobic liquid containers that may be extracted from a variety of plant parts, including flowers, seeds, and stems. Essential oils are employed as flavoring agents in the cosmetic and food sectors due to their fragrant properties (Yilmazel al., 2019). Additionally, it has biologic properties like antibacterial, anti-diabetic, and anticancer activity (Adesetan*et al*.,2020). The benefits of essential oils as natural antimicrobialshave thus been demonstrated. By altering membrane permeability, denaturing proteins, and inhibiting enzymes, these oils' diverse chemical compositions or individual components at various quantities have various inhibitory mechanisms that can affect a range of pathogens. Additionally, it has shown useful in combating drug-resistant types of bacteria (Algabri*et al*., 2018). The aim of this study is to determine the antibacterial activities of bay leaf (*Laurusnobilis*) on some selected bacterial isolates.

MATERIALS AND METHODS

Collection of Bay Leaves (*Laurusnobilis*)

The plant material for the study was purchased from the local markets in Bida, Niger state. It was cleaned and washed with running tap water so that all the dust and dirt are removed. At room temperature, leaves were air-dried and then dried in a hot air oven for 2 hours. The weight of dried leaves was taken and recorded. Thereafter, it was crushed by the mixer to get a fine powdered form and the weight of the powder was also taken and recorded (Bharadwaj*et al*., 2022).

Extraction of Active Components of Bay Leaf (*Laurusnobilis*)

Extraction was carried out using the Soxhlet apparatus. About 30g of leaves powder was taken for each aqueous, acetone, and methanol solvent. Powdered plant material (30g) were uniformlypacked in the respective thimbles and covered .with cotton wool, the thimble having

sample wasthen placed in the extraction jacket and the continuous extraction was carried out by using respective solvents, i.e., double distilled water (700ml), acetone (500ml) and methanol (500ml). The extraction was done till the color of the solvent in the siphon tubechanged to colorless.

The temperature of Soxhlet was maintained at approximately 60-70°C for aqueous extraction, 30-40°C for acetone, and 50°C for methanol extraction. After the extraction is complete, the thimble was removed and again switched on the Soxhiet, and the purified solvent was extracted out from the extract which will be used for the further study. The extract was then poured on a 200nm Petri dish and put overnight in an incubator to incubate it at 50°C till the solventevaporate, and nal1y, the extract was collected by scratching it from the plate. Then the weight of the extract was obtained and stored in a sterile vial or sample container (Bharadwaj*et al*., 2022).

Phytochemical Screening of (*Laurusnobilis*)

Test for Alkaloids

Hager's test was used for screening the presence of alkaloids in the extract. A saturated solution of picric acid (TNP) was made and then mixed with 2 ml of the respective extracts. The appearance of yellow ppt. indicates the presence of alkaloids (Bharadwaj*et al*., 2022).

Test for Cardiac Glycosides

For this, 2ml of glacial acetic acid was collected, and a drop of $FeC1_3$ solution was added into it. In this solution, 2ml of plant extract and 1ml of concentrated H_2SO_4 was also added. The formation of a brown ring at the interface specify the presence of de-oxy sugar i.e. thecharacteristics of cardenolide (Eze*et al*., 2021).

Test for Saponins

2m1 of water was added to the dried extract and shake vigorously. The appearance of foam indicates the presence of saponins (Taoheed*et al*., 2020).

Test for Tannins

2ml of extract was mixed with a few drops of 1% $FeCI_3$, and the appearance of blue-black precipitate in the solution confirms the presence of tannins (Bharadwaj*et al*., 2022).

Test for Steroids

The presence of steroids was screened by Salkowski Test. For this, chloroform was added into the crude extract and this was followed by the addition of a few drops of concentrated H_2SO_4, it was mixed and allowed to stand for some time. The presence of steroids confirms the appearance of red color at the lower layer while the presence of tri-terpenoids confirmed by yellow color layer formation (Taoheed*et al*, 2020).

Carbohydrates

For carbohydrate screening, Molisch's Test was carried out, in this, 2ml of sample was taken in a test tube and a small amount of Molisch's reagent (∞ naphthol dissolved in ethanol) was mixed. This was followed by the addition of concentrate H_2SO_4 slowly through the wall of the test tube, so a ring was formed at the bottom layer; formation of a bluish-violet ring at the junction confirms its carbohydrates (Taoheed*et al*, 2020).

Proteins

2ml of extract was taken in a test tube and boiled with Ninhydrin, development of a violet color confirms the presence of proteins (Bharadwaj*et al*., 2022).

Phenolic Compound

2ml of extract was mixed with the Bromine water and the presence of yellow precipitate confirms the presence of phenolic compounds (Taoheed*et al*, 2020).

Terpenoids

2ml of chloroform was taken and concentrated H_2SO_4 was carefully added to form a layer. 2ml of extracts was added to it. The appearance of reddish-brown color at the interface authenticates the presence of terpenoids (Bharadwa.j*et al*., 2022).

Flavonoids

2ml of 2% NaOH was added to the extract which produces yellow color which get disappeared when 2-3 drops of dilute acid was added, this is the best test for the screening of the presence of flavonoids (Taoheed el al, 2020).

Antibacterial Activity of Different Bay Leaf Extracts

Test Microorganisms

Escherichia coli, *Salmonella typhi* and *Staphylococcus aureus* are the bacterial strains that were used for the analysis of antimicrobial activity of bay leaf.

Preparation of different Concentrations of Bay Leaf Extracts Different extracts (acetone, methanol, and aqueous) was prepared. The dried extracts will be diluted with appropriate solvents to make different final concentrations (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) respectively (Bharadwaj*et al*., 2022).

Preparation of Bacterial Inoculum

A loopful culture of each bacterium was inoculated into 4-5ml peptone water and incubated at 37^{o}C for 24 hours. The bacterial growth was matched with that of 0.5 McFarland standards thatare formulated by adding 99.5ml of 1% (y/y) H_2SO_4 in 0.5ml of 1.75 % (w/v) $BaC1_2.2H_2O$. If the bacterial growth is dense then it will be diluted by adding more peptone water to match exactly with the McFarland standard. This concentration is equivalent to 1-2 x 108CFU/mlapproximately (Bharadwaj*et al*., 2022).

Statistical Analysis

Results (in terms of zone of inhibition) was subjected to statistical analysis that was performed by one-way analysis (ANOVA) through SPSS ver. 20.0 software at $p < 0.05$ by determining the significant variation in mean values between the experimental and control values. These values were defined as mean ± S.E.M (standard error mean) (Bharadwaj*et al*., 2022).

RESULT AND DISCUSSION

Result

Table 1: shows the phytochemical screening of crude extract of *L. nobilis*

Phytochemicals	**Bark**	**Leaf**
General glycosides	+	+
Tannins	+	+
Steroids	-	+
Saponins	+	+
Carbohydrates	+	+
Alkaloids	+	+
Flavonoid	+	-
Terpenoids	+	+

KEY: + = Present, - = Absent

Antimicrobial Properties of*L. nobilis* Extract

The result of the antimicrobial properties of extracts of *L. nobilis* are shown in table 2. The mean diameter of growth of *Staphylococcus aureus* when assayed with ethanol extract of *L. nobilis* was 12.8±0.58mm while a value of 10.2±0.1mm was recorded against distilled water. A value of 11.0±0.58mm was recorded against *Salmonella typhi* when ethanol extract of *L. nobilis*was assayed against the test bacteria and a value of 9.3±0.2mm was recorded against distilled water. A value of 11.0±0.58mm was recorded against *Escherichia coli* when ethanol extract of *L. nobilis* was assayed against the test bacteria and a value of 7.30±0.1mm was recorded against distilled water.

Table 2: Antimicrobial Properties of Extract of *L. nobilis*

Mean diameter of growth (mm) +SD		
Organism	Gentamicin	Ethanol extract n=3
S. aureus	16.3±1.5	25.6±05
S. typhi	14.5±0.5	20.6±1.1
E. coli	11.3±1.1	19±0.5

Values including diameter of the well (6mm), are means of three replicate ± SD

Minimum Inhibitory Concentration of Extracts of *L. nobilis*

The result of the minimum inhibitory concentration of extracts of *L. nobilis* against *Staphylococcus aureusSalmonella typhi, Escherichaia coli* shown in Table 3.

Minimum inhibitory concentration values recorded against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, when distilled water of *L. nobilis* was assayed against the bacterial were 40mg/ml, 30mg/ml and 40mg/ml respectively while Minimum inhibitory concentration values of 40mg/ml, 30mg/ml and 40mg/ml were recorded against *Staphylococcus aureus*, *Salmonella typhi*,*Escherichia coli* respectively when ethanol extract of *L. nobilis* was assayed against the bacteria. The result of the minimum inhibitory concentration of the extracts against the test organisms correlate with report that microorganisms varied in the degree of their susceptibility to antibacterial agents.

Table 3: Minimum Inhibitory Concentration of Extracts *L. nobilis*

Minimum inhibitory concentration (mg/ml)		
Organism	Ethanol extract	Distilled water
Staphylococcus aureus	40	40
Salmonella typhi	30	30
Escherichia coli	40	40

Table 4: Shows Minimum Bactericidal Concentration (MBC) of extracts (mg/ml)

Organism	**Leaf**
Staphylococcus aureus	40
Salmonella typhi	30
Escherichia coli	40

Discussion

From the result of the experiment displayed in Table 2 The inhibition zone (IZ) *of L. nobilis*extract to*S. aureus* (16.3 ± 1.5mm) followed by *S. typhi* (14.5 ± 0.5mm) and *E. coli* (11.3 ± 1.1mm). The results illustrates that *S. aureus* were more sensitive against *L. Nobilis* leaves ethanol extract. This finding was in tandem with the results published by Al-Ogaili (2020) which highlighted the great inhibition activity of *L. Nobilis* leaves ethanol extract to this Gram positive bacterium. As reported by Otsuka*et al*., 2018. The *L. Nobilis* had antimicrobial activity against methicillin resistant *S. aureus* (MRSA) through purified two compounds Flavonoid and kaempferol, that both compounds showed strong antimicrobial activity.

The active compound was seen against *S. aureus, S. typhi*and *E. Coli*. One from this Flavonoid compound has antibacterial properties because it has the capability to produce transduction energy that will affect the cytoplasm of the bacteria and slow down it motility since it has an ability to interact directly with the Deoxyribonucleic acid (DNA) of the bacteria. The type ofsolvent used for extracting *L. Nobilis* leaves had a major impact on their antibacterial activity.Extraction of *L. nobilis* leaves with ethanol resulted in a product with greater overall antibacterial activity of Libya bay leaf extracted with methanol and n- hexane, it was observed that the hexane showed no antibacterial activity but the methanol extract had good inhibitory activity against *S. aureus*. Also, El Malti and Amarouch (2019) found that the bay leaf extract had a significant antimicrobial activity against wide a range of human pathogens.

Therefore, the result from this study confirmed that *L. nobilis* leaves ethanol extract has antimicrobial activity against micro-organisms. It was further observed that the antimicrobial activity during Agar Well diffusion and antibactericidal activity development, the resultconcurred with the result of Aldhaher*et al*., (2020) that found aqueous extract to be good inhibitory agent against *Staphylococcusmutants* with MBC's range 30 - 50mg/ml. Also, the study of Sirikene*t al*., (2018)who demonstrated that the essential oil of *L. Nobilis* had strong antibacterial activity against Gram negative and Gram positive food borne pathogens. Study of Aljindan and Alkharsah(2020) shows the resistance of Salmonella species to antimicrobial drugs increasing from 24.6% in 2013 to 37.9% in 2019. The studies of Patil and Mule (2019) found *S. typhi* to be sensitive to Cefixime, Ceftriaxone and Azithromycine based on average minimal inhibitory concentration and MIC breakpoints. Studies of Nafis*et al*., (2020) exhibited notable potency regarding antimicrobial activity of *L. Nobilis* leaves which had the highest activity against *E. coil* and *Staphylococcus aureus* with MIC: 40mg/ml while it had activity against *S. typhi* with MIC: 30mg/ml.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The result from this study demonstrated the antibacterial effects of L. *Nobilis* leaves ethanol extract to be strong antibacterial agent against bacterial infections as they exhibited antimicrobialactivity against *Staphylococcus aureus,Salmonella typhimurium* and weak activity in *Escherichia coli*, so

that it is considered a kind of drug development substance especially for multi-drug resistant bacteria.

Recommendations

i. More research should be made on the effectiveness of the extract of *L. nobilis* against other micro-organism that were not tested in this work.
ii. Studies on the other part of the plant should be investigated for antibacterial activities.
iii. Purification of the extract should be carried out in further work to expose more active ingredients.
iv. Studies on anti-fungal properties of *L. nobilis* should be carried out to ascertain the broad spectrum nature of the plant as claimed.

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