Evaluation of Chemical Composition, Antibacterial, Antifungal, and Cytotoxic Activity of Laurus nobilis L Grown in Saudi Arabia

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Abstract

Present study aimed to evaluate the chemical composition, antibacterial, antifungal, and cytotoxic activity of Laurus nobilis grown in Tabuk region of Saudi Arabia. Dried leaves of L. nobilis were extracted with various solvent with increasing polarities. Solvent extracts exhibited variable inhibition zones against bacterial pathogens, however all the solvent extracts showed significant inhibition against fungal pathogens. Acetone extracts had the largest inhibition zone against Streptococcus pneumoniae (37.16 ± 0.23 mm) while ethanol and methanol extract showed the most efficient percentage inhibition against mycelial growth of Alternaria alternata (91.33 ± 0.47; 90.66 ± 0.94). High cytotoxicity was demonstrated by methanol and aqueous extracts (IC50 14.90µg/ml, 24.56 µg/ml), while acetone extracts showed moderate effects on cell inhibition (IC50 41.43µg/ml). The significant activity shown by the bay leaf extracts could be attributed to monoterpene hydrocarbons, oxygenated monoterpenes, phenylpropanoids, phenols, and other important phytoconstituents identified in GC-MS and FTIR studies. Our findings clearly show significant antifungal, antibacterial and cytotoxic activity of solvent extracts of bay leaves, which could be attributed to the presence of wide range of phytochemicals. Since plants derived natural products are less toxic, cheaper, and have negligible side effects, they would serve as an excellent alternative to antimicrobial and chemotherapeutic drugs.

Keywords: Laurus nobilis, solvent extracts, GC-MS, cytotoxic, antibacterial, antifungal.
INTRODUCTION

Medicinal plants are used worldwide to cure many diseases. Due to the undesirable and the simultaneous increase in microbes with multidrug resistance, traditional herbal approach to cure diseases is in vogue. Saudi Arabia represents one of the most biodiverse and the hospital isolate, Tabuk, is located in the Northwest region of Saudi Arabia and is a herbal medicine (both native and non-native plants) and European region. The leaves are called bay leaves or bay laurel and used as a spice and flavoring agent since ages because of their immense benefits. Plants are well known for their diverse flora and fauna, and their different parts possess a wide array of properties.

Based on previous reports and literature collected, L. nobilis contains several important and chemical components, which are used in ethano extracts. The leaves of Laurus nobilis leaves growing in Tabuk region spread on Muller Hinton agar plates and allowed to dry. After drying, the agar was aseptically punched with cork borer (6 mm) to make wells. Each well was filled with 50µl of the crude solvent extract volume (10µl) of the quantified broth was evenly distributed on the amended plate. Mycelial plugs (6 mm) were allowed to solidify, gently swirled to allow thorough mixing. Plates were antibiotic disc of Tetracycline (30µg), while was as negative control. The antibacterial activity of the crude solvent extracts served as negative control.

MATERIALS AND METHODS

Plant material

The leaves of L. nobilis were collected from Tabuk region during the month of February and dried using rotary evaporator, after which residues were reconstituted with respective mother solvents to obtain a fixed final volume of 200 ml. Twenty grams of powdered leaves or bay laurel and used as a spice were added to 100 ml of water, separately in the extracting solvents. The effect of solvent extracts on mycelial growth of test fungi was determined using well diffusion method.

Preparation of Extracts

W (v/v) v following the method of Do et al., with minor modifications. Various solvents (Water, ethanol, methanol, acetonitrile) were used for extraction purposes. Twenty grams of powdered leaves were added to 100 ml of water, separately in the mother solvents to obtain a fixed final volume of 200 ml. Twenty grams of powdered leaves were added to 100 ml of water, separately in the mother solvents to obtain a fixed final volume of 200 ml.

Microorganisms

All the bacterial and fungal isolates used in the study were procured from King Khaled hospital, Riyadh, Saudi Arabia. The bacterial strains screened were Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), Streptococcus pneumoniae (hospital isolate), and Proteus mirabilis (hospital isolate). Fungal strains screened were Fusarium solani, Fusarium oxysporum, Alternaria alternata, and Bipolaris sp. Nutrient agar was used to maintain all the stock cultures of bacteria, while PDA was used for fungi. Nutrient agar was used to maintain all the stock cultures of bacteria, while PDA was used for fungi.

Antibacterial Activity

dZ vfo on}{z R} j(4)j(5)j(6)j(7)j(8)j(9)j(10)j(11)j(12)j(13)

Antifungal Activity

dZ v j(f)j(v)j(u)j(o)j(o)j(o)j(o)j(o)j(o)
actively growing mycelia (10 days). Culture plates were then incubated at 25 ± 2°C. Measurements were taken when the control plate showed growth covering the entire plate. Control plate contained media without any treatment (without extract). The assay was performed in triplicates and their mean values were noted.

The following formula was used to calculate the percentage inhibition of mycelial growth.

\[ PI = \frac{C_o - T_e}{C_o} \times 100 \]

where PI stands for percentage inhibition, \( T_e \) is the treatment and \( C_o \) denotes the mean growth rate of control.

**Minimum Inhibitory Concentration (MIC)**

Broth dilution method was used to determine the Minimum Inhibitory Concentration of both fungi and bacteria, by following the standard method of CLSI with slight modification.\(^{14,15}\) Muller Hinton broth (bacteria) and Potato dextrose broth (fungi) were used to prepare a double fold dilution of extracts in the range 0.125mg/ml -32mg/ml. Equal amount of broth and extract were added to a sterile test tube containing 5X10\(^5\) CFU/ml bacterial spore suspension and 2X10\(^6\) CFU/ml of fungal spore suspension separately. The tubes were incubated at 37°C for 24h (bacteria) and 25 ± 2°C for 7-9 days (fungi). The lowest concentration showing no visible growth was regarded as its MIC. The assay was performed in triplicates and their mean values were noted.

**Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)**

The Minimum bactericidal (MBC) concentration and Minimum fungicidal concentration (MFC) tests were conducted as an adjunct to MIC assay.\(^{16}\) The lowest concentration inhibiting the growth of test pathogens in tubes were transferred on the respective agar plates, Muller Hinton Agar for bacteria and Potato Dextrose agar for fungi. The subcultured plates were incubated at 37°C for 24h (bacteria) and 25 ± 2°C for 7-9 days (fungi). Concentrations that did not show any growth on respective agar medium were regarded as their MBC and MFC.

**MTT Assay and IC50 Values**

Cell viability was evaluated by MTT \( \rightarrow \) [Purpurogallin] D\(^{17}\) well plate, 100\( \mu \)l of Hela cells was added at a concentration of 2x10\(^5\) cell/well and allowed to settle for 24 h. Cells were maintained in their respective medium. After 24 h incubation, various concentrations of solvent extracts were added to the wells and further incubated for 24 h. DMSO served as negative control and Doxorubicin was positive control.

**FTIR**

Nicolet 6700 spectrometer (Thermo Scientific, USA) equipped with DTGS detector and beam splitter was used in studying the IR spectrum of methanol and aqueous extracts. The IR spectrum obtained was analysed (OMNIC software) and scanned in the range of 400-4000 cm\(^{-1}\).

**Statistical Analysis**

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as percentage cell viability using dose dependent curves. Graphpad prism (Version 7.00 for Windows GraphPad Software Inc.) was used in calculating the IC$_{50}$ by generating standard curve with regression analysis.

RESULTS

Extraction

Solvents with increasing polarity were used for extraction purpose. Methanol extracts gave the highest yield (52%) followed by ethanol (49%), water (27%), acetone (12%), ethyl acetate (12%) and chloroform (10%).

Antibacterial Activity

Solvent extracts of L. nobilis examined in the present study exhibited significant antibacterial activity except the aqueous extracts (Table 1). Acetone extracts followed by ethyl acetate and methanol displayed significant inhibition against all the bacterial isolates tested. However, aqueous extracts were least inhibitory. Maximum zone of inhibition was observed with acetone extracts (37.16 ± 0.23) and ethyl acetate extracts (25.00 ± 0.81) against S. pneumonia. Proteus mirabilis was also strongly inhibited by acetone extracts (24.00 ± 0.81) against E. coli and P. mirabilis and S. pneumoniae.

Antifungal Activity

Bay leaf extracts demonstrated significant inhibition against radial mycelial growth of test fungi (Table 2). It was noticed that methanol and ethanol extract effectively inhibited mycelial growth of A. alternata, F. solani and F. oxysporum. Highest inhibitory activity was observed by ethanol and methanol extracts against A. alternata (91.33 ± 0.47; 90.66 ± 0.94). Aqueous extracts exhibited least antifungal activity amongst all the extracts screened.

MIC, MBC and MFC of L. nobilis extract against test isolates

MBC values for both bacteria and fungi ranged from 0.125 - 0.25 mg/ml while the MFC was 0.125 - 0.5 mg/ml. Bipolaris sp was inhibited by all the extracts in the range of 0.125 -16 mg/ml. Aqueous extracts could not inhibit F. solani and F. oxysporum at 32 mg/ml.

Cytotoxic Activity of Extracts and IC$_{50}$

All the crude extracts were tested for their in vitro anticancer activity against HeLa cell through MTT assay. Fig. 1 shows the cell viability of extracts (in a dose dependent manner). All the extracts inhibited HeLa cells, except ethyl acetate extracts. Cell viability data is presented on a graph, at the highest concentration of 100µg/ml. Aqueous extracts, acetone, and methanol extracts caused 86%, 58%, and 76% inhibition respectively. The IC$_{50}$ for methanol, aqueous, and acetone extracts were 9.33 ± 0.47, 11.66 ± 0.94, and 16.00 ± 0.62 mg/ml respectively.

Table 1. Inhibitory Activity of Laurus nobilis solvent extracts against pathogenic bacterial strains

| Solvent Extract | Zone of Inhibition (mm) |
|-----------------|-------------------------|
| Aqueous         | 8.66 ± 0.47             |
| Ethanol         | 23.33 ± 0.94            |
| Acetone         | 24.00 ± 0.81            |
| Chloroform      | 17.00 ± 0.62            |
| Ethyl Acetate   | 35.66 ± 0.47            |
| Control Tetracycline | NI 28.00 ± 0.00 |

Table 1. /vZ]/[\]p\{ Laurus nobilis\}o\{v][v]v\}v\}

| Solvent          | Aqueous | Methanol | Ethanol | Acetone | Chloroform | Ethyl Acetate | Control |
|------------------|---------|----------|---------|---------|------------|---------------|---------|
| Escherichia coli | E/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Proteus mirabilis| K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Staphylococcus   | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| aureus           | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Streptococcus    | E/      | K/       | K/      | K/      | K/         | K/            | K/      |
| pneumoniae       | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Enterococcus     | K/      | K/       | K/      | K/      | K/         | K/            | K/      |

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| Solvent | Aqueous | Methanol | Ethanol | Acetone | Chloroform | Ethyl Acetate | Control |
|---------|---------|----------|---------|---------|------------|---------------|---------|
| Escherichia coli | E/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Proteus mirabilis | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Staphylococcus | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| aureus | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Streptococcus | E/      | K/       | K/      | K/      | K/         | K/            | K/      |
| pneumoniae | K/      | K/       | K/      | K/      | K/         | K/            | K/      |
| Enterococcus | K/      | K/       | K/      | K/      | K/         | K/            | K/      |

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FKU K [k X [\]p\{ A/\} o\{v][v]v\}v\}v/ screened.
extracts was 14.90 µg/ml, 24.56 µg/ml, and 41.43 µg/ml respectively.

GC-MS

Crude acetone extract exhibited significant antimicrobial and cytotoxic activities, hence was subjected to GCMS analysis. Acetone extracts showed the presence of monoterpene hydrocarbons, oxygenated monoterpenes, phenylpropanoids, phenols and many others important compounds as shown in Table 5.

Chemical composition of acetone extracts showed the presence of compounds such as Bicyclo (3.1.1) hept-2-ene,2,6,6-trimethyl, 6,6-Dimethyl-2-methylenebicyclo[3.1.1] heptane (α and β pinene), o-xylene, p-xylene, 3-carene, terpenyl acetate, γ terpinene, eugenol, phenol, 2-methoxy-4(2-propenyl)-acetate, Camphene, and 9-Hexadecen-1-ol, (Z), Phenol, 2-methoxy-4-(2-propenyl)-acetate, Cyclohexene.1-methyl-4-(1-methylethylidene) and Terpineol acetate.

FTIR

FTIR of methanol and aqueous extract was performed, as these extracts demonstrated significant antifungal and cytotoxic activity. IR spectroscopy of methanol and aqueous extract show a broad peak at 3295 cm⁻¹ and 3285 cm⁻¹, these peaks denotes the presence of phenolic compounds such as eugenol and terpineols.

The two peaks in methanol extract at 2946 cm⁻¹ and 2834 cm⁻¹ are due to C-H asymmetric and symmetric stretching of proteins, lipids and carbohydrates, while the peaks at 2169 cm⁻¹ and 2018 cm⁻¹ are due to C=C stretches of alkyne molecules, similar peaks were also observed in aqueous extracts at 2026 and 2217 cm⁻¹. The peak at 1647 cm⁻¹ & 1634 cm⁻¹ in methanol and aqueous shows a broad peak at 3295 cm⁻¹ and 3285 cm⁻¹, these peaks denotes the presence of phenolic compounds such as eugenol and terpineols.

Table 2. /vZ]\{\(\text{Laurus nobius}\) extracts of the test strains.

| Fungi Tested   | Aqueous | Methanol | Ethanol | Acetone | Chloroform | Ethyl Acetate | Control |
|----------------|---------|----------|---------|---------|------------|---------------|---------|
| Fusarium solani | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Fusarium oxysporum | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Alternaria alternata | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Bipolaris sp    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |

All data are expressed as mean ± standard deviation where n = 3. Control- Voriconazole

Table 3. \(\text{Laurus nobius}\) extracts of /\text{E}, \text{P}, \text{S}, \text{E}

| Strain          | A/| B/| C/| D/| E/| F/| G/| H/| I/| J/| K/| L/|
|-----------------|---|---|---|---|---|---|---|---|---|---|---|---|
| E. coli         | E/| E/| E/| E/| E/| Ed| K/| K/| O/| O/| E/| E/|
| P. mirabilis    | E/| E/| U/| U/| U/| K/| K/| K/| O/| E/| Ed| Ed|
| S. aureus       | E/| E/| O/| Ed| O/| E/| K/| K/| O/| E/| E/| E/|
| S. pneumoniae   | E/| E/| K/| K/| O/| U/| K/| K/| K/| K/| K/| K/|
| E. faecalis     | E/| E/| K/| K/| K/| K/| K/| K/| K/| K/| O/| O/|

E/\text{E} /vZ/} E. coli- Escherichia coli, P. mirabilis- Proteus mirabilis, S. aureus- Staphylococcus aureus, S.pneumoniae- Streptococcus pneumoniae, E. faecalis - Enterococcus faecalis

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|----------------|---------|----------|---------|---------|------------|---------------|---------|
| Fusarium solani | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Fusarium oxysporum | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Alternaria alternata | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |
| Bipolaris sp    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32    | ≈ 32       | ≈ 32          | Control |

All data are expressed as mean ± standard deviation where n = 3. Control- Voriconazole

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Table 5. GC-MS Analysis of Laurus nobilis

| Name of Compound                          | Retention time | Molecular formula | Peak area |
|-------------------------------------------|----------------|-------------------|-----------|
| 2,4-dichlorophenoxyacetic acid, methyl ester | 5.061          | C₁₁H₁₂Cl₂O₃      | 1.293     |
| 2(Methoxycarbonyl)benzoic acid           | 5.234          | C₉H₈O₄           | 1.004     |
| Bicyclo (3.1.1) hept-2-ene,2,6,6-trimethyl | 6.359          | C₂₀H₃₂           | 11.914    |
| Camphene                                  | 7.149          | C₁₀H₁₆           | 0.434     |
| o-Xylene                                  | 7.606          | C₈H₁₀             | 1.025     |
| p-Xylene                                  | 8.564          | C₈H₁₀             | 0.931     |
| γ-Terpinene                               | 31.485         | C₁₀H₁₆           | 1.422     |
| Terpinyl acetate                          | 44.638         | C₁₂H₂₀O₂          | 13.209    |
| 3 Carene                                   | 48.502         | C₁₀H₁₆           | 1.020     |
| 6,6-Dimethyl-2-methylenebicyclo(3.1.1)heptane | 54.372         | C₁₀H₁₆           | 2.380     |
| Eugenol                                    | 56.045         | C₁₀H₁₂O₂          | 2.031     |
| Phenol,2-methoxy-4-(2-propenyl)-acetate   | 59.421         | C₁₂H₁₄O₃          | 0.610     |
| Cyclohexene.1-methyl-4-(1-methylethylidene) | 74.390         | C₁₀H₁₆           | 3.010     |
| Terpineol, acetate                        | 75.641         | C₁₂H₂₀O₂          | 5.511     |
| 1,8-cineole                                | 76.523         | C₁₀H₁₈O₂          | 6.313     |
| 1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-| 77.307         | C₁₅H₂₂O₃          | 35.739    |
| 2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-| 81.447         | C₁₄H₂₀O₂          | 2.474     |
| (1,1-dimethylethyl)-1,8-cineole           | 82.181         | C₁₆H₃₂           | 0.986     |
| Oleyl alcohol                              | 84.413         | C₁₈H₃₆O            | 0.610    |
| Cyclopentadecanol                          | 86.107         | C₁₅H₃₀O          | 1.221     |
| Ricinoleic acid                            | 87.324         | C₁₈H₃₄O₃          | 0.200    |

Table 4. Minimum Inhibitory and Minimum Fungicidal Concentration (MIC and MFC) of Laurus nobilis extracts

| Solvent extract | Aqueous MIC | Methanol MIC | Ethanol MIC | Acetone MIC | Chloroform MIC | Ethylacetate MIC |
|-----------------|-------------|--------------|-------------|-------------|----------------|------------------|
| F. solani       | >32         | 0.125        | 0.125       | 0.125       | 0.25           | 0.5              |
| F. oxysporum    | NI          | 0.125        | 0.25        | 0.125       | 0.125          | 4                |
| A. alternata    | 4           | 8            | 0.125       | 0.125       | >32            | NI               |
| Bipolaris sp.   | 8           | 16           | 0.25        | 0.5         | 0.25           | 0.5              |

NI - Not Inhibited, F. solani - Fusarium solani, F. oxysporum - Fusarium oxysporum, A. alternata - Alternaria alternata
are in agreement with previous reports which increasing polarity of solvents\textsuperscript{18,19} show that the extraction yields increased with increasing polarity of solvents\textsuperscript{18,19}. It is known that plants possess a wide variety of bioactive compounds, which exhibit different solubility properties with different solvents; hence screening with different solvents helps identify the most appropriate solvent for biological studies\textsuperscript{19,20}.

In the present study, bacterial isolates showed varied inhibitory activities against \textit{L. nobilis} extracts, some isolates were significantly inhibited, while others responded poorly. In general, the Gram positive bacteria were inhibited with significant inhibition zones and exhibited low MIC values when compared to Gram negative bacteria.

The two gram positive bacteria \textit{S. pneumoniae} and \textit{E. faecalis} showed least MIC values of 0.125 mg/ml while \textit{E. coli} was not inhibited at the highest test concentration of 32 mg/ml. Similar to our findings, Malti and Amarouch, 2009 and Ramos et al., (2012), reported that ethanolic extracts of \textit{L. nobilis} extracts were potent in inhibiting the Gram positive bacteria than the Gram negative isolates\textsuperscript{21,22}.

The variation in the inhibition zones and MIC values shown by Gram positive and negative bacteria is attributed primarily to the chemical composition and morphology of the bacterial cell membrane. The pronounced antibacterial activity shown by Gram positive bacteria could be due to the peptidoglycan layer which is permeable to the hydrophobic compounds present in the extract\textsuperscript{23}. The resistance shown by Gram negative bacteria could be due to the lipopolysaccharide layer present in the cell membrane. The outer membrane of Gram negative bacteria is made up of lipopolysaccharides and proteins that form a barrier to the penetration of hydrophobic compounds.

### Table 6.

| Peak value (frequency, cm\textsuperscript{-1}) | Functional group |
|---------------------------------------------|------------------|
| 3285 | Hydrogen bond |
| 2217 | C=C stretching |
| 2026 | C=C stretch |
| 1990 | C=C bending |
| 1966 | C≡C stretch |
| 1947 | C≡C stretch |
| 1634 | C=C Vibrations |

### Table 7.

| Peak value (frequency, cm\textsuperscript{-1}) | Functional group |
|---------------------------------------------|------------------|
| 3295 | Broad peak of OH stretch |
| 2946, 2834 | Two peaks due to CH\textsubscript{2} asymmetry stretching and symmetry stretching |
| 2169 | C≡C stretch |
| 2018 | C≡C stretch |
| 1647 | C≡C stretch |
| 1449 | CH\textsubscript{2} stretches |
| 1111 | C-O stretches |
| 1015 | C-O stretches |
up of tough and rigid lipopolysaccharide bilayer, with porins and uptake channels embedded in them, this kind of an arrangement is very efficient in resisting the entry of antimicrobial compounds. According to Zgurskaya et al., the lipopolysaccharide layer is rigid and impermeable to hydrophobic compounds. The porins restrict the entry of hydrophilic compounds while the multidrug transporter pumps help in extruding the bioactive compounds. Hence, together they serve as an excellent permeability barrier to antimicrobial compounds across the cell membrane.

All the fungal isolates screened interestingly exhibited strong inhibition by methanol extracts. *Alternaria alternata* showed maximum inhibition with methanol extracts, its inhibition was quite close to the antifungal voriconazole, indicating the potency of the extracts. However, there are very few reports that show the inhibition of emerging fungal pathogens by *L. nobilis* solvent extracts, especially the pathogens used in the present study (*A. alternata*, *F. solani*, *F. oxysporum*, and *C. gleosporoides*).

The potent antimicrobial activity shown by acetone, methanol and ethanol extracts in a variable manner against a wide array of microorganisms in this study could be attributed to the richness of phenolic compounds along with monoterpenes hydrocarbons and oxygenated monoterpenes. Based on the antimicrobial activity we can deduce that methanol and acetone were efficient in extracting phenols and terpenes, which was further authenticated by GCMS and FTIR findings shown in Table 5,6,7. According to Bakari et al., phenolic compounds are more soluble in polar organic solvents than in non-polar solvents. Similarly, methanol and ethanol were reported as better solvents in extracting various phenolic compounds. Recently promising antibacterial activity of *L. nobilis* extracts was reported by Fernandes et al., and they attributed it to the presence of phenolic compounds, like flavonoids. Therefore, variable inhibition of test isolates is due to their sensitivity towards the bioactive compounds present in the solvent extracts which is influenced by the polarity of the extracting solvent. Phenolic compounds have the ability to interfere and inhibit the synthesis of peptidoglycan, modify the hydrophobicity of the membrane and cause damage to the membrane structures. Though the exact mechanism and mode of action of phytochemicals on microbes is not fully understood. However, studies indicate that phytochemicals like terpenes and terpenoids are known to alter membrane integrity by causing expansion of the cytoplasmic membrane, changing the fatty acid composition, and hyperpolarization due to lowering of pH internally, thereby destabilizing the membrane leading to cell membrane disruption. Furthermore, the interaction of phytocompounds causes inhibition.
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GCMS analysis of acetone extracts shows presence of compounds like α-pinene and β-pinene, 3 carene, terpinene, Terpinyl acetate, 1,8-cineole, 1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonapthathalen-7(4aH)-one and eugenol besides others. Interestingly, previous reports show the presence of all the aforementioned compounds in various solvent extracts of L. nobilis and exhibited antimicrobial activity. Recently Vardapetyan et al., examined bay leaves from two different origins of South Caucasus (Armenia and Georgia). The ethanol extracts of the leaves from both regions showed the presence of o-Cymene, β-pinene, and D-Limonene. However, the major component was 1,8-cineole, and minor quantities of acyclic and cyclic monoterpene phellandrene, γ-terpinene, and camphene were present. Similarly, 1,8-cineole, terpinyl acetate, linalool, R-pinene, and sabiene were identified as the major compounds in bay leaves growing in Spain and Italy. These findings are in accordance with our results as most of the above-listed chemical constituents were present in the extracts evaluated in our study, denoting the richness of the extracts of plants grown in Saudi Arabia.

Some studies have reported the presence of certain sesquiterpenes and guaianolides such as phellandrene, β-eudesmol, α-tocopherol, β-sitosterol and dehydrocostus lactone, which are missing in our findings. Recent studies have shown that pinene, eugenol and terpenene have shown antibacterial, antifungal, antiviral, and insecticidal properties. Monoterpenes hydrocarbons and oxygenated monoterpenes like α-pinene and β-pinene, 3 carene, terpinene and Terpinyl acetate are in accordance with our results as most of the above-listed chemical constituents were present in the extracts evaluated in our study, denoting the richness of the extracts of plants grown in Saudi Arabia.

**Fig. 3.** Cytotoxic activity of L. nobilis extracts against HeLa cell line (MTT assay). The graph shows the % cell viability. All data are expressed as mean ± standard deviation (n = 3).

| % Cell Viability | Water | Methanol | Acetone |
|------------------|-------|----------|---------|
| 0                | 100   | 100      | 100     |
| 20               |       |          |         |
| 40               |       |          |         |
| 60               |       |          |         |
| 80               |       |          |         |
| 100              |       |          |         |

**Table 1.** Effect of different concentrations of L. nobilis extracts on HeLa cell line.

Figure 3. Graph showing the percentage cell viability of L. nobilis extracts against HeLa cell line (MTT assay). The graph shows the % cell viability. All data are expressed as mean ± standard deviation (n = 3).
methanol, ethanol and water extracts. As stated above, these solvents are excellent in extracting various phenols, flavonoids, terpenes, terpenoids and alkaloids, all of which are known for their antimicrobial, anti-inflammatory, antimutagenic and anticancer activities. In fact, the presence of diverse terpenes and phenolic compounds present in the extracts actually prompted us to investigate their cytotoxic activities.

Plant derived compounds have different modes of action; however, most of them induce apoptosis without cytotoxic effect on normal cells. Recently it was reported that in phenols, the number of substitutes in the OH-ring, increases its anti-proliferative and cytotoxic activities. Similarly, terpenes, terpenoids, alkaloids and flavonoids induce cytotoxicity of breast cancer cells by activating the intrinsic pathway resulting in apoptosis. Significant cytotoxic and antitumor activity of methanol and aqueous extracts of L. nobilis leaves towards a number of cancer cell lines, like NCI-H460-lung cancer cells, cervical carcinoma, HCT15-colon carcinoma, breast adenocarcinoma and HepG2-hepatocellular carcinoma have been reported earlier. High amounts of phenols and flavanols were responsible for the activity. Similarly, Berrington and Lall, reported acetone extracts of L. nobilis to inhibit HeLa cells strongly with an IC_{50} values of 34.46 ± 0.48µg/mL. Our results are in agreement with the all the above-mentioned findings as phenolic compounds, flavanones and terpenes were present in the extracts screened and could have contributed to the promising cytotoxic activity.

CONCLUSION

Our findings clearly show the presence of diverse phytochemicals in bay leaves grown in Saudi Arabia. The significant antimicrobial and cytotoxicity could be attributed to phenolic compounds along with Terpenes. Since drugs derived from plants are less toxic, cheaper, and with negligible side effects, they would serve as excellent alternatives to antimicrobial and chemotherapeutic drugs. Recently, several herbal extracts have been approved by FDA for clinical trials. An IC_{50} that is lower than 30µg/ml is considered a very strong cytotoxic agent (American National Cancer Institute). The IC_{50} of some extracts in our study was lower than this limit.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS’ CONTRIBUTION

HR designed the experimental work. NNA and NMSM carried out the experimental work. HR, NNA and NA analyzed the GCMS and IR analysis. GA assisted in analyzing the Cytotoxicity results. HR wrote the manuscript. NA reviewed and edited the manuscript.

DATA AVAILABILITY

The data that support the findings of this study are available with the corresponding author.

ETHICS STATEMENT

No animal or human subjects were involved in the work related to this manuscript.

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