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Research Article

Antibacterial and antioxidant properties and phytochemical screening of *Laurus nobilis* L. extract from Ethiopia

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Abstract: Microbial resistance to antibiotics and the shortage of efficient antimicrobial agent has necessitated the search for a better antimicrobial agent from various sources. Plants secondary metabolites are the major sources for discovery of new bioactive chemical compounds. The objective of this study was to determine the antibacterial and antioxidant activities of *Laurus nobilis* leaf extract and its essential oil against human pathogenic microorganisms and to analyse its chemical composition. The leaf of *L. nobilis* (500 g) was air-dried, powdered and extracted using four different solvents. The crude extract and the essential oil were tested against four Gram-negative and two Gram-positive bacterial strains. The radical scavenging activity of the crud extract was examined using DPPH assay. Bacterial inhibition activity of the crude extract increased with increased concentration from 25 mg/mL to 200 mg/mL. The maximum inhibition zone was recorded against *Enterococcus faecalis* 13.33±1.52 mm, *Escherichia coli* 14.33±1.53 mm and *Salmonella typhimurium* 16.00±1.00 mm, respectively. MeOH extract (1000 µg/mL) showed superior radical scavenging property (0.02) than ascorbic acid (0.05). The analysis of the oil using GC-MS indicated the presence of 48 chemical substances accounting for 91.4 % of the total compositions. The finding of this study showed that bay leaf has considerable antimicrobial and antioxidant activities. Further evaluation of this plant is recommended with particular focus on the mechanisms of action of the antimicrobial substance.

1. INTRODUCTION

Bay leaf (*Laurus nobilis* L.) is a perennial aromatic evergreen tree or large shrub with smooth leaves classified in the laurel family (Lauraceae). The plant is an important component in culinary and many traditional practices (Parthasarathy *et al*., 2008). The antimicrobial activity of this plant has been reported against several infections including fungi, viruses, bacteria, and protozoa (Fukuyama *et al*., 2011). *L. nobilis* has been used as herbal preparation to increase perspiration for diseases like rheumatism, sprains, dyspepsia, and earaches (Fang *et al*., 2005). The juice of this plant is an efficient treatment for sore eyes and night blindness resulted from vitamin A deficiency. The seeds of bay plant are also reported to have relieved indigestion, sore

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throat, constipation, and diarrhoea (Batool *et al*., 2019). Additionally, the cosmetic industry employs its essential oil in creams, perfumes, and soaps (Sharmeen *et al*., 2021).

In Ethiopia, there is no sufficient information on the antibacterial and antioxidant activities and phytochemical analysis of *L. nobilis.* Thus, the objective of this work was to investigate the antibacterial and antioxidant activities of *L. nobilis* leaf extract against selected human pathogenic microorganisms.

2. MATERIAL and METHODS

2.1. Plant Material

The *L. nobilis* leaf was collected in January 2021 from Addis Ababa, Ethiopia, and brought to Adama Science and Technology University. Voucher sample of the plant was prepared and pressed on a newspaper separately for taxonomic identification. The taxonomy of the plant was determined at the Department of Plant Biology and Biodiversity Management, Addis Ababa University.

2.2. Preparation of Plant Extract

In this study, the solid-liquid extraction technique was employed as a general extraction method. Total extraction of plant material was made by mixing the dried and grounded *L. nobilis* sample with organic solvents. Air-dried and powdered plant material (0.5 kg) was extracted with methanol (2.5 L), macerated at ambient temperature by placing it on a shaker for 24 h and filtered with Whatman No. 1 filter paper. Then, using rotary evaporator, the filtrate was concentrated and dried. The dried methanol extract was suspended and consecutively partitioned twice with equal volumes of n-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc). All solvent fractions were filtered and the filtrates were dried at 40 ^oC using rotavap and maintained in a vial in a refrigerator until further use.

2.3. Phytochemical Screening

The preliminary screening of phytochemical constituents of crude extract of the *L. nobilis* was done to investigate compounds including alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, and steroids using the standard procedures.

2.3.1. *Test for steroid*

Two mL of acetic anhydride was mixed with *L. nobilis* extracts previously dissolved in 2 mL of sulphuric acid. The change of appearance from violet to green confirmed the existence of steroids.

2.3.2. *Test for terpenoid*

Five mL of the extract and 2 mL chloroform were mixed and then combined with with 3 mL concentrated H_2SO_4 (Salkowski test). The formation of reddish-brown color at the interface indicated the existence of terpenoids.

2.3.3. *Test for saponins*

Two to three drops of distilled water were mixed with one millilitre of the crud extract and the mixture was violently agitated. The presence of saponins was determined by persistent foaming.

2.3.4. *Test for flavonoids*

Three mL of *L. nobilis* crude extract and fractions were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which becomes colorless with the addition of dilute acid indicates the presence of flavonoids.

2.3.5. *Test for tannins*

The leaf extract (about 0.25 g) of was heated in 20 mL of water in a test tube. Then, the solution was filtered and mixed with 2-3 drops of 0.1% Iron III chloride. The existence of tannins was confirmed by the observation of a green-blackish color.

2.3.6. *Alkaloids test*

One mL hydrochloric acid (1%) was added to 3 mL of the leaf extract. The solution was boiled for 20 minutes and filtered after cooling. Then one millilitres of the filtrate was mixed with 0.5 mL of Mayer's reagent. The appearance of a yellow precipitate reveals the existence of alkaloids.

2.3.7. *Test for phenol*

The solution prepared from the leaf extract (2-3 drops) was mixed with a few drops of ferric chloride reagent. The appearance of a bluish-black color indicates the existence of phenols.

2.4. Extraction of essential oils (EOs)

L. nobilis leaf (60 g) was powdered by an electrical grinder and extracted for 2 h by hydro-distillation method using 500 mL distilled water in a Clevenger apparatus. The EOs were taken and dried using anhydrous Na_2SO_4. The yield of the oil was determined as follows and kept in sealed vials at 4 °C in a refrigerator until further analysis.

$$Yieald\ /\%) = \frac{Amount\ of\ extracted\ oil\ (g)}{Amount\ of\ dry\ plant\ material} x100$$

2.5. GC-MS Analysis

Investigation of the EOs was done by GC-MS; GC (7890B, Agilent Technologies) coupled with an MS (5977A Network). The GC had an HP-5MS column (30 µm × 250 µm (i.d.) and 0.25 µm). The GC-MS method of Hanus *et al*., 2008 was used for the analysis. Helium used as a carrier gas (flow rate 1 mL/ min). The initial oven temperature was 100 °C for 2 min and raised from 100 to 280 ºC at 10 ºC/min (inlet 250 °C; detector 280 °C; split less injection/purge time 1.0min), solvent delay 4.00 min. Mass spectra were recorded in electron-impact mode, with ionization energy of mode at 70 eV, scanning the 33-550 m/z range. Identification of the components in the oils carried out by comparing the mass spectra of the samples with the database of NIST11 GC-MS libraries.

2.6. Antibacterial Assay

Antibacterial assay of *L. nobilis* leaf extract and essential oil was performed by disk diffusion method in Mueller Hinton Agar (MHA) plates. Four different concentrations were prepared from the extract (200, 100, 50, and 25 mg/mL) and the oil (45, 22. 5, 11.25, and 5.6 µg/mL) using DMSO as a solvent. Bacterial cultures of *S. aureus* (ATCC25923), *E. faecalis* (ATCC29212), *P. aeruginosa* (ATCC27853), *E. coli* (ATCC25922), *S. typhimurium* (ATCC13311) and *K. pneumonia* (ATCC700603),obtained from Adama Public Health Research and Referral Laboratory (Ethiopia) were grown in blood agar media at 37 °C for 24 h. The colonies were adjusted to 1.5 $\times 10^8$ CFU/mL (Andrews, 2002) using 0.5 McFarland standard (Saeed & Tariq, 2007) and maintained in a flask to compare the bacterial turbidity.

2.6.1. *Disk diffusion assay*

Two hundred microliter of the cultures of the test bacteria (1.5 $\times 10^8$ CFU/mL) was inoculated on to MHA medium. Presterilized 6 mm filter paper disks were soaked in each concentration of the extract and essential oil and carefully put on the agar media inoculated with the test bacteria. Tobramycin 10 µg/mL and DMSO 10% were used as positive and negative controls, respectively. The plates were subsequently incubated at 37 °C for 24h. After incubation, the growth inhibition zones were recorded using a transparent ruler (mm) and the readings were interpreted as mean value ± standard deviation.

2.6.2. *Minimum inhibitory concentration (MIC)*

Extract samples that showed significant antimicrobial activity with disk diffusion assay were selected to determine MIC using the broth dilution method. Four different concentrations were made from the extract and the oil using two-fold serial dilutions technique by transferring

appropriate concentrations from the stock solutions. The obtained concentrations were incubated with 0.02 mL bacterial cultures adjusted to 1.5 x 10^8 CFU/mL, and incubated at 37 ^oC for 24h. The growth of the bacteria was assessed by comparing the turbidity of the nutrient broth that contains plant extract and bacterial strains, with the controls. The smallest concentration where no turbidity was seen was considered as the extract's MIC value (Khalil *et al*., 2010; Radojevic *et al*., 2012).

2.6.3. *Minimum bactericidal concentrations (MBC)*

A loopful of the broth was taken from the test tubes in which growth was not visually detected and was streaked on nutrient agar (Muller Hinton Agar). The growth of bacterial cells was determined after 24 h incubation period at 37 °C. MBC was considered as the concentration of samples that didn't support the growth of cells on a fresh medium.

2.7. Antioxidant Assay

The antioxidant activity of the sample was evaluated by using DPPH assay. The crude extracts obtained using methanol, chloroform, and ethyl acetate were dissolved in four set of vials containing methanol to give 1000, 500, 250, and 125 µg/mL. While ascorbic acid was used as a positive control, a sample-free DPPH solution in methanol was utilised as a negative control. Four millilitres of DPPH solution (0.04 mg/mL) was mixed with 1 mL of extract preparations and then incubated for 30 min at room temperature. The absorbance of the samples was measured at 517 nm and the radical scavenging activitis were interpreted as present inhibition (IP) of free radicals using the formula:

$$IP\ (\%) = ([A_{control} - A_{test})/A_{control}]) \times 100\%.$$

Where $A_{control}$ is the absorbance of the control reaction, and A_{test} is the absorbance of the extracts (Suprava, 2012). The extracts' radical scavenging activity was also determined based on the percentage of the DPPH reduction by calculating the IC50 values.

2.8. Data Analysis

Microsoft excel and SPSS version 22 software were used to analyse the antibacterial activity of the crud extracts and essential oils against the tested bacterial species. The results were presented using mean value ± standard deviation

3. RESULTS and DISCUSSION

3.1. Phytochemical Screening

The phytochemical screening of methanol extract was positive for all tested phytochemicals and showed the existence of tannins, flavonoids, saponins, alkaloids, phenol, terpenoids, and steroids. However, the phenol and tannin constituents were absent in chloroform, and alkaloids were absent in ethyl acetate extracts (Table 1). Previous report (Mursyida *et al*., 2021) showed the presence of tannins, flavonoids, saponins, alkaloids, and essential oils, in *L. nobilis* leaf ethanol extract, which is consistent with the finding of this study. The result of this study was also compatible with the finding of Zuraida, 2018 and Algabri *et al*., 2018 who reported the availability of bioactive secondary metabolites in the leaf extracts of *L.nobilis*. Furthermore, the study of Onuminya *et al*. (2017) showed methanol extract of *L. nobilis* contains active compounds similar to those reported in the current study, except tannin. Variations in the composition of the secondary metabolites might be due to the difference in the genetics of the plant, climatic conditions of the environment of the plants, and the part of the plant examined (Dewijanti *et al*., 2019).

Table 1. Phytochemical compositions of *L. nobilis* leaf extracts.

Phytochemical Screening	Extracts		
	Methanol	Chloroform	Ethyl acetate
Alkaloids	+	+	_
Flavonoids	+	+	+
Saponins	+	+	+
Phenol	+	_	+
Tannin	+	_	+
Terpenoids	+	+	+
Steroids	+	+	+

Key: (+) presences (-) absences

3.2. *Laurus nobilis* EOs

The chemical content of the EOs from *L. nobilis* leaves are listed in Table 2. Investigation of the EOs by GC-MS (Figure 1) revealed 48 chemicals contributed for 91.4 % of the total compositions. The major constitutes were 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-(2-Hydroxy-1,8-cineole) (26.3%), 3-Cyclohexene-1-methanol,.α.,.α.,4-trimethyl, acetate (α-terpinyl acetate) (17.1%), Benzene, 1,2-dimethoxy-4-(2-propenyl)-(Methylisoleugenol) (9.1%), α.-Terpineol [.α.,α.,4-trimethyl-3-cyclohexene-1-methanol] (5.2%), and 1,6-Octadien-3-ol, 3,7-dimethyl- -(Linalool) (4.6 %). The remaining constituents ranged from 0.3 to 3.5%. In general, the plant is composed of monoterpenes and sesquiterpenes or their derivatives.

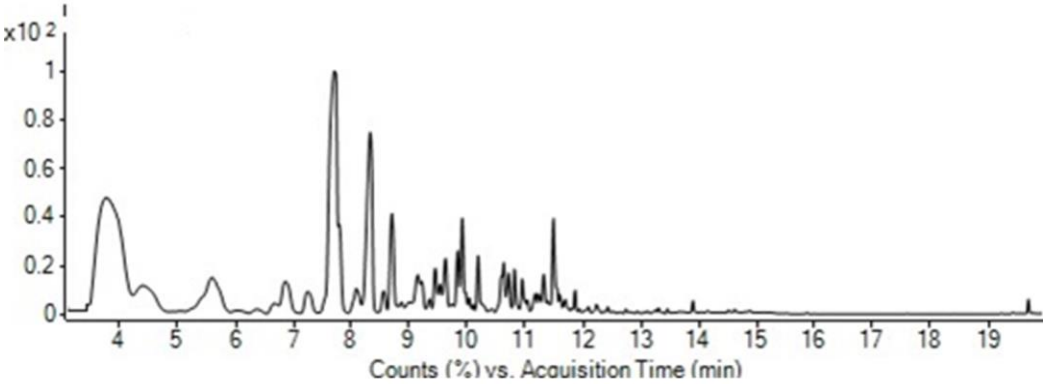

Figure 1. GC-MS chromatogram of essential oil of leaves of *L. nobilis.*

The comparison of chemical content of the oil of *L. nobilis* obtained in this study showed appreciable variation in 1,8-cineole percentage. The value of this finding (26.3%) is lower than the reported values of 44.97%, 56.0%, 58.59%, 52.43% and 34.62% from Türkiye, Tunisia, Cyprus, Morocco, and Algeria respectively (Sıdıka *et al*., 2013; Snuossi *et al*., 2016; Yalçın *et al*., 2007; Nabila *et al*., 2022; Mediouni Ben Jemâa *et al*., 2012). The amount of α-terpinyl acetate obtained in this study (17.1%) was greater than the value obtained by Fidan *et al*. (2019) (14.4%). However, it was comparable with the values reported previously by some investigators (Mediouni Ben Jemâa *et al*., 2012).

Essential oils of *L. nobilis* obtained from various locations were investigated by several evaluators to identify the chemical components of the plant. The results of their studies showed 1,8-cineole was the dominant component with a range of 26.70% to 68.48% (Özcan & Chalchat, 2005). Moreover, α-terpinyl acetate varies from 0.65-25.70% (Sangun *et al*., 2007; Sellami *et al*., 2011) and terpinen-4-ol from 1.50-4.56% (Di Leo Lira *et al*., 2009; Sellami *et al*., 2011) was found as major components. According to this aforementioned studies, the amount of 1, 8-cineole and α-terpinyl acetate recorded in this current study was compatible with previous reported amounts.

Table 2. GC-MS analysis result of EOs of *L.nobilis.*

Compound Name	RT	Formula	%
2(10)-Pinene	3.168	$C_{10}H_{16}$	1.3
2-Oxabicyclo[2.2.2]octane,1,3,3-trimethyl-(2-Hydroxy-1,8-cineole)	3.763	$C_{10}H_{18}O$	26.3
1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool)	4.392	$C_{10}H_{18}O$	4.6
α-Terpineol[.α.,α.,4-trimethyl-3-cyclohexene-1-methanol]	5.605	$C_{10}H_{18}O$	5.2
3-isopropyl-6-methyl-7-oxabicyclo[4.1.0]hept-4-ene	6.685	$C_{10}H_{16}O$	0.5
1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate	6.87	$C_{12}H_{20}O_2$	2.3
3-Cyclohexene-1-methanol,.α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate)	7.713	$C_{12}H_{20}O_2$	17.1
Eugenol	7.788	$C_{10}H_{12}O_2$	2.3
Copaene	8.089	$C_{15}H_{24}$	1.3
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	8.325	$C_{11}H_{14}O_2$	9.1
1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	8.702	$C_{15}H_{24}$	3.5
(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene	9.007	$C_{15}H_{24}$	0.5
(-)-Germacrene D	9.452	$C_{15}H_{24}$	1.3
β-Selinene	9.532	$C_{15}H_{24}$	0.7
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.α.,4a.β.,8a.α.)-	9.839	$C_{15}H_{24}$	1.6
δ-Cadinene	9.919	$C_{15}H_{24}$	2.4
3a(1H)-Azulenol,2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-,[3R-(3.α.,3a.α.,8a.α.)]-	9.971	$C_{15}H_{26}O$	0.3
(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	10.041	$C_{15}H_{26}O$	0.3
Benzene,1,2,3-trimethoxy-5-(2-propenyl)-(Methylisoleugenol)	10.191	$C_{12}H_{16}O_3$	1.3
α.-Santalol	10.254	$C_{15}H_{24}O$	0.3
1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.α.,4a.α.,7.α.,7a.β.,7b.α.)]-	10.636	$C_{15}H_{24}O$	1.8
1,1,7-trimethylspiro[2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene-4,2'-oxirane]	10.722	$C_{15}H_{24}O$	1.1
1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	10.82	$C_{15}H_{26}O$	1
γ.-HIMACHALENE	11.178	$C_{15}H_{24}$	0.6
Guaiol	11.236	$C_{15}H_{26}O$	0.5
.tau.-Cadinol	11.323	$C_{15}H_{26}O$	0.9
2-Naphthalenemethanol,decahydro-.α.,.α.,4a,8-tetramethyl-,didehydroderiv.,[2R-(2.α.,4a.α.,8a.β.)]-	11.49	$C_{15}H_{26}O$	2.5
2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	11.698	$C_{15}H_{24}O$	0.4
(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	11.866	$C_{15}H_{24}O$	0.4
Total			91.4

3.4 Biological Activity of Crude Extract and Essential Oil

3.4.1 Antibacterial activity of L. nobilis

The extract and the EO of the *L. nobilis* were evaluated at four concentration levels against two Gram-positive and four Gram-negative bacteria using tobramycin and DMSO as a positive and negative controls respectively. Overall antibacterial activities of *L. nobilis* extract inhibition zone ranged from 7.00± 0.00mm to 16.0±1.00mm (Table 3). The maxim inhibition zone was recorded by the ethyl acetate extract against *S. typhimurim* (16.0±1.00 mm). While the chloroform extract showed highest inhibition against *E. coli* (14.33±1.53mm), and methanol showed high activity on *S. aureus* (13.67±0.58mm) at 200 mg/mL compared to tobramycin (16.90±0.80mm and 19.16±0.58mm respectively) at 10 µg/mL. However, the crude extract of methanol showed no inhibiton against *K. pneumonia, S. typhimurium and E. faecalis*, and the chloroform extract showed no inhibit against *E. faecalis* at a concentration of 25-200 mg/mL, respectively (Table 3).

In another study, the extracts of *L. nobilis* were found to be prominently active against *B. subtilis, S. aureus, S. epidermidis, E. coli* and *P. aeruginosa* at concentrations less than 5 mg/mL

(Algabri *et al*., 2018), and the methanol extract of bay leaves showed inhibition against *S. aureus* (18 ± 0.8 mm). In contrast, there was no antibacterial activity against bacteria like *P. aeruginosa and E. coli* (Algabri *et al*., 2018). The difference may be due to multipolar factors such as the type and origin of the *L. nobilis*, drying and extraction methods, and differences in the crude extract concentration.

Table 3. Antibacterial activities of extracts from *Laurus nobilis.*

Extract type	Conc. (mg/mL)	Inhibition Diameter (mm)±*SD*					
		E. coli	*P. aeruginosia*	*K. pneumonia*	*S. typhimurium*	*E. faecalis*	*S. aureus*
Methanol	200	NI	9.00±0.00	NI	NI	NI	13.67±0.58
	100	NI	8.67±0.58	NI	NI	NI	13.33±1.52
	50	NI	7.00±0.00	NI	NI	NI	12.33±0.58
	25	NI	7.00±0.00	NI	NI	NI	12.67±0.58
Chloroform	200	14.33±1.53	13.0±0.00	14.00±0.00	11.67±1.15	NI	12.33±1.53
	100	14.00±1.00	12.67±0.58	12.67±0.57	11.33±0.58	NI	11.0±1.73
	50	12.67±0.58	12.0±1.0	11.67±1.15	10.33±0.58	NI	10.0±1.73
	25	11.33±0.58	8.0±1.0	6.33±0.58	8.67±1.15	NI	9.0±1.73
Ethyl acetate	200	14.33±0.58	14.0±0.00	13.0±0.00	16.0±1.00	14.67±0.58	13.33±1.53
	100	14.33±0.58	13.0±1.00	13.0±0.00	15.33±1.53	13.67±0.58	13.33±1.53
	50	14.0±1.00	12.33±0.58	11.67±0.58	14.0±1.00	13.0±0.00	8.33±0.58
	25	14.0±1.00	11.67±0.58	10.67±1.15	13.0±1.00	12.33±2.08	7.33±0.58
Tobramycin	10µg/mL	16.90±0.80	20.84±0.80	11.33±0.80	17.30±0.50	13.83±1.15	19.16±0.58
Negative control		6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Key: NI= No Inhibition

The essential oil of *L. nobilis* demonstrated antibacterial activities against both types of bacteria at 0.045 mg/mL. Among this, maximum zone of inhibition (12.33±0.58 mm) was measured in *P. aeruginosa* and *K. pneumonia.* The positive control tobramycin showed an inhibitory zone of 20.00 ± 1.73 mm and 12.33 ± 0.58 mm against the tested bacteria pathogens, respectively. The antibacterial investigation of this work revealed the activities of the extracts and the oil of *L. nobilis* (Tables 3 and 4) increase with increased concentration. This observation agrees with previously reported findings, and antibacterial activities were increased with increasing the concentration of extract (Jahangirian *et al*., 2013).

Different types of antimicrobial testing method can be used to evaluate the biological activities of plants and their constituents. The variation of the method may influence the inhibition levels. Additionally, other factors like season of sample collection, plant part analysed, and the composition within the plant material may result in the differences of antimicrobial activity of plant material (Fidan *et al*., 2019). The three extracts of *L.nobilis* obtained in this study exhibited different zone of inhibition towards the tested bacteria (Table 3). This difference may be due to the variation of the solvents used to extract the plant material. The extract obtained by using ethyl acetate as an extraction solvent showed the best activity compared to others. The antibacterial activity of *L. nobilis* EO may be attributed to its ability to disrupt cell membrane and affect the semipermeable property of bacterial cells. The oil may also alter the proteins attached to the cell membrane and disturb the transport of nutrients in and out of the cell. Previous reports showed that terpenes are the components accounted for the antibacterial activity of *L. nobilis* essential oil (Siriken *et al*., 2018). Similar to this study finding, the main component of *L. nobilis* oil (1, 8-cineole) has been also implicated in previous studies, inhibited the growth of several microorganisms (Caputo *et al*., 2017).

The variation of inhibition zones displayed by different group of bacteria attributed primarily to the difference chemical composition of the sample and morphology of the bacterial cell membrane. The pronounced antibacterial activity seen in Gram-positive bacteria might be because of the peptidoglycan layer permeating the hydrophobic components in the extract (Rameshkumar *et al*., 2007). The resistance in Gram-negative bacteria could be due to the lipopolysaccharide layer in the cell membrane. The outer membrane of Gram-negative bacteria is made up of hydrophobic lipopolysaccharides, efficient in resisting the entry of hydrophilic compounds (Zgurskaya *et al*., 2015).

In this study, the EOs of *L. nobilis* exhibits greater inhibition effect towards Gram-negative bacteria (Table 4). This finding did not agree with the previous report (Fidan *et al*., 2019) that claimed that Gram-positive bacteria are generally more susceptible to the oils' action than Gram-negative ones. Contrary to our current finding, the EO of *L. nobilis* didn't show inhibitory activity against *P. aurognosa* and *E. coli*. The main EOs constituent of this *L. nobilis* was 1,8-cineole, which has shown good inhibition against many microorganisms, and this constituent has also been reported by previous studies (Caputo *et al*., 2017). Each EO contains a varied mixture of constituents that may play a role in the extended spectrum of antimicrobial activity.

Table 4. Antibacterial activities of essential oil from *L. nobilis*.

Compound	Conc. (mg/mL)	Inhibition Diameter (mm)±*SD*					
		E. coli	*P. aeruginosia*	*K. pneumonia*	*S.typhimurium*	*E. faecalis*	*S.aureus*
Essential oil	0.045	12.33±1.15	12.33±0.58	12.33±0.58	NI	11.33±1.15	11.67±1.15
	0.022	10.60±0.58	11.67±0.58	10.33±0.58	NI	9.33±1.53	10.67±1.15
	0.011	10.33±0.58	10.00±0.00	10.33±1.15	NI	7.00±0.00	7.00±0.00
	0.005	10.00±0.00	9.00±0.00	9.67±1.52	NI	7.0±0.00	7.00±0.00
Tobramycin	10µg/mL	16.67±1.15	20.00±1.73	12.33±0.58	14.33±1.53	10.67±0.58	20.00±1.00
Negative control	10% DMSO	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Key: NI=No Inhibition

3.4.2 The MIC and MBC

The MIC value for the EtOAc and chloroform extracts was 2.5 mg/mL (except *P. arognosa* and *S. aureus*, 5 mg/mL) (Table 5). The MIC was 1.25 mg/mL for the Methanol extract and 0.11 mg/mL for the essential oil against all the tested bacteria except for *S. aureus* (0.22 mg/mL). *Ethyl acetate and chloroform* extract revealed bactericidal activity against the tested organisms with MBC of 5 mg/mL. However, chloroform showed MBC against *P. arognosa* and *S. aureus* at 10 mg/mL. In contrast, the MBC of methanol extract was 2.5 mg/mL against all of the tested bacteria. The MBC results for the EO was 0.22 mg/mL except for *S. aureus*, which was less sensitive, and its minimal bactericidal concentration was 0.44 mg/mL (Table 6).

Table 5. The MIC and MBC values for the extracts in mg/mL.

Bacteria	Methanol		Chloroform		Ethyl acetate	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	_	_	2.5	5	2.5	5
P. aeruginosia	1.25	2.5	5	10	2.5	5
K. pneumonia	_	_	2.5	5	2.5	5
S.typhimurium	_	_	2.5	5	2.5	5
E. faecalis	_	_	_	_	2.5	5
S. aureus	1.25	2.5	5	10	2.5	5

Table 6. The MIC and MBC values of the EO in mg/mL.

Bacteria	Essential oil	
	MIC	MBC
E. coli	0.11	0.22
P. aeruginosia	0.11	0.22
K. pneumonia	0.11	0.22
S.typhimurium	NA	NA
E. faecalis	0.11	0.22
S. aureus	0.22	0.44

3.4.3 Antioxidant activity of L. nobilis

The DPPH radical scavenging activities of the crude extracts (%) were 94.75, 89.12, and 96.11 for the ethyl acetate, chloroform and methanol extracts, respectively (Table 7). Among the studied crude samples MeOH extract of 1000 µg/mL had the same radical scavenging activity with the standard ascorbic acid (96.11). For the EO the free radical scavenging activity was 24.40 at 100 µg/ml (Table 8). Our finding implied the activity of the extracts against DPPH radical increase with increased dosage or concentration of the extract (Elmastaş *et al*., 2006).

IC_{50} values for the ethyl acetate, chloroform, and methanol extracts obtained in this study were 45.5, 153.2, and 0.02, respectively. Since low IC_{50} values correspond to a higher antioxidant capacity, compared to previous studies, the value obtained in this study (45.5) is better than the earlier values (75.65 µg/mL and 83.24 µg/mL) reported by Conforti *et al*., 2016. The effective antioxidant activities of *L. nobilis* seen in this study ars related to the phenolic compounds present in the plant (Renuka *et al*., 2018) which have a high degree of hydroxylation, manifested in the increased ability to donate protons and hence, stabilize the DPPH radical. A specific correlation of the antioxidant properties and the amount of phenols and flavonoids existed in the plant material was reported by some investigators (Larissa da Silva *et al*., 2017). *L. nobilis* leaves are good source of natural phenols and flavonoids which have antioxidant activists that benefit public health. Indeed, using these substances helps to minimize chemical products which usually initiate some secondary undesirable impacts (Taroq *et al*., 2018).

Table 7. Antioxidant activities of crude extract from *L. nobilis*.

Conc. (µg/mL)	Control	Crude Extracts						Positive control	
		Ethyl acetate		Chloroform		Methanol		Ascorbic Acid	
		A	%RSA	A	%RSA	A	%RSA	A	%RSA
1000	1.029	0.054	94.75	0.112	89.12	0.04	96.11	0.04	96.11
500	1.029	0.055	94.66	0.279	72.89	0.049	95.24	0.045	95.63
250	1.029	0.181	82.41	0.428	58.41	0.056	94.56	0.047	95.43
125	1.029	0.358	65.21	0.543	47.23	0.142	86.2	0.05	95.14
IC_{50}		45.5		153.2		0.02			

According to Ibrahim *et al*., 2020, the potency of *L. nobilis* EOs to scavenge free radicals is attributed to the components present in the oil (Cherrat *et al*., 2014), particularly to the high proportion of 1.8-cineole (26.3%) and α-terpinyl acetate (17.1%). Furthermore, other compounds such as methylisoleugenol (9.1%), α.-terpineol (5.2%) and linalool (4.6 %) may be involved. Our results are aligned with previous findings (Celikel & Kavas, 2008; Cherrat *et al*., 2014), and further confirm the antioxidant capacity of EOs from *L. nobilis* and its potential as a natural preservative in food and pharmaceutical industries.

Reports showed that oxidative stress, which occurs when free radical generation in the body exceeds the the defines system, the biological basis of chronic conditions such as arteriosclerosis can be formed (Elmastaş *et al*., 2006). Oxidative stress is recognized as a disproportionate production of reactive species compared to the antioxidant defines system and

according to some studies (Carocho & Ferreira, 2013) an enhanced production of the antioxidants has direct association with decreased degenerative diseases including cancer, diabetes, neurodegenerative and cardiovascular diseases. This study revealed the crude extracts and essential oils showed free radical inhibition activity and may limit free radical damage occurring in the human body.

Table 8. Antioxidant activities of the EO from *L. nobilis*,

Concentration (µg/mL)	Control	Essential oil		Positive control Ascorbic acid	
		A	%RSA	A	%RSA
100	1.275	0.964	24.4	0.028	97.8
50	1.275	1.027	19.45	0.028	97.8
25	1.275	1.059	16.94	0.032	97.49
12.5	1.275	1.086	14.82	0.035	97.25
IC_{50}		339.96			

4. CONCLUSION

This study showed the antibacterial and antioxidant activities of crude extracts and essential oil of *L.nobilis* on different bacterial strains through an in vitro experiment appeared exciting and promising. The crude extract of ethyl acetate showed the highest zone of inhibition against *S. thyphimurium* (16.00± 1.00mm) at 200 mg/mL compared to the positive control tobramycin (17.30±0.50mm) at 10 µg/mL. GC-MS analysis of essential oils obtained from *L.nobilis* leaves revealed a total of 48 chemicals accounted for 91.4 % of the whole. The major constitutes were 2-oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- (2-hydroxy-1,8-cineole) (26.3%), 3-cyclohexene-1-methanol, .α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate) (17.1%), benzene, 1,2-dimethoxy-4-(2-propenyl)- (methylisoleugenol) (9.1%), α.-terpineol [.α.,α.,4-trimethyl-3-cyclohexene-1-methanol](5.2%), and 1,6-octadien-3-ol, 3,7-dimethyl- -(linalool) (4.6 %). *L.nobilis* EOs showed maximum inhibition against *P. aeruginosa and K. pnumonia* (12.33±0.58mm) at 0.045 mg/mL compared to tobramycin (24.0±1.73 and 12.33±0.58mm) respectively at 10 µg/mL. The finding of this study showed that bay leaf has considerable antimicrobial and antioxidant properties.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Rebecca Beyene: accomplihsed the laboratory experimental work and prepared the original draft manuscript. **Teshome Geremew** and **Aman Dekebo** supervised the experimental work and edited the manuscript.

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