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Phytochemical Profiling and In Vitro Antibacterial Activity of *Leonotis ocymifolia* (Burm. f.) and *Laggera tomentosa* (Sch. Bip. ex A. Rich) Leaf Extracts Against Clinically Relevant Human Pathogenic Bacteria

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Posted Date: 19 March 2026

doi: 10.20944/preprints202603.1494.v1

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Phytochemical Profiling and In Vitro Antibacterial Activity of *Leonotis ocymifolia* (Burm. f.) and *Laggera tomentosa* (Sch. Bip. ex A. Rich) Leaf Extracts Against Clinically Relevant Human Pathogenic Bacteria

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Abstract

Background: *Leonotis ocymifolia* and *Laggera tomentosa* are traditionally used in Ethiopian folk medicine to treat various ailments. This study evaluated the phytochemical constituents and antibacterial activity of their leaf extracts against selected human pathogenic bacteria. Methods: Crude extracts were prepared using six solvents (chloroform, ethanol, methanol, hexane, ethyl acetate, and water) via maceration. Phytochemical screening employed standard procedures. Plant activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* was assessed using disc diffusion (100 mg/mL), with MIC and MBC determinations. Results: *L. ocymifolia* contained flavonoids, tannins, alkaloids, and phenols; *L. tomentosa* additionally contained saponins. Chloroform extracts exhibited the highest activity, with inhibition zones of 20 ± 1 mm (*L. ocymifolia* against *P. aeruginosa*) and 20 ± 4.6 mm (*L. tomentosa* against *P. aeruginosa*). Water extracts showed minimal or no activity against several strains. MIC values ranged from 1.56–12.5 mg/mL (*L. ocymifolia*) and 3.13–6.25 mg/mL (*L. tomentosa*); MBC values ranged from 3.13–12.5 mg/mL and 3.13–6.25 mg/mL, respectively. Conclusion: Chloroform extracts of both plants demonstrated significant broad-spectrum antibacterial activity, validating their traditional use and highlighting their potential as sources of novel antibacterial agents.

Keywords: antibacterial; MBC; MIC; phytochemical screening

1. Introduction

The use of medicinal plants has acquired renewed interest in developed countries and constitutes the first therapeutic strategy for 80% of developing countries [1]. According to the World Health Organiza-tion, as many as 80% of the world's population depends on traditional medicine for their primary healthcare needs [2]. Medicinal and aromatic plants can play an important role in enhancing the sub-sistence livelihood of rural communities in an environmentally sustainable manner while maintaining biodiversity [3].

The medicinal value of plants lies in chemically active compounds that produce definite physiological effects on the human body [4]. Plants possessing phytochemicals (secondary metabolites) have the po-tential for use as drugs, and the known pharmacological activities of these biochemical substances form the scientific basis for their applications in modern medicine, when scientifically validated [5].

In Ethiopia, approximately 80% of the human population and 90% of livestock rely on traditional medicine [6,7]. Due to community trust in medicinal plants, culturally associated traditions, and

 doi:10.20944/preprints202603.1494.v1

relatively low cost, medicinal plants are highly demanded in Ethiopia. Inadequate health centers, shortage of medicines, and limited healthcare personnel in clinics may be additional reasons driving communities toward traditional health centers, thereby increasing the demand for medicinal plants.

Several traditional medicinal plants are used for various human and animal treatments in North Shoa Zone. Among these, Laggera tomentosa in Kuyu district and Leonotis ocymifolia in Girar Jarso district are major traditional medicinal plants. Both plants are used as antibacterial, antioxidant, and an-ti-inflammatory agents [8]. Local elders in these districts continue to use these plants as antibacterial agents.

Bacterial diseases are widespread and dramatically increasing worldwide. Although treatment primar-ily relies on commercial antibiotics, numerous antibiotics have lost effectiveness due to the develop-ment of resistant strains. Additionally, safety concerns exist regarding antibiotic use, including hyper-sensitivity, immunosuppression, and allergic reactions. Therefore, there is a need to develop alternative antibacterial drugs for treating common infectious diseases.

This study aimed to identify the major secondary metabolites and evaluate the antibacterial activities of Laggera tomentosa and Leonotis ocymifolia leaf crude extracts against four human pathogenic bacterial strains: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae.

2. Materials and Methods

2.1. Description of the Study Area

The research was conducted in the Microbiology Laboratory of Salale University. Laggera tomento-sa and Leonotis ocymifolia plants were collected from Kuyu and Girar Jarso districts, respectively. Both districts are located in North Shoa Zone, Oromia Regional State, Ethiopia. Kuyu district is situated at 9°52′0″ N latitude and 38°21′0″ E longitude, while Girar Jarso district is located between 9°05′ and 10°23′ N latitude and 37°57′ and 39°28′ E longitude.

2.2. Study Design

An experimental study design with three replications was conducted to test the phytochemical constit-uents and antibacterial activity of Leonotis ocymifolia and Laggera tomentosa against human pathogenic bacterial strains.

2.3. Plant Material Collection and Identification

About 5 kg of fresh leaves from each plant species (Leonotis ocymifolia and Laggera tomentosa) were col-lected from Kuyu and Girar Jarso districts, respectively. The plant leaves were properly screened to re-move unwanted woody parts, yielding 2.5 kg final weight per species. The screened leaves were dried at room temperature for 20 days. Plant identification was performed by botanists, and voucher specimens were deposited at the Department of Biology, Salale University.

2.4. Preparation of Plant Extracts

The dried leaves were ground into fine powder using a grinder machine and passed through a sieve (30 µm pore size). Fifty grams of powdered leaves were weighed and mixed with 150 mL (1:3 ratio) of six different solvents: distilled autoclaved water, chloroform, ethyl acetate, hexane, methanol, and ethanol. Extraction was performed using a shaker with continuous shaking for 8 hours per day for three consec-utive days. The extracts were filtered using Whatman No. 1 filter paper with a vacuum pump. The fil-trates were evaporated under vacuum using a rotary evaporator, and the remaining extracts were placed in an oven at 40 °C to evaporate residual solvent. The dried extracts were weighed using an elec-tronic balance. Stock solutions were prepared at 100 mg/mL concentration using 50% dimethyl sulph-oxide (DMSO) [9], vortexed thoroughly, labeled, and stored at 4 °C until use. Chloroform crude extract required microwave treatment for 3 minutes with one-minute intervals and continuous vortexing to dissolve in DMSO.

Figure 1. Plant crude extraction process.

2.5. Phytochemical Screening

Preliminary phytochemical screening was conducted to determine the presence of alkaloids, saponins, tannins, flavonoids, and phenols using standard procedures. Alkaloids were tested using Dragendorff's reagent, where the formation of a prominent yellow precipitate indicated a positive result [10]. Saponin detection involved diluting extracts with distilled water to 20 mL and shaking in a graduated cylinder for 15 minutes, with the formation of a 2 cm foam layer indicating presence [11]. For tannin analysis, approximately 0.5 mg of crude extract was boiled in 20 mL water, filtered, and treated with few drops of 0.1% ferric chloride; brownish-green or blue-black coloration confirmed tannins [12]. Flavonoid testing was performed by adding few drops of dilute NaOH to 1 mL of stock solution; intense yellow color that became colourless upon acid addition indicated flavonoids, further confirmed by yellow precipitate formation with lead acetate solution [13]. Phenol detection involved treating aqueous extract solutions with 10% ammonium hydroxide solution, where yellow fluorescence indicated phenol presence [13].

2.6. Test Organisms

The plant extracts were tested for antibacterial activity against two Gram-positive bacteria (Staphylococ-cus aureus, Streptococcus pneumoniae) and two Gram-negative bacteria (Escherichia coli, Pseudomonas aeru-ginosa) using the disc diffusion assay which is standard method for this like

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test [14]. The bacterial strains were obtained from the Ethiopian Public Health Institute and stored at -20 °C until use.

2.7. Media Preparation

Mueller Hinton agar (38 g/L) was prepared according to manufacturer instructions. The pH was ad-justed to 7.0, and the media were autoclaved at 121 °C for 15 minutes. After autoclaving, the media were incubated at 37 °C for 24 hours to check sterility. Test microorganisms were grown on Mueller Hinton agar at 37 °C for 24 hours.

Figure 2. Media preparation process.

2.8. Preparation of Standard Inoculum

The 0.5 McFarland turbidity standard (1.5 × 10^8 CFU/mL) was prepared by mixing 1% sulfuric acid and 1% barium chloride to achieve specific optical density. Bacterial strains were cultured in nutrient agar and incubated at 37 °C for 24 hours. Pure colonies were picked and diluted in sterile saline water, and absorbance was adjusted to 580 nm to match the 0.5 McFarland standard.

2.9. Antibacterial Activity Assay

The antimicrobial activities of chloroform, ethyl acetate, water, methanol, ethanol, and hexane extracts were determined using the agar well diffusion method [15]. Bacterial inoculum was prepared by ad-justing overnight culture to 0.5 McFarland standard in 0.9% sterile normal saline. Mueller Hinton agar was poured into sterilized Petri dishes with uniform thickness and allowed to set. The inoculum was spread evenly on the agar surface using a sterile cotton swab. Diffusion discs (6 mm diameter) were prepared from Whatman No. 1 filter paper, sterilized at 120 °C for 1 hour, and dried. One hundred mi-croliters of each plant extract (100 mg/mL) were aseptically added to respective discs. Amoxicillin (30 µg/disc) and vancomycin (30 µg/disc) were used as positive controls, while 50% DMSO served as a negative control. Plates were allowed to sit for 30 minutes under a fume hood and then incubated at 37 °C for 24 hours. An inhibition zone of ≥7 mm around the disc was considered significant susceptibil-ity. All experiments were performed in triplicate.

2.10. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (lowest concentration inhibiting bacterial growth) was deter-mined for extracts showing >7 mm inhibition zones at 100 mg/mL concentration using the agar dilu-tion method with three-fold serial dilutions from 50 to 0.78 mg/mL [16]. Double serial dilution was em-ployed from 50 mg/mL to obtain 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 mg/mL concentrations in 50% DMSO. One hundred microliters of each diluted extract were added to prepared discs on Mueller Hin-ton agar as described for the sensitivity test. Control plates without test organisms were

 doi:10.20944/preprints202603.1494.v1

also prepared. Plates were incubated at 37 °C for 24 hours, and MIC was recorded as the lowest concentration with no visible bacterial growth.

2.11. Minimum Bactericidal Concentration (MBC)

For MBC determination, dilutions showing no visible growth were sub cultured on Mueller Hinton agar and incubated for 24 hours at 37 °C. The lowest concentration showing no visible growth after sub cul-turing was taken as the MBC value.

2.12. Data Analysis

All assays were conducted in triplicate. Data were analyzed using SPSS version 20 and presented as mean ± standard deviation (SD). Duncan's test was used to compare means of antibacterial activity among extraction solvents and test microorganisms. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical Analysis

The preliminary phytochemical screening of Leonotis ocymifolia and Laggera tomentosa leaf extracts re-vealed varying presence of secondary metabolites depending on the extraction solvent (Table 1).

For Leonotis ocymifolia, flavonoids were absent in ethanol and water extracts; tannins were absent in ethyl acetate extract; alkaloids were absent in ethanol, chloroform, and hexane extracts; phenols were absent in ethyl acetate and hexane extracts; and saponins were absent in all solvent extracts. For Laggera tomentosa, flavonoids were present only in ethanol extract; tannins were present in ethanol, methanol, and water extracts; alkaloids were present only in hexane extract; phenols were present in methanol and water extracts; and saponins were present in methanol, ethanol, hexane, and water extracts.

Table 1. Phytochemical Analysis of Leonotis ocymifolia and Laggera tomentosa Leaf Extracts.

S. No	Tested secondary metabolites	***Leonotis ocymifolia* leaf crude extract** solvents					
		Ethanol	Methanol	Chloroform	Ethyl acetate	Hexane	Water
1	Flavonoids	x	√	√	√	√	x
2	Tannins	√	√	√	x	√	√
3	Alkaloids	x	√	x	√	x	√
4	Saponin	x	X	x	x	x	x
5	Phenols	√	√	√	x	x	√
	Tested secondary metabolites	***Laggera tomentosa* Leaf crude Extract**					
		Ethanol	Methanol	Chloroform	Ethyl acetate	Hexane	Water
1	Flavonoids	√	X	x	x	x	x
2	Tannins	√	√	x	x	x	√
3	Alkaloids	x	X	x	x	√	x
4	Saponin	√	√	x	x	√	√
5	Phenols	x	√	x	x	x	√

Note: (+) indicates presence; (-) indicates absence.

3.2. Antibacterial Activity of Leonotis ocymifolia

The antibacterial effects of Leonotis ocymifolia crude extracts (100 mg/mL) from six solvents were tested against four bacterial strains (Table 2). Water extract showed no antibacterial activity against P. aeru-ginosa and E. coli. However, all other solvent extracts inhibited all tested bacterial strains. Chloroform crude extract demonstrated significantly higher zone of inhibition (20 ± 1 mm) against P. aeruginosa, while water extract showed the minimum inhibition zone (3 ± 5.2 mm) against S. aureus. Amoxicillin (30 μg/disc) and vancomycin (30 μg/disc) were used as positive controls.

Table 2. Mean Inhibition Zones of Leonotis ocymifolia Extracts against Test Bacteria.

Test organism	Inhibition zone (mm) M+S.D							
	Solvents crude extracts						Control	
	Water	Ethanol	Methanol	Chloroform	Ethyl acetate	Hexane	A30	V30
E. coli	-	11.7±1.5	8 ±1	18.3+1.15	15.7+1.53	19+1	20	24
S. aureus	3±5.2	9+2	10.7±1.53	19±1	13±2	11±1	15	16
S. pneumonia	3.7±6.4	9±2	12±3	15.3±1.15	10±1	11.7±1.53	20	18
P.aeruginosa	-	6.3±.58	7.3+.58	20+1	11±2	8±1	17	20

NB: A30 = Amoxicillin (30 μg/disc); V30 = Vancomycin (30 μg/disc).

3.3. Minimum Inhibitory Concentration and Minimum Bac-tericidal Concentration of Leonotis ocymifolia

Among the four tested bacterial strains, MIC values ranged from 1.56 mg/mL (chloroform extract against E. coli) to 12.5 mg/mL (hexane extract against P. aeruginosa). Hexane extract showed the highest MIC value compared to other solvents (Table 3). MBC values ranged from 3.13 to 12.5 mg/mL. The low-est MBC (3.13 mg/mL) was recorded for ethanol extract against E. coli, S. aureus, and S. pneumoniae; methanol extract against S. aureus and S. pneumoniae; chloroform extract against all bacterial strains; ethyl acetate extract against E. coli and S. aureus; and hexane extract against E. coli, S. aureus, and S. pneumoniae. The highest MBC (12.5 mg/mL) was recorded for hexane extract against P. aeruginosa.

Table 3. MIC and MBC Concentrations of Leonotis ocymifolia Extracts (mg/mL).

Test organism	Gram type	Ethanol		Methanol		Chloroform		Ethyl acetate		Hexane	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	-	3.13	3.13	3.13	6.25	1.56	3.13	3.13	3.13	3.13	3.13
S. aureus	+	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13
S. pneumonia	+	3.13	3.13	3.13	3.13	3.13	3.13	3.13	6.25	3.13	3.13
P. aurongousa	-	3.13	6.25	3.13	6.25	3.13	3.13	6.25	6.25	12.5	12.5

3.4. Antibacterial Activity of Laggera tomentosa

The antibacterial effects of Laggera tomentosa crude extracts (100 mg/mL) from six solvents were tested against four bacterial strains (Table 4). Water extract showed no antibacterial activity against E. coli, S. aureus, and P. aeruginosa. However, all other solvent extracts inhibited all tested bacterial strains. Chlo-roform crude extract demonstrated the highest zone of inhibition (20 ± 4.6 mm) against P. aeruginosa, while water extract showed the minimum inhibition zone (2.7 ± 4.6 mm) against S. pneumoniae.

Table 4. Mean Inhibition Zones of Laggera tomentosa Extracts against Test Bacteria.

Test organism	Inhibition zone (mm) M+S.D						Control	
	Water	Ethanol	Methanol	Chloroform	Ethyl acetate	Hexane	A30	V30
E. coli	-	8.3±1.53	9.7+2.1	13.3+1.53	5.7+.58	13.3+2.1	20	24
S. aureus	-	10.3±1.53	14.7±2.1	16.7±.58	13.3±1.53	10.7+1.15	15	16
S. pneumonia	2.7±4.6	9.3±.58	9.7±1.53	11.7±1.53	10±1	9±0	20	18
P.aeruginosa	-	15.7±3.8	15.3±4.0	20+4.6	19.3±4.16	10.3±1.53	17	20

NB: A30 = Amoxicillin, V30 = vancomycin.

 doi:10.20944/preprints202603.1494.v1

3.5. Minimum Inhibitory Concentration and Minimum Bac-tericidal Concentration of Laggera tomentosa

Among the four tested bacterial strains, MIC values ranged from 3.13 to 6.25 mg/mL. The highest MIC (6.25 mg/mL) was recorded for chloroform extract against S. pneumoniae, ethyl acetate extract against E. coli, and hexane extract against P. aeruginosa and S. pneumoniae (Table 5). MBC values ranged from 3.13 to 6.25 mg/mL. The highest MBC (6.25 mg/mL) was recorded for ethanol extract against E. coli, chloro-form extract against S. pneumoniae, ethyl acetate extract against E. coli, and hexane extract against S. pneumoniae and P. aeruginosa.

Table 5. MIC and MBC Concentrations of Laggera tomentosa Extracts (mg/mL).

Test organism	Gram type	Ethanol		Methanol		Chloroform		Ethyl acetate		Hexane	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	-	3.13	6.25	3.13	3.13	3.13	3.13	6.25	6.25	3.13	3.13
S. aureus	+	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13
S. pneumonia	+	3.13	3.13	3.13	3.13	6.25	6.25	3.13	3.13	6.25	6.25
P. aurongousa	-	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	6.25	6.25

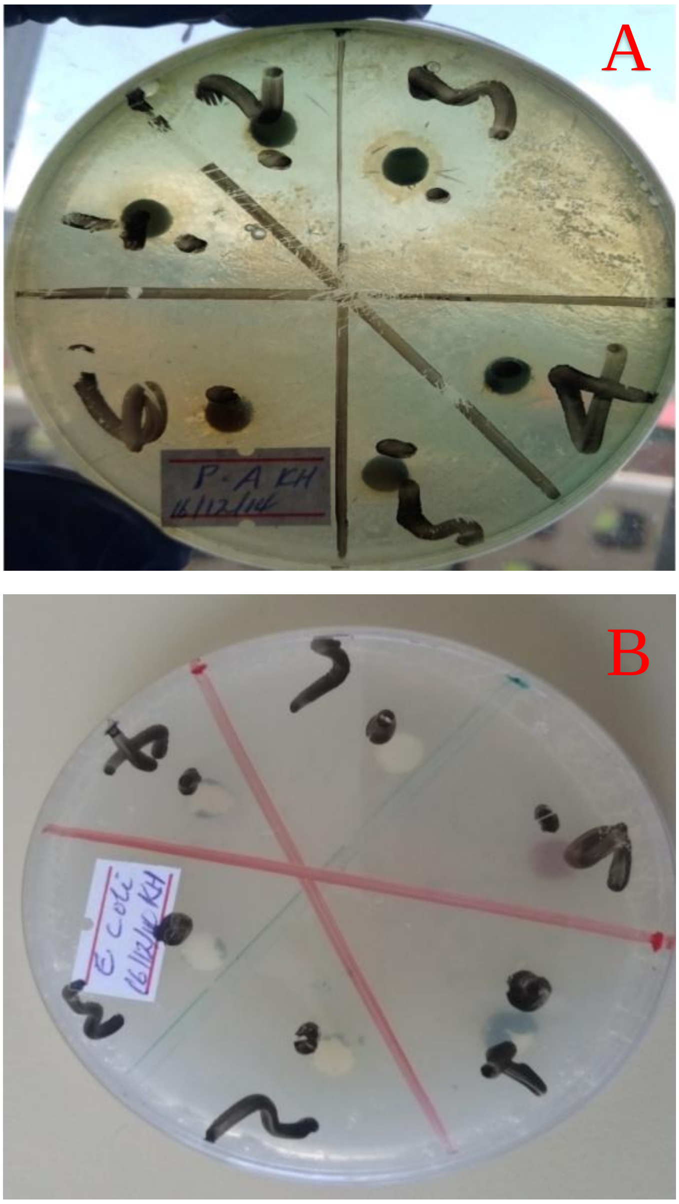

Figure 3. Bacterial strains response to the Leonotis ocymifolia (A) & Laggera tomentos (B) crude extracts .

 doi:10.20944/preprints202603.1494.v1

4. Discussion

Ethnobotanical screening provides valuable information on the importance of traditional medicines and contributes to modern drug formulation. This study on traditionally well-known herbal medicines of Ethiopian local communities provides scientific evidence for their antibacterial activity against selected human pathogenic bacteria. To the best of our knowledge, no previous studies have investigated the antibacterial activity of these plants against the tested bacterial strains, and limited information is available on these indigenous herbs.

The current study clearly demonstrated that water extracts of Leonotis ocymifolia failed to inhibit Pseudomonas aerugino-sa and Escherichia coli, while water extracts of Laggera tomentosa could not inhibit P. aeruginosa, Staphylococcus aureus, and E. coli. This indicates that Gram-negative bacterial strains can tolerate water crude extracts of both plants, suggest-ing that polar solvent extracts may be less effective against these organisms. The observed differences in antibacterial activity between bacterial strains can be attributed to variations in the composition and structure of bacterial outer membranes and cell walls, which are primary sites of drug action [17]. The outer membranes of Gram-negative bacteria are rich in lipopolysaccharides, which can hinder the penetration of different antibiotic molecules [17,18].

Notably, chloroform extracts of both plants demonstrated broad-spectrum antibacterial activity, inhibiting all tested Gram-negative and Gram-positive bacteria with the highest zones of inhibition. This finding aligns with previous stud-ies reporting that organic solvent extracts generally provide more consistent antimicrobial activity compared to aqueous extracts [19]. The limited activity of water extracts in this study confirms that water is not an optimal solvent for extract-ing antibacterial compounds from medicinal plants, consistent with findings reported by El-Safey and Ali [20].

The antimicrobial activity of these plants may be attributed to the presence of various bioactive compounds identified through phytochemical screening. Leonotis ocymifolia contained flavonoids, tannins, alkaloids, and phenols, while Laggera tomentosa contained flavonoids, tannins, alkaloids, phenols, and saponins. These secondary metabolites are known to possess antimicrobial properties through various mechanisms. Flavonoids have been reported to form complexes with bacterial cell walls and proteins, while tannins can inhibit bacterial enzyme production and disrupt cell membrane function [21].

The activity of both plant extracts against Gram-positive and Gram-negative bacteria suggests the presence of broad-spectrum antibiotic compounds [22]. Similar studies on other medicinal plants have shown comparable antibac-terial activity to positive controls, sometimes with even higher efficacy [23]. The fact that these plant extracts were active against both standard strains indicates their potential as sources of potent antibiotic substances that could be effective against multi-drug resistant microorganisms.

The MIC values obtained in this study (1.56-12.5 mg/mL for L. ocymifolia and 3.13-6.25 mg/mL for L. tomentosa) demon-strate significant antibacterial potential. The MBC values, ranging from 3.13-12.5 mg/mL and 3.13-6.25 mg/mL respec-tively, indicate that these extracts possess bactericidal rather than merely bacteriostatic properties against most tested strains. The similarity between MIC and MBC values for several extract-bacterium combinations suggests strong bacte-ricidal activity.

The variation in antibacterial activity among different solvent extracts highlights the importance of solvent selection in phytochemical studies. Chloroform, being a moderately polar solvent, appeared most effective in extracting bioactive compounds from both plants. This may be due to its ability to extract a wide range of secondary metabolites with vary-ing polarities, as supported by the phytochemical screening results showing the presence of multiple compounds in chloroform extracts.

5. Conclusions

This study demonstrates that Leonotis ocymifolia and Laggera tomentosa possess significant antibacterial activity against human pathogenic bacteria, including both Gram-positive (S. aureus, S.

pneumoniae) and Gram-negative (E. coli, P. aeru-ginosa) strains. The chloroform extracts of both plants exhibited the highest and broadest antibacterial activity, with in-hibition zones comparable to standard antibiotics. The presence of various phytochemicals, including flavonoids, tan-nins, alkaloids, and phenols, likely contributes to the observed antimicrobial effects.

The study also revealed that water extracts of both plants showed limited antibacterial activity, particularly against Gram-negative bacteria, indicating that organic solvents are more suitable for extracting antibacterial compounds from these medicinal plants. The MIC and MBC values obtained provide quantitative evidence of the bactericidal potential of these extracts.

These findings provide scientific validation for the traditional use of Leonotis ocymifolia and Laggera tomentosa in treating bacterial infections and suggest their potential as affordable, natural sources of antibacterial substances. In an era of in-creasing antibiotic resistance, these plants may offer alternative therapeutic options and serve as sources for novel drug development.

6. Recommendations

Based on the study findings, bioassay-guided fractionation is warranted to identify the specific active compounds re-sponsible for the observed antibacterial effects. Comprehensive toxicity profiling is essential before clinical applications, while mechanism of action studies would elucidate bactericidal pathways. Conservation strategies for endemic Laggera tomentosa must accompany these efforts to ensure sustainable utilization.

Author Contributions: Kefale Techane Biru conceived the study, collected plant materials, performed laboratory ex-periments, analyzed data, and drafted the manuscript. Deribew Girma Tola contributed to laboratory investiga-tions, manuscript preparation, and edition. Abreham Bekele Alemu supervised the research, participated in study design, and reviewed the manuscript. Zewdie Kassa provided bacterial strains, assisted in microbiologi-cal techniques, and reviewed the manuscript. All authors read and approved the final manuscript.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgments: The authors thank Salale University, College of Natural Sciences, Department of Biology, for their support. We are grateful to the Ethiopian Public Health Institute for providing bacterial strains. The authors also acknowledge the local communities in Kuyu and Girar Jarso districts for sharing their traditional knowledge.

Conflicts of Interest: The authors declare no conflict of interest.

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