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Phytochemical Compositions and Antimicrobial Activities of the Leaves Extract of *Ocimum lamiifolium* (Damakesse) from Debre Berhan, Ethiopia

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Abstract

Medicinal plants exhibit activity against pathogenic microbes owing to their bioactive constituents. The antimicrobial potenti of both crude extracts and essential oils of *Ocimum lamiifolium* was evaluated using the disc diffusion method. Phytochemic components were screened, and the essential oils' chemical composition was analyzed through a Gas Chromatogram-Ma Spectrometer. The leaf extracts of *O. lamiifolium* demonstrated antibacterial and fungicidal activities. The ethanol extract, at concentration of 20mg/ml, exhibited superior antibacterial effects compared to the n-hexane extract at the same concentratio Essential oil extract demonstrated a significant antimicrobial activity, particularly against *S. aureus* (13.75±0.35). GC/M analysis identified five constituents, constituting 97.8% of the total oil: 3, 3 Dimethyl-4-methylamino-butan-2-one (27.5% Hydroxyurea (14.2%), 2-Amino-1-(O-hydroxyphenyl) propane (25.8%), phenylephrine (18.0%), and Ethylamine, (adamantan-1yl)-1-methyl- (12.3%). These findings suggest that *O. lamiifolium* leaf extract possesses antidiarrheal activit supporting traditional medicinal practices. However, further studies are necessary to assess safety and efficacy.

Keywords: Antimicrobial activities, *Ocimum lamiifolium*, Phytochemical screening

1. Introduction

Drug resistance poses a significant global challenge. Traditional plant-based medicines have been widely relied upon for primary healthcare, especially in Ethiopia, where numerous medicinal plants are used in their raw forms to address various diseases. A substantial portion, up to 80%, of the population in many developing countries relies on traditional medicine for primary healthcare [1].

Biologically active compounds found in plants have been applied as drug entities or served as model compounds for drug synthesis. These compounds, including alkaloids, saponins, glycosides, flavonoids, tannins, phenols, terpenoids, and steroids, are utilized by pharmaceutical industries in drug manufacturing. The utilization of natural products as sources for new drugs has seen increased recognition [2]. Medicinal plants produce active compounds crucial for treating illnesses [3]. The antimicrobial activity of plants varies based on the oils' composition, structure, and functional groups. Aromatic plants, traditionally used in folk medicine exhibit inhibitory effects against bacteria and fungi, contributing to food preservation [4].

O. lamiifolium, a widely recognized indigenous plant in Ethiopia, has been traditionally utilized in various regions of the country in its raw form for treating diverse ailments. Currently, there is a growing interest in identifying traditional medicinal plants with potent antimicrobial properties. The screening of essential oils and extracts from plants can contribute to the quest for new, safe, and effective antibiotics, with a focus on researching their active ingredients. *O. lamiifolium*, belonging to the aromatic genus *Ocimum* in the Lamiaceae

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family, is renowned for its medicinal attributes and economically valuable essential oils.

Traditionally, the leaves of *O. lamiifolium* have been employed to address conditions such as coughs, colds, diarrhea, amoebas, pain, and fever, as corroborated by scientific studies [5]. In the study region, the plant's water extract is consumed to treat various ailments, including diarrhea and amoebas, and its distilled water extracts have demonstrated the ability to shorten barbiturate-induced sleep-in animals by 88% [6]. *Ocimum* species, including *O. lamiifolium*, have traditionally been used as insect repellent, flavoring foods, fragrances, folk medicine, and as condiments. In the study area, *O. lamiifolium* is employed medicinally to address infections around reproductive organs and urinary tracts in women during childbirth, with the juice used as an eye rinse for eye infections. Additionally, crushed leaves are applied in the nostrils to halt nose bleeding [7]. This study aimed to explore the chemical composition and antimicrobial properties of crude leaf extracts and essential oils from *O. lamiifolium* against selected pathogenic microorganisms.

2. Materials and Methods

2.1. Collection and Identification of the Plant

The fresh leaves of *O. lamiifolium*, also known as “Dama-kesse”, were gathered in January 2019 in the vicinity of Debre Berhan town, Amhara Regional State, Ethiopia. Debre Berhan town experiences relatively colder weather, with a mean annual temperature of 20°C. The average annual rainfall in the area is 897.8 mm. Upon collection, the plant material was transported to Debre Berhan University (DBU), where it was identified by a plant taxonomist at DBU. The samples were carefully cleaned with distilled water to eliminate any dust particles and then left to dry in a shaded area to prevent the loss of volatile compounds due to direct sunlight exposure. Subsequently, the dried material was ground into a fine powder using a grinder and sieved through a 0.5 mm mesh size. Approximately, 500 g of the resulting powder was obtained and stored in an airtight container until further use.

2.2. Preparation of Crude Extracts of *Ocimum lamiifolium*

The dried leaves were subjected to grinding using an electrical grinder (Fritsch analysts 3) at the chemical engineering laboratory of Debre Berhan University. The ground leaves underwent further sieving through an electrical sieving machine, targeting a particle size of 0.5 [8]. Following the grinding process, the powdered leaves were immersed in 1000 ml flasks, each containing ethanol 80% v/v, absolute n-hexane 99%, and distilled water. Each flask held an equivalent volume of 100 g of the samples and underwent maceration for five days with continuous handshaking. Upon completion of the maceration process, the samples were filtered using filter paper (Whatman filter paper no.1). The filtrates from ethanol 80% and n-hexane 99% were subjected to drying via a rotary evaporator (DW-RE-3000), with adjustments made to maintain a temperature of 45°C to prevent the loss of certain volatile compounds. The resulting dry residues were measured, and labeled, and the requisite amounts of crude hexane and ethanol extracts were dissolved in 10% DMSO [9]. The filtrate obtained from distilled water was directly utilized for antimicrobial activity.

2.3. Preparations of Essential oil of *Ocimum lamiifolium*

The essential oil of *O. lamiifolium* was obtained through hydro-distillation, involving 100 g of freshly chopped leaves of *O. lamiifolium*. The chopped leaves were finely cut to enhance the extraction process and were combined with 1000 ml of distilled water in a 1000 ml conical flask within a Clevenger-type apparatus. This mixture was then transferred to a 1000 ml borosilicate flask containing an additional 1000 ml of water. Boiling chips were introduced into the mixture to prevent over-boiling. The concoction was subjected to heating at 100°C for 2 hours, utilizing a 1000 ml heating mantle. Following the heating process, the oil was separated from the water using separator funnels, and the resulting yield was measured. The obtained oil yield was then stored at 4°C for subsequent analysis involving GC/MS and antimicrobial activity testing.

Yield Estimation

The concentrated extracts from both samples were quantified by weight, and the resulting yields were transferred to a vial, preserved at 4°C, and earmarked for future applications. The calculation of the yield for the extracted samples was determined using the following formula:

Percent of yield extraction= (Final extract weight(g))/(Initial dry weight(g)) X100%

2.4. Phytochemical test

Phytochemical screening of the crude ethanol extracts of *O. lamiifolium* was conducted using various chemical assays to detect the presence or absence of phytochemical components in the plant extracts. This was accomplished through qualitative chemical tests, following the detailed procedures outlined by Belay Zelalem [10] to test Phenols [11], Flavonoids [12], Tannins[13], Terpenoids[14], Alkaloids[15], Saponins[16], Glycosides[17], and Steroids[18].

2.5. Gas Chromatograph/Mass Spectroscopy Analysis

The analysis of FAMEs was conducted using GC/MS (Mass Hunter GC/MS Acquisition 1989-2016 Agilent Technologies) at Addis Ababa Science and Technology University, Department of Food Science. The GC/MS was equipped with a non-polar ZB-5 fused bonded column (Phenomenex) and MS detector. The column parameters were as follows: 30 mm length, 0.25 mm inner diameter, and 0.25 µm film thicknesses. The identification of constituents was accomplished by comparing the retention times and mass spectra of the chromatographic peaks with those of standards analyzed under the same conditions. Peak assignment for other components relied on computer matching of mass spectra obtained with the WILEY 275, NIST 08, and ADAMS libraries, considering the coherence of the retention indices of the analyzed compounds with those reported by Adams and NIST08 libraries.

2.6. Sources of test organisms and Inoculum preparation

The standard strains, including *Staphylococcus aureus* ATCC 5923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 5922, *Salmonella typhimurium* ATCC 13311, and *Candida albicans* ATCC 10231, were acquired from Debre Berhan University, Microbiology Laboratory. Before using, all test organisms were stored at 4°C. To ensure purity and prevent contamination, these bacteria were streaked on sterilized nutrient agar media inside a laminar airflow hood and then incubated at 37°C for 24 hours. A loop full of bacterial pure colonies was transferred to a test tube containing sterilized nutrient broth and incubated at 37°C for 24 hours to activate the test organisms. For the standard *C. albicans* strain, it was streaked on sterilized Potato Dextrose Agar (PDA) media inside a laminar airflow hood and incubated at 37°C for 24 hours to obtain a pure culture and prevent contamination. Similarly, a loop of the fungal strain was transferred to the sterilized nutrient broth and incubated at 37°C for 24 hours to prepare the yeast for fresh use.

2.7. Test Organism Growth Measurement

The test organisms derived from cultured nutrient broth underwent serial dilution in a test tube containing nine ml of sterile normal saline solution (0.85% NaCl). Subsequently, one ml of each bacterial and fungal strain was transferred to a prepared plate count agar media, gently spread, and then incubated at 37°C for 24 hours. Following the completion of the incubation period, the colonies were enumerated [19].

2.8. Preparation of stock solution

The preparation of stock solutions involved dissolving 100mg samples extracted from (80%) ethanol and 99% n-hexane with 5 ml of 10% DMSO solution to achieve a stock solution concentration of 20 g/l. However, the essential oil and water extract were used directly as stock solutions since they were in liquid form. Various concentrations (20, 10, 5, 2.5, and 1.25) g/l were then prepared from the stock solution using the formula C1V1 = C2V2, where C1 represents the stock solution concentration, C2 is the final concentration, V1 is the volume obtained from the stock solution, and V2 is the final volume.

2.9. Antimicrobial Activity tests of plant crude extracts and essential oils

The evaluation of the antimicrobial activities of the three crude extracts (ethanol, hexane, and water) and essential oil from *O. lamiifolium* involved employing the disc diffusion method, as outlined by Pramila et al. [15]. Muller Hinton agar served as the chosen culture medium for this method. The agar media, Petri dishes, forceps, and paper discs (5mm in diameter) were subjected to sterilization through autoclaving at 121°C for 15 minutes. Following sterilization, the plates were placed in the laminar airflow hood until completely dry. These sterilized plates were then divided into four equal sections, with one designated for positive control, another for negative control, and two for the samples to avoid interference in the inhibition zone, each section appropriately labeled. Subsequently, 25 ml of

sterilized Muller Hinton Agar media were poured into each sterilized Petri plate and allowed to solidify.

The microbial strains, including *S. aureus* ATCC 5923, *S. epidermidis* ATCC 12228, *S. typhimurium* ATCC 13311, *E. coli* ATCC 5922, and *C. albicans* ATCC 10231, were obtained from nutrient broth (100 µl) containing 1.45×10^9 cfu/ml. These strains were transferred onto the media and gently spread across the entire Petri plate. Filter paper discs (5 mm in diameter) were impregnated with the crude extract (20 µl) at a concentration of 20 g/l from the stock solution. The impregnated discs were then placed on the Muller Hinton agar using sterile forceps.

Similar to the crude extract, filter paper discs (5mm in diameter) were impregnated with essential oil extract (20µl) at a concentration of 20 g/l from the stock solution. These impregnated discs were also placed on the Muller Hinton agar using sterile forceps. The antibacterial activity was assessed by measuring the diameter of the inhibition zones around both the treated and control discs. The measurements were recorded to the nearest whole millimeter using a ruler. The standard antibiotic discs (SAD) such as chloramphenicol (30µg/mL) and nitroglycerin (20µg/mL) discs were used as a positive control for bacteria and yeasts respectively whereas 10% DMSO was used as a negative control. To ensure reliability, all tests were conducted twice, and the average of the two replicates was considered for each extract and standard antibiotic used.

2.10. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of *O. lamiifolium* plant extracts (g/l) was determined using the disc diffusion method, following the procedure outlined by Pramila et al. [15]. An inoculum density, prepared in sterile saline solution (0.85% NaCl) containing 100 µl and having a concentration of 1.45×10^9 cfu/ml, was transferred onto the media and gently spread across the entire Petri plate, which had been previously divided into four equal parts. Various concentrations (10, 5, 2.5, and 1.25 g/l) for ethanol and hexane extracts, as well as different amounts (10, 5, 2.5, and 1.25 µl) of *O. lamiifolium* plant extracts prepared from the stock solution, were applied onto the discs. The plates were then incubated at 37°C for 24hrs, and the zones of inhibitions were subsequently measured using a ruler. To ensure reliability, all tests were conducted twice, and the average of the two replicates for each extract and antibiotic was calculated.

2.11. Statistical Analysis

The experimental results were presented as means ± standard deviation, and the data analysis was conducted using descriptive statistics with Microsoft Office Excel 2007.

3. Results

3.1. Yield of Extracts

The percentage yield of essential oil, n-hexane, ethanol, and distilled water extracts from the leaves of *O. lamiifolium* is detailed in Table 1. Notably, the ethanol extract exhibited a higher yield.

Table 1. Yield of *O. lamiifolium* leaves constituent extracted by various solvents.

Extraction methods	Extraction	Yield (%)
Hydro-distillation	Essential oil	0.032
Solvent extraction (Maceration)	n-Hexane	2.3
	Ethanol	5.2
	Distilled water	0.5

3.2. Phytochemical compositions of the crude extract of *O. lamiifolium*

The qualitative phytochemical screening of ethanolic *O. lamiifolium* leaf extracts revealed the presence of eight phytochemicals: alkaloids, saponins, glycosides, flavonoids, tannins, phenol, terpenoids, and steroids (Table 2).

Table 2. Results of phytochemical screening for the ethanol extracts of *O. lamiifolium*

No	Phytochemical test	Indicator identification	Results
1	Alkaloids	Formation of colorless	+
2	Steroids	yellow with green fluorescence	+
3	Terpenoids	The deep red color at the junction of 2 layers	+
4	Saponins	Formation of foam	+
5	Tannins	Formation of a yellowish precipitate	+
6	Flavonoids	Intense yellow color to colorless	+
7	Glycoside	Formation of brown color at the junction	+
8	Phenol	Formation of great intense blue color	+

3.3. Antibacterial Activities

The antimicrobial properties of *O. lamiifolium* leaf were investigated through various extraction methods, and the essential oils obtained through hydro-distillation exhibited antibacterial and antifungal activities against the tested organisms (Table 3). The ethanol extract, at a concentration of 20 g/l, demonstrated superior antibacterial inhibition zones compared to the distilled water and n-hexane extracts at the same concentration, particularly against *S. aureus* (14.75±0.35), *S. typhimurium* (12.15±0.21), and *S. epidermidis* (11.25±0.35). Notably, *S. aureus* exhibited greater susceptibility to water extract, n-hexane extract, ethanol extract, and essential oil. *S. aureus* displayed higher susceptibility to water extract compared to the other test organisms in this study. *C. albicans* exhibited lower susceptibility to water extracts than both gram-negative and gram-positive test organisms. The minimum inhibitory concentration of the water extract against *S. au*reus, *S. epidermidis, E. coli*, and *S. typhimurium* was consistent, while the minimum inhibitory concentration against *C. albicans* was higher than the rest in this study.

The ethanol extract demonstrated the maximum inhibition zone against *S. aureus* (14.75±0.51), followed by *S. typhimurium* (12.15±0.21), *S. epidermidis* (11.25±0.35), *C. albicans* (11.9±0.14), and *E. coli* (10.25±0.35) at a concentration of 20 g/l (Table 3). The minimum inhibitory concentration of the ethanol crude extract against *S. epidermidis*, *E. coli*, *S. typhimurium*, and *C. albicans* was 5 g/l, while the minimum inhibitory concentration value against *S. aureus* was 2.5 g/l. *S. typhimurium* (10.1±0.28) exhibited higher susceptibility to hexane extract than *S. aureus* (9.2±0.28), *E. coli* (8.9±0.14), *S. epidermidis* (8.55±0.21), and *C. albicans* (7.85±0.21). However, the hexane extract demonstrated lower efficacy in killing the test organisms compared to the ethanol extract, and the minimum inhibitory concentration of hexane extract against all test organisms was 10 g/L (Table 3).

Table 3. Inhibition zones of *O. lamiifolium* leaf extracts at various concentrations for antibacterial activities. NA - No activity; SAD - the standard antibiotic disc (30µg/mL Chloramphenicol for bacteria and 20µg/mL Nitroglycerin for yeast test).

		Mean Inhibition Zone (mm) ±SEM				
Extracts	Conc. (mg/ml)	*S. aureus*	*S. epidermidis*	*E. coli*	*S. typhimurium*	*C. albicans*
Water	20	12.5 ±0.71	11±0.00	10.33±0.35	10.33±0.35	9±0.00
	10	10±0.00	8±0.73	8.67±0.33	7.83±0.44	7.5±0.71
	5	7.83±0.33	7±0.00	6.00±0.29	7.3±0.33	NA
	2.5	NA	NA	NA	NA	NA
Ethanol	20	14.75±0.51	11.25±0.35	10.25±0.35	12.15±0.21	11.9±0.14
	10	11.25±0.35	10.25±0.35	9.15±0.21	9.33±0.21	7.85±0.21
	5	8.5±0.78	6.15±0.21	6.1±0.14	5.8±0.28	5.7±0.21
	2.5	5.5±0.71	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA
n-Hexane	20	9.2±0.28	8.55±0.21	8.9±0.14	10.1±0.28	7.85±0.21
	10	6.85±0.21	6.75±0.07	6.2±0.28	7±0.28	5.8±0.14
	5	NA	NA	NA	NA	NA
Controls	SAD	28	25	28	29	21
	DMSO	NA	NA	NA	NA	NA

3.4. Essential Oils

The essential oil extract exhibited the highest antimicrobial activity against *S. aureus* (13.75±0.35). The antimicrobial impact of the essential oil was consistent for *S. epidermidis* and *E. coli*. The minimum inhibitory concentration of the essential oil against the test organisms was 5.4±0.71 at a concentration of 2.5 µl, 6.15±0.2, 6.1±0.14, 5.4±0.28 at the same concentrations of 5 µl, and 6.15±0.21 at the concentrations of 10 µl for *S. aureus*, *S. epidermidis*, *E. coli*, *S. typhimurium* and *C. albicans*, respectively (Table 4).

Table 4. Zone of inhibition of the essential oils extract against the test organisms. NA - No activity; SAD - the standard antibiotic disc (30µg/mL Chloramphenicol for bacteria and 20µg/mL Nitroglycerin for yeast test)

Conc. (µl)	***S. aureus***	***S. epidermidis***	***E. coli***	***S. typhimurium***	***C. albicans***
16	13.75±0.35	10.25±0.35	10.21±0.35	11.15±0.21	11±0.00
8	10.25±0.35	8.25±0.35	9.15±0.21	9.33±0.21	6.15±0.21
4	7.8±0.78	6.15±0.2	6.1±0.14	5.4±0.28	NA
2	5.4±0.71	NA	NA	NA	NA
1	NA	NA	NA	NA	NA
SAD	28	25	28	29	21

3.5. GC-MS Characterization of the essential oil extracted from *Ocimum lamiifolium*

The compounds namely: 3,3-Dimethyl-4-methylamino-butan-2-one, Hydroxyurea, 2-Amino-1- (o-hydroxyphenyl) propane, Phenylephrine, and Ethylamine, 2-(adamantan-1-yl)-1 -methyl were detected in the essential oil of *O. lamiifolium* with different probabilities (Table 5 and Figure 1- 5).

Table 5. The constituents identified from the leaves EO of *O. lamiifolium* by GC/MS.

S. No	Compound Name	Retention Time	Area in (%)	Molecular Formula	Probability (%)
1	3,3-Dimethyl-4-methylamino-butan-2-one	12.573	3.06	$C_7H_{15}NO$	27.5
2	Hydroxyurea	13.224	1.24	$CH_4N_2O_2$	14.2
3	2-Amino-1-(o-hydroxyphenyl) propane	19.494	3.59	$C_9H_{13}NO$	25.8
4	Phenylephrine	24.261	1.83	$C_9H_{13}NO_2$	18.0
5	Ethylamine, 2-(adamantan-1-yl)-1-methyl-	28.059	1.98	$C_{13}H_{23}N$	12.3

The mass spectra of the compound 3,3-Dimethyl-4-methylamino-butan-2-one, as illustrated in Figure 1, reveal a molecular formula of C7H15NO, with a molecular weight of 129 g/mol. The peak at 44 m/z was identified as the most abundant ion of the compound.

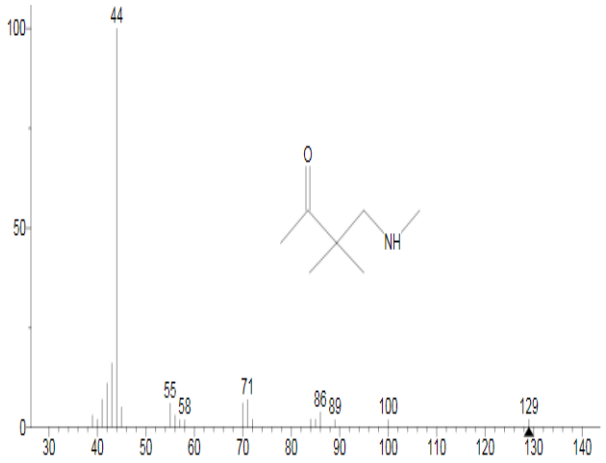


Figure 1. 3, 3-Dimethyl-4-methylamino-butan-2-one detected by GC/MS.

The mass spectra of the compound Hydroxyurea, as depicted in Figure 2, exhibit a molecular formula of CH4N2O2, with the peak at 44 m/z representing the most abundant ion of the compound. The molecular weight was determined to be 76 g/mol.

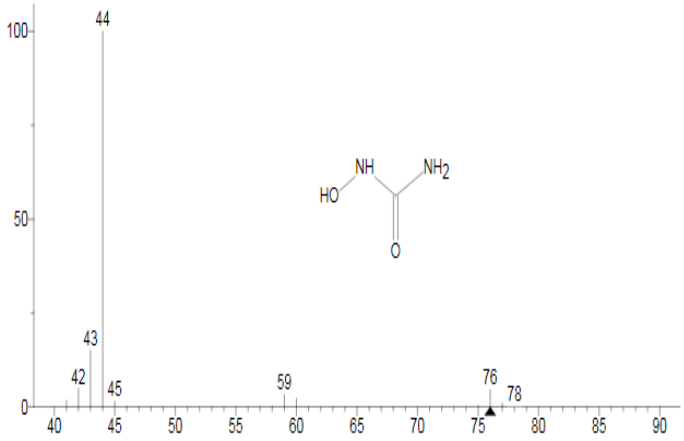
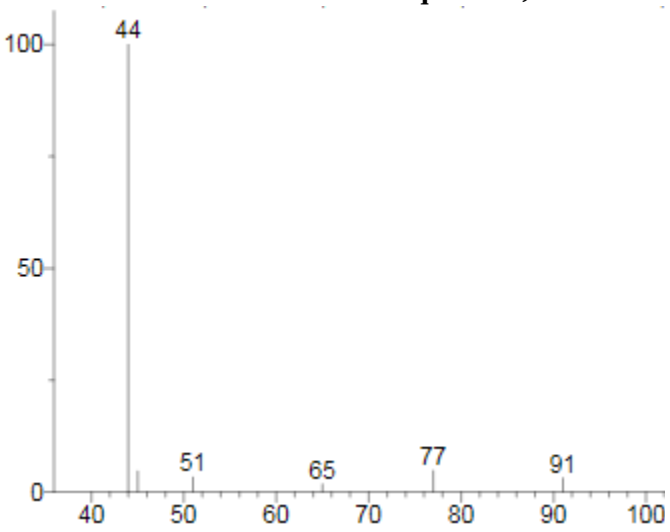


Figure 2. Hydroxyurea detected by GC/MS.

The mass spectra of the compound 2-Amino-1-(o-hydroxyphenyl) propane, depicted in Figure 3, exhibited a molecular formula of C9H13NO, with a molecular weight of 151 g/mol. The peak at 44 m/z was identified as the most abundant ion of the compound.



The mass spectra of the compound phenylephrine, illustrated in Figure 4, reveal a molecular formula of C9H13NO2, with a molecular weight of 167 g/mol. The peak at 44 m/z was recognized as the most abundant ion of the compound.

The mass spectra of the compound phenylephrine, illustrated in Figure 4, reveal a molecular formula of C9H13NO2, with a molecular weight of 167 g/mol. The peak at 44 m/z was recognized as the most abundant ion of the compound.

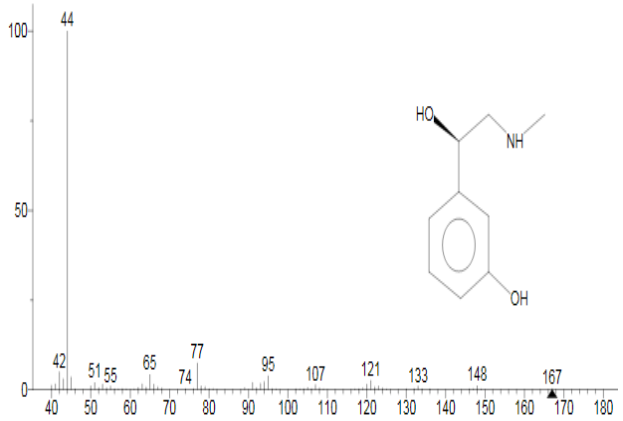


Figure 4. Phenylephrine detected by GC/MS.

The mass spectra of the compound Ethylamine, 2-(adamantan-1-yl)-1-methyl- displayed in Figure 5, indicate a molecular formula of C13H23N, with a molecular weight of 193 g/mol. The peak at 44 m/z was identified as the most abundant ion of the compound.

peak at 44 m/z was recognized as the most abundant ion of the compound.

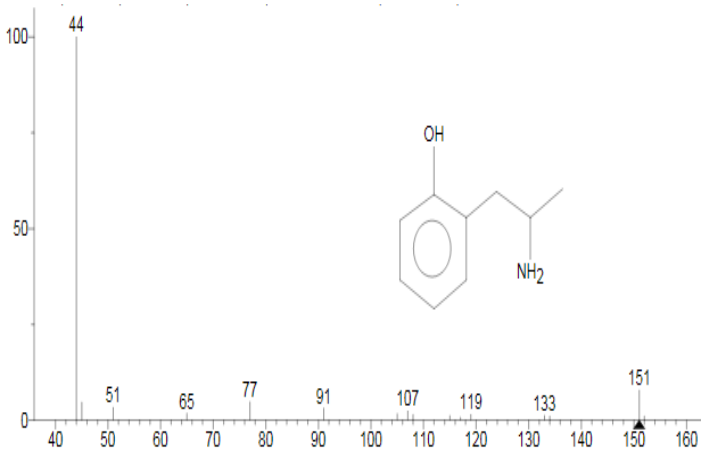


Figure 3. 2-Amino-1-(o-hydroxyphenyl) propane detected by GC/MS.

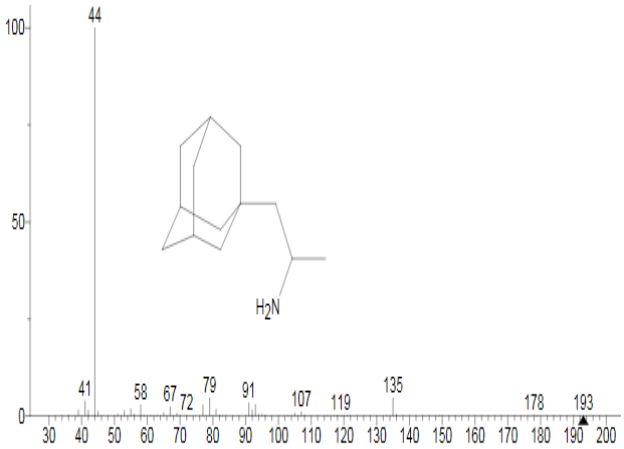


Figure 5. Ethylamine, 2-(adamantan-1-yl)-1-methyl- detected by GC/MS.

4. Discussion

The ethanol extracts of *O. lamiifolium* leaves were analyzed for phytochemical composition, revealing the presence of eight constituents: alkaloids, saponins, glycosides, flavonoids, tannins, phenol, terpenoids, and steroids. This finding contrasts with the methanol extract obtained from *O. lamiifolium* in Adama, Oromia Region, Ethiopia, which lacked flavonoids [20]. Phenolic compounds, one of the

largest and most widespread groups of plant metabolites, exhibit diverse biological properties, including anti-apoptotic, antiaging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protective, and endothelial function-improving activities, as well as the inhibition of angiogenesis and cell proliferation. Phenolic compounds are known for their potential to scavenge free radicals. Flavonoids have been reported to possess various therapeutic properties, including antimicrobial, anti-cancer, antiviral, anti-allergic, antitumor, and anti-inflammatory effects [21]. Tannins, in general, can inactivate and eliminate microorganisms that cause diseases [13].

Terpenoids exhibit activity against bacteria, fungi, and viruses, with reports indicating that 60% of examined essential oil derivatives inhibit fungi, while 30% show inhibitory effects on bacteria. Terpenes, including monoterpenes, sesquiterpenes, triterpenes, and sterols, have demonstrated various biological activities in animals and microorganisms, such as anti-inflammatory, antimicrobial, and hormonal effects. Some tannins have been identified as having antiviral and antitumor properties, along with diuretic effects. Additionally, saponins and glycosides have been reported to possess antifungal activity. The presence of tannins and flavonoids in *O. lamiifolium* leaves suggests potential antibacterial and antifungal activities. The results of various phytochemical tests indicate that plants are rich in biologically active compounds, serving as a promising source for drug discovery [20].

The efficacy of the active compounds in *O. lamiifolium* plant extracts is evident in the formation of growth inhibition zones. Among all the extracts, the ethanolic crude extract of leaves exhibited higher activity against all organisms (Table 3). The choice of ethanol as a solvent for extraction proved advantageous, not only yielding better results than n-hexane but also being less toxic for bioactive compounds. The ethanol extract demonstrated the maximum inhibition zone against *S. aureus* (14.75±0.51), followed by *S. typhimurium* (12.15±0.21), *S. epidermidis* (11.25±0.35), *C. albicans* (11.9±0.14), and *E. coli* (10.25±0.35) at the concentration of 20 g/l. E. coli exhibited the least susceptibility among the tested organisms.

The hexane extract derived from *O. lamiifolium* in this study demonstrated antimicrobial activities against all test organisms, albeit with lower efficiency compared to the ethanolic extract (Table 4). The hexane extract exhibited higher antimicrobial activity against *S. typhimurium* (10.1±0.28), followed by *S. aureus* (9.2±0.28), *E. coli* (8.9±0.14), *S. epidermidis* (8.55±0.21), and the lowest antimicrobial activity was observed against *C. albicans* (7.85±0.21) at the concentration of 20 g/l (Table 4). However, the hexane extract was less effective in eliminating the test organisms compared to the ethanol extract.

The minimum inhibitory concentration of the ethanol extract against *S. epidermidis*, *E. coli*, *S. typhimurium*, and *C. albicans* at a concentration of 5 g/l was 6.15±0.21, 6.1±0.14, 5.8±0.28, and 5.7±0.21, respectively. In contrast, the minimum inhibitory concentration against *S. aureus* at the concentration of 2.5 g/l was 5.5±0.71. The ethanolic extract demonstrated greater effectiveness and a more pronounced inhibitory effect compared to the hexane extract [22]. The similar efficacy observed in the antimicrobial activities of ethanol and hexane extracts from some plants against certain bacterial species might be attributed to the extraction capabilities of active ingredients responsible for antibacterial activity by both extraction systems [19]. This suggests that the crude extract from the leaf parts of *O. lamiifolium* can be employed to combat selected test organisms.

The essential oil exhibited higher activity against bacterial test organisms compared to the water extract. The essential oil extract displayed maximum antimicrobial activity against *S. aureus* (13.75±0.35), while *C. albicans* showed less susceptibility compared to bacteria (Table 3). The minimum inhibitory concentration against test organisms was 5.4±0.71 at the concentrations of 2.5 mg, 6.15±0.2, 6.1±0.14, 5.4±0.28 at the same concentrations of 5 mg, and 6.15±0.21 at the concentrations of 10 mg for *S. aureus*, *S. epidermidis*, *E. coli*, *S. typhimurium*, and *C. albicans*, respectively.

The aqueous extract exhibited lower activity against bacterial test organisms and the fungal strain. The water extract demonstrated a maximum inhibition zone against *S. aureus* (12.5 ±0.71), *S. epidermidis*

(11±0.00), *E. coli* (10.33±0.35), *S. typhimurium* (10.33±0.35), with *C. albicans* being the least susceptible among the tested organisms (9±0.00) at the same concentration of one drop (10 µl) from the concentrations of 20 mg. The minimum zone of inhibitory concentration of the water extract against S. aureus (7.83±0.33), *S. epidermidis* (7±0.00), *E. coli* (6.00±0.29), and *S. typhimurium* (7.3±0.33) remained the same at concentrations of 5 g/l, and the MIC of *C. albicans* (7.5±0.71) was higher than all the others in this study at the concentrations of 10 g/l. This indicates that the essential oil (EO) obtained from the leaf parts of *O. lamiifolium* can be utilized to combat the selected test organisms. In contrast, the antimicrobial activities of both EO and aqueous extracts from some plants showing similar efficacy against certain bacterial species could be attributed to the extraction ability of active ingredients responsible for antibacterial activity by the two extraction systems [23].

Identification of numerous constituents present in the essential oil (EO) obtained through hydrodistillation techniques revealed that five major compounds, constituting 97.8% of the total oil, were identified in the essential oil extracted from the leaves of *O. lamiifolium* (Table-7, Figures 2-6). These compounds include 3,3 Dimethyl-4-methylamino-butan-2-one (27.5%), Hydroxyurea (14.2%), 2-Amino-1-(o hydroxyphenyl) propane (25.8%), phenylephrine (18.0%), and Ethylamine, 2-(adamantan-1yl)-1-methyl- (12.3%). The chemical constituents identified in the EO from the leaf parts of *O. lamiifolium* by GC/MS are reported to possess a high level of potent antimicrobial properties.

The compound 3,3-Dimethyl-4-methylamino-butan-2-one (Figure 2), with the molecular formula (C7H15NO, MW 129 g/mol) obtained from the leaf part of *O. lamiifolium*, plays a significant role in exhibiting antimicrobial properties [24]. Hydroxyurea (Figure 3), with a molecular formula CH4N2O2 and a weight of 76 g/mol, obtained from *O. lamiifolium* leaf part, plays a relevant role in the treatment of sickle cell anemia and induces fetal hemoglobin production [25]. The compound 2-Amino-1-(o-hydroxyphenyl) propane, with the molecular formula C9H13NO and a molecular weight of 151 g/mol (Figure 4), plays a significant role in antimicrobial properties [24]. Phenylephrine (Figure 5), identified with a molecular formula C9H13NO2 and a molecular weight of 167 g/mol, obtained from *O. lamiifolium* leaf part, is relevant in leading to short-lived changes in blood pressure and heart rate. The compound Ethylamine, 2-(adamantan-1-yl)-1-methyl- (Figure 6), with the molecular formula C13H23N and weight 193 g/mol, obtained from *O. lamiifolium* leaf part, plays a relevant role in the treatment for reducing viral load. All the chemical constituents identified in EO from the leaf parts of *O. lamiifolium* by GC/MS are reported to exhibit very high levels of potent antimicrobial properties.

5. Conclusion and recommendations

The current investigation demonstrated that the ethanol extract exhibited superior antimicrobial activity in the leaf part of *O. lamiifolium* compared to the hexane extract. The ethanol extract displayed the maximum inhibition zone against *S. aureus*, followed by *S. typhimurium*, *S. epidermidis*, *C. albicans*, and *E. coli* at a concentration of 20 g/l. Among the test organisms, *E. coli* showed the least susceptibility. Phytochemical analysis of the ethanol extracts of *O. lamiifolium* leaf revealed the presence of eight phytochemicals, namely alkaloids, saponins, glycosides, flavonoids, tannins, phenol, terpenoids, and steroids. The primary chemical compounds identified in the essential oil from *O. lamiifolium* leaf constituted 97.8% of the total oil and included 3,3 Dimethyl-4-methylamino-butan-2-one, Hydroxyurea, 2-Amino-1-(o hydroxyphenyl) propane, phenylephrine, and Ethylamine, 2-(adamantan-1yl)-1-methyl-.

Based on the findings of this study, the following recommendations are proposed: Further chemical analysis of crude extracts is highly recommended to identify the specific compound responsible for antibacterial activity. The results support the traditional use of *O. lamiifolium* plant extracts, which contain antibacterial compounds, suggesting their potential as antibacterial agents in the development of new drugs for treating infectious diseases caused by pathogens. Subsequent research may involve pharmacological evaluations to further explore the therapeutic potential of these compounds.

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