


Survey and Antimicrobial Activity Study of Ethnomedicinal Plants in Selected Districts of North Shewa Zone, Oromia, Ethiopia

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Background: The emergency of multidrug resistance due to the global burden of infectious diseases and drug misuse leads to an urgent identification of new medicines from medicinal plants. The study was designed to perform the documentation of ethno-medicinal plants usage, extraction, phytochemical screening and antimicrobial activities of the herbal extracts.

Methods: A cross-sectional study design was conducted in this study. Maceration of plant extraction, phytochemical screening and disc diffusion method for antimicrobial activity tests were employed.

Results: The results of the study indicated that *Euphorbiaceae*, *Lamiaceae* and *Sapindaceae* plant families have commonly used for the treatment of infectious diseases in the study areas. The antimicrobial activity test results indicated that *Ostegia integrifolia Benth* which was extracted with chloroform solvent showed equivalent inhibition zone as compared to standard drug, gentamicin. All the extracts could be considered as broad spectrum, have strong inhibition against *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *C. albicans*. All extracts against *C. albicans* were also revealed strong inhibition activity as compared to fungal standard drug, contamazole.

Conclusion: Phytochemical screening showed the existence of secondary metabolites in the studied plants and thus, supports the traditional healers' claim of such plants use for the treatment of diseases. The study also concluded that chloroform extracts of all plants showed promising antimicrobial activities against *K. pneumoniae*, and *C. albicans*. Therefore, further in vitro and vivo antimicrobial activity study of chloroform extracts against these bacterial and fungal strains were recommended. Moreover, isolation and structural elucidation of bioactive compounds from chloroform extracts were also recommended.

Keywords: antimicrobial activity, Ethiopia, maceration method, medicinal plants, North Shewa zone, phytochemical screening

Introduction

Plants are a good source of new medicines so that searching for the medicines are well justified. Plants are the only sources of therapeutics for nearly 80% of human population in developing country.¹ Hence, human being has used plants to treat common infectious diseases, and some of the traditional medicines are still part of the habitual treatment of various diseases.² Ethiopia has diverse flora and about 80 to 90% of its population relies on traditional medicine to meet their primary health care needs.¹⁻³ Traditional health practitioners have made remedies from plants that play an important role in the health of millions of people. Ethno-medicinal studies on traditional knowledge, folk beliefs, skills, methods and practices used for the

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treatment of ailments.⁴ It offers medicines due to cultural acceptability, efficacy against certain diseases, economic affordability and locally available than pharmacotherapy.⁵

The medicinal value of plants like antimicrobial, anticancer, anti-inflammatory, antidiabetic, antioxidant, anti-diuretic and etc. mainly depends on the secondary metabolites and the most important bioactive compounds of plants are alkaloids, saponins, flavonoids, tannins, sterols and phenolic compounds.⁶ In general, natural products are particularly good at providing many new chemical structures that no chemist would dream of synthesizing. For example, the anti-malarial drug artemisinin containing an extremely unstable-looking trioxane, ring-one of the most unlikely structure to have appeared in recent years.⁷

The emergence of drug resistance becomes the global health threat that involves all major microbial pathogens and antimicrobial drugs.⁸ It is difficult to treat and is responsible for a variety of infectious diseases. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to older and newly produced drugs is on the rise. As a result; scientific interest in medicinal plant has multiplied in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine. Hence, the need to looking for new molecular structures as lead compounds from the plant kingdom.⁹

A few studies conducted in some African countries also indicated that herbal medicines used to treat infectious diseases.^{10–13} Similar studies that were also conducted on a wider range of Ethiopian plants revealed that are being used in the treatment of wounds, malaria and other diseases in the traditional health care system of the country.^{14–18} The studies which were conducted around the border area of North Shewa zone, Oromia, Ethiopia like Ankober district,¹⁹ Ada'a district,²⁰ and around Fitch town,²¹ indicated the existence of large number of ethno medicinal plants associated with indigenous knowledge.

However, there was no study conducted in North Shewa zone of Oromia Region, Ethiopia in this regard. Hence, this study was designed to document the knowledge of medicinal plants usage, extraction, phytochemical screening, and assessment of antimicrobial activities of the herbal extracts.

Materials and Methods

Study Areas and Design

A cross sectional study was conducted from October, 2018 to June, 2019 at three selected districts of North Shewa

zone, Oromia, Ethiopia. The zone is found at about 112 km north of Addis Ababa to Gojam main road. It is located between 9°05' and 10°23'N latitude and 37°57' and 39°28'E longitude. Its altitude ranges from about less than 1000 m to over 3540 m. It has average annual rainfall ranging from 600–2000 mm. The average minimum temperature is 10°C and maximum temperature is 32°C [10].

The total population of Fiche is 27,493. The number of female and male population is 14,560 and 12,933, respectively giving sex ratio of 113 females to 100 males.²² The average annual rainfall and temperature of the zone are 1075 mm and 20.3 °C, respectively. The vegetation of the area is dominated by xerophytic plants. The population is highly used the traditional medicines to treat different diseases and disease conditions in the areas. It is also called the milk belt area where 206,000,000 liters of milk per year is produced and more 60% is supplied to Addis Ababa; because there are huge numbers of dairy cattle population with local (352,216) and cross (1,324,532) breed.

Study Participants

The study participants were traditional health practitioners/healers who varied with age, sex, marital status, occupation, educational level and location in the selected districts.

Sampling Methods

A purposive sampling technique was used to select study districts, participants and plants based on the availability of traditional healers, proximity and agro-climatic conditions of the areas. In this study, 14 commonly used medicinal plant species were collected from the three districts.

Key Informants

In this study, 18 key informants (28–81 years old) were selected for in depth interview in the study districts based on the recommendations of local community leaders and knowledgeable elders. The selected healers were well known in the community due to experienced community based traditional health care services. Prior to the interview process, discussion was done with the informants through assistance of clan and local leaders to elaborate clearly the objective of the study. In depth interview of the key informants was done in the study areas. The interview was ensured by preparing key informants guide.^{17,19}

Observation

In this survey, the key informants were also come together with the researchers for field guided walk in order to identify the origin of the plants where it grows, and provide local names. Then after, particular medicinal plants were collected with all ethno botanical data. Moreover, informant consensus was given to confirm the reliability of the information. Hence, each key informant was contacted at least twice and if the responses that were given at different time contradict to each other. Unreliable and not accepted or reliability of the information was confirmed by similar information given by different informants on the same issue.

Plant Specimen Collection and Identification

In this study, the collected plant specimens with the necessary recorded information both from wild and home garden were documented. Then the plants were taken to National Herbarium, Addis Ababa University for plant species identification using taxonomic keys of Ethio-Eritrea Flora. The voucher specimen has been given starting from SIU Ethno Med/01-14/2018) as indicated in Table 1 and deposited in the National Herbarium. The identification was conducted by the botanist, Mr. Meleku Wondafrash Aytigid (Melaku.wondafrash@aau.edu.et), Addis Ababa University.

Plant Material Preparation

The fresh plant materials were collected from three selected districts of North Shewa zone, Oromia, Ethiopia;

namely Girar Jarso, Degam and Warra Jarso. The leaf parts of *Dodonea angustifolia* L.f., *Croton marostachyus* Del., *Calpurnia aurea* (Ait.) Benth and *Ostegia integrifolia* Benth; and root part of *Echinops kebericho* Mesfin were collected, chopped into smaller pieces and shade dried in laboratory at room temperature without any exposure to direct sunlight. The dried plant materials were then powdered to facilitate easy solvent penetration. The powdered plant materials were stored in suitable containers until used for extraction.^{23,24}

Extraction of the Plant Material

About 250 g of each powdered plant materials were sequentially extracted with an equal volume of about 2.5 liter hexane, chloroform and methanol using maceration technique for 24 hrs with shaking. The extracts of each solvent was filtered first through a fresh cotton plug and then through fluted filter paper. The filtrates were concentrated by evaporation under reduced pressure using a Rota vapor at 40 °C. The semidried extracts of each solvent were weighed and stored in refrigerator below 4 °C until used for antimicrobial activity.

Phytochemical Screening of Secondary Metabolites

Phytochemical screening tests for alkaloid, terpenoids, phenolic, flavonoid and saponin were conducted in all extracts by using standard procedures.^{25,26} Wagner's and Heger's reagents were used for alkaloid foam test for saponins, Magnesium chloride and Zinc chloride for

Table 1 Family, Scientific and Local Names of Ethno-Medicinal Plants Used for Treatment of Infectious Diseases in North Shewa Zone, Oromia, Ethiopia

S.No	Specimen Voucher No	Family	Scientific Name	Local Name
1.	SIU Ethno Med/01/2018	Fabaceae	<i>Acacia abyssinica</i> Hochst. Ex Benth.	Qoree
2.	SIU Ethno Med/02/2018	Fabaceae	<i>Calpurnia aurea</i> (Ait.) Benth	Ceekaa
3.	SIU Ethno Med/03/2018	Asteraceae	<i>Laggera tomentosa</i> (Sch.Bip.ex A Rich.) Olivo & Hien	Dama Kasee
4.	SIU Ethno Med/04/2018	Urticaceae	<i>Urtica simensis</i> steudel	Doobbii
5.	SIU Ethno Med/05/2018	Euphorbiaceae	<i>Croton marostachyus</i> Del.	Bakkanniisa
6.	SIU Ethno Med/06/2018	Polygonaceae	<i>Rumex nervosus</i> Vahl	Embuachoo
7.	SIU Ethno Med/07/2018	Loganiaceae	<i>Buddleja polystachya</i> Fresen	Adaaddii
8.	SIU Ethno Med/08/2018	Myrtaceae	<i>Eucalyptus globulus</i> Labill	Baargamoo adii
9.	SIU Ethno Med/09/2018	Lamiaceae	<i>Leonotis ocyimifolia</i> (Burm.f) Lwarsson	Bokkolluu
10.	SIU Ethno Med/10/2018	Euphorbiaceae	<i>Euphorbia tetraacantha</i> Rendle	Qincib
11.	SIU Ethno Med/11/2018	Sapindaceae	<i>Dodonea angustifolia</i> L.f.	Kitkittaa
12.	SIU Ethno Med/12/2018	Ranunculaceae	<i>Clematis simensis</i> Fresen	Hidda fitii
13.	SIU Ethno Med/13/2018	Lamiaceae	<i>Ostegia integrifolia</i> Benth	Xunjittii
14.	SIU Ethno Med/14/2018	Asteraceae	<i>Echinops kebericho</i> Mesfin	Qabarichoo

flavonoids, acetic anhydride, and Ferric chloride for phenol test.

Antimicrobial Activity

The antimicrobial activity was carried out for five medicinal plant crude extracts. In this activity study, four reference bacterial strains, and one fungal strain were used. The antimicrobial assay was conducted using Agar disc diffusion method,²⁷ and micro dilution broth assay.²⁸ The microbial standard strains were obtained from Jimma University. In this study, bacterial strains such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*; and fungal strain such as *Candida albicans* (spp) were considered for antimicrobial activity tests.

Antimicrobial activities of the crude extract were assessed using the disk diffusion method and the results were interpreted as per the Standards.²⁹ Test solutions were prepared by dissolving 0.2, 0.1 and 0.05 g of plant extracts in Dimethylsulfoxide (DMSO) to achieve final stock concentrations of 200, 100 and 50 mgmL⁻¹ respectively. Comparable microbial turbidity with standard 0.5 McFarland were prepared.³⁰ The McFarland was prepared by mixing two solutions (solution A and B). Solution A has 1% v/v of sulphuric acid (H₂SO₄) and solution B has 1% w/v of barium chloride (BaCl₂).

To get 0.5 McFarland standards, concentration equivalents to cell density of about 10⁷–10⁸ cells mL, an amount of 0.5 mL BaCl₂ of 1% solution A was mixed with 99.5 mL H₂SO₄ of 1% solution B. A small volume of the turbid solution was transferred to a screw-cap bottle of the same types to use for preparing test and control inoculums. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards was used for inoculation of media. The standard was used immediately, after shaking, and stored in a well-sealed container in a dark place at room temperature (20–28 °C) when not use. When match with the standard, the standardize suspension and local isolates strains was swabbed by cotton swab onto the Muller-Hinton Agar (MHA) for bacteria, and Potato Dextrose agar for fungi (Oxoid) and allow to dry.

Sterilize standard paper discs (6 mm) was soaked with 30 µL respective solution concentration of the extracts and air dry to evaporate the solvent and the discs were placed over the seeded plates at equidistance using forceps. Standard antibiotics disc, such as Gentamycin, and Contamazole were used as positive controls for microbial

cultures. Thereafter, the plates were incubated at 37 °C for 18–24 hrs, room temperature and 48 hrs for pathogenic fungi. The clear zones forming around each disc was measured using transparent ruler. Each experiment was carried out in triplicates.

The results of the antimicrobial susceptibility were interpreted according to the guidance described by Taylor et al.³¹ The mean ± SD of the inhibition zone of each test sample was taken for evaluating the antimicrobial activity of the extracts.

Data Analysis

The collected data both from field and laboratory were entered and analyzed by using SPSS version 23. Descriptive statistics like percent, figures and tables were used to summarize the collected ethno-medicinal data.

Ethical Approval

This study was approved by the Research Review Ethical Board of Salale University. The confidentiality of traditional property owners were completely maintained during processing of the data. Moreover, all the collected data from this study were not shared with third party out of researchers. Informed consent and permission were also obtained from the study participants and North Shewa zone Administration, respectively.

Results

A total of 10 major considerable medicinal plant families, and 14 plant species were identified and documented during the period in the study areas. Most of the habits of medicinal plants were shrub (64.3%) followed by tree (21.4%) and herb (14.3%). The results indicated that the widely used plant parts for the preparation of the remedies were leaves (71.4%) followed by root (14.3%), shoot bud and bark with the same proportion (7.14%). The traditional healers revealed that topical application (42.9%) was most commonly used route of administration followed by inhalation (28.6%). The majority of traditional healers were used plant remedies for the treatment of wound (35.7%) followed by abdominal pain in the study areas. The healers indicated that *Fabaceae* *Asteraceae*, *Lamiaceae* and *Euphorbiaceae* plant families were the most commonly used plant remedies for the treatment of infectious diseases with equal proportion (14.3%) (Tables 1 and 2).

Table 2 Summary of Plant Families, Habits, Parts, Administration Routes and Diseases Treated Using Medicinal Plants in North Shewa Zone, Oromia, Ethiopia

S.No	Description of Items		Frequency	Percent (%)
1.	Plant family	<i>Fabaceae</i>	2	14.3
		<i>Asteraceae</i>	2	14.3
		<i>Lamiaceae</i>	2	14.3
		<i>Euphorbiaceae</i>	2	14.3
		<i>Utriticaceae</i>	1	7.14
		<i>Polygonaceae</i>	1	7.12
		<i>Loganiaceae</i>	1	7.12
		<i>Myrtaceae</i>	1	7.14
		<i>Sapindaceae</i>	1	7.14
		<i>Ranunculaceae</i>	1	7.14
2.	Plant habits	Shrub	9	64.3
		Tree	3	21.4
		Herb	2	14.3
3.	Plant parts	Leaf	10	71.4
		Root	2	14.3
		Bark	1	7.14
		Shoot bud	1	7.14
4.	Administration routes	Topical	6	42.9
		Inhalation	4	28.6
		Oral	4	28.5
5.	Diseases treated	Wound	5	35.7
		Abdominal pain	3	21.4
		Headache	2	14.3
		Ring worm	2	14.3
		Pneumonia	2	14.3

Note: Each percentage was calculated from the total 14 documented family plants.

Phytochemical Screening Tests

The phytochemical screening test results revealed that all solvent extracts of *Calpurnia aurea* (Ait.) Benth, *Dodonea angustifolia* L.f., *Croton marostachyus* Del., *Echinops kebericho* Mesfin and *Ostegia integrifolia* Benth contain at least one secondary metabolite. The phytochemical screening test of *Dodonea angustifolia* L.f.'s hexane and methanol extract contain excess amount of terpenoids and phenolic compounds, respectively. On the other hand, *Ostegia integrifolia* Benth's chloroform extract also contains excess amount of phenol. But, the alkaloid was negative in all the plant extracts (Table 3).

Antibacterial Activity Test

The antibacterial activity results of *Dodonea angustifolia* L.f., *Croton marostachyus* Del. and *Calpurnia aurea* (Ait.) Benth extracted with chloroform solvent showed

equivalent inhibition zone against all bacterial strains as compared to standard drug, gentamicin. When compared with the inhibition zone of other extracts, *Croton marostachyus* Del.'s extract with chloroform showed highest antibacterial activity of 22.5 ± 0.50 mm inhibition zone against *K. pneumonia*. On the other hand, the antibacterial activity of *Echinops kebericho* Mesfin and *Ostegia integrifolia* Benth extracted with chloroform showed less antibacterial activity than the antibacterial standard drug. However, the extracts of hexane and methanol solvent showed low inhibition zone as compared to chloroform solvents extracts (Table 4).

Antifungal Activity Test

The antifungal activity results of all extracts against *C. albicans* were revealed strong activity. *Ostegia integrifolia* Benth's extracted with chloroform showed inhibition zone of 23.3 ± 0.29 mm which is greater than the antifungal activity of the standard drug, contamazole with 20.7 ± 0.58 mm diameter of inhibition zone; and the antifungal activity results of *Dodonea angustifolia* L.f. and *Calpurnia aurea* (Ait.) Benth extracted with chloroform showed equivalent inhibition zone as compared to antifungal standard drug. However, the chloroform extracts of *Croton marostachyus* Del. and *Echinops kebericho* Mesfin showed less antifungal activity than the antifungal standard drug (Table 5).

Discussion

The current study documented the knowledge of traditional healers, and plant remedies used for the treatment of different diseases in the study areas. *Euphorbiaceae*, *Lamiaceae* and *Sapindaceae* were plant families that commonly practiced. This finding was in agreement with other studies that were conducted in different parts of Ethiopia.^{3,5,32-34}

To assure the traditional usage of these plants, the phytochemical screening tests were conducted and the results indicated that *Dodonea angustifolia* L.f.'s hexane and methanol extract contain excess amount of terpenoids and phenolic compounds, respectively. *Ostegia integrifolia* Benth's chloroform extract also contains excess amount of phenol. This indicates that the secondary metabolites exist in the plant extracts are responsible for defenses against pathogenic microbes, and thus, support the traditional usage of the plant remedies for the treatments of infectious diseases.³⁵⁻³⁸

Table 3 Phytochemical Screening Test Results Selected Ethno-Medicinal Plants

S.No	Scientific Name	Phytochemicals	Tests	Hexane extract	Chloroform extract	Methanol extract
1	<i>Calpurnia aurea (Ait.)Benth</i>	Alkaloid	Wagner's test	–	–	–
		Terpenoids	Salkowski's test	+	–	–
		Phenolic	Ferric chloride	–	–	+
		Flavonoid	NaOH test	–	+	–
		Saponin	Foam test	–	+	+
2	<i>Dodonea angustifolia L.f.</i>	Alkaloid	Wagner's test	–	–	–
		Terpenoids	Salkowski's test	++	–	–
		Phenolic	Ferric chloride	–	–	++
		Flavonoid	NaOH test	+	+	–
		Saponin	Foam test	–	–	+
3	<i>Croton marostachyus Del.</i>	Alkaloid	Wagner's test	–	–	–
		Terpenoids	Salkowski's test	+	+	–
		Phenolic	Ferric chloride	–	–	+
		Flavonoid	NaOH test	–	–	+
		Saponin	Foam test	–	+	–
4	<i>Echinops kebericho Mesfin</i>	Alkaloid	Wagner's test	–	–	–
		Terpenoids	Salkowski's test	+	–	–
		Phenolic	Ferric chloride	–	–	+
		Flavonoid	NaOH test	+	–	–
		Saponin	Foam test	+	+	–
5	<i>Ostegia integrifolia Benth</i>	Alkaloid	Wagner's test	–	–	–
		Terpenoids	Salkowski's test	+	–	–
		Phenolic	Ferric chloride	–	++	+
		Flavonoid	NaOH test	–	–	+
		Saponin	Foam test	–	–	–

Notes: – = Not present; + = Present; ++ = highly present.

All the plant extracts showed antimicrobial activities against all bacterial and fungal strains. Chloroform extracts of *Croton marostachyus Del.*'s showed higher antibacterial activity than the standard drug against *K. pneumonia*. On the other hand, chloroform extracts of *Dodonea angustifolia L.f.*, *Croton marostachyus Del.* and *Calpurnia aurea (Ait.) Benth* showed equivalent inhibition zone against all bacterial strains as compared to standard drug, gentamicin. However, *Echinops kebericho Mesfin* and *Ostegia integrifolia Benth* extracts had lower

antibacterial activity. All the extracts could be considered as broad spectrum, and had promising inhibition against *E. coli*, *K. pneumoniae*, *S.aureus*, and *P. aeruginosa*. Similarly, the antifungal activities of all chloroform extracts were also found to be comparable with standard antifungal drug. The antifungal activities of all extracts against *C. albicans* were also revealed promising activity as compared to antifungal standard drug, contamazole. The antibacterial activity of all chloroform extracts against *K. pneumonia* were found to be similar. The

Table 4 Antibacterial Activity Results of Plant Extracts Against Bacterial Species (200 mg mL⁻¹)

Bacterial Species	Diameter of Inhibition Zone (mm) (Mean ± S.D)											
	Dodonea angustifolia L.f.						Echinops kebericho Mesfin					
	Hexane	Chloroform	Methanol	G	DMSO		Hexane	Chloroform	Methanol	G	DMSO	
<i>E.coli</i>	10.3± 0.29	13.1 ± 0.29	9.83±0.29	13.2 ± 0.29	NI		11.7±0.58	13.2±0.29	12.7±0.58	15.3±0.58	NI	
<i>K. pneumoniae</i>	8.83 ±0.29	21.7±0.58	17.7±0.58	21.7±0.58	NI		9.0±0.0	21.7±0.58	15.8±0.29	22.5±0.50	NI	
<i>S.aureus</i>	11.7 ± 0.58	12.3±0.58	10.8±0.29	12.3±0.58	NI		14.0±0.0	12.3±0.58	11.5±0.50	20.3±0.58	NI	
<i>P. aeruginosa</i>	11.7 ± 0.58	11 ± 0.0	12.7± 0.58	11.0±0.0	NI		10.7±0.58	11.0±0.0	12.7±0.58	12.7±0.58	NI	
Bacterial Species	Croton marostachyus Del.						Calpurnia aurea (Ait.) Benth					
<i>E.coli</i>	11.7±0.58	15.3±0.58	10.8±0.29	15.3±0.58	NI		12.7±0.58	11.7±0.58	12.0±0.0	11.7±0.58	NI	
<i>K. pneumoniae</i>	16.7 ±0.58	22.5±0.50	20.0±0.0	22.5±0.50	NI		12.7±0.58	22.3±0.58	11.7±0.58	22.3±0.58	NI	
<i>S.aureus</i>	13.9±0.06	20.3±0.58	12.7±0.58	20.3±0.58	NI		12.3±0.58	11.7±0.58	10.8±0.29	11.7±0.58	NI	
<i>P. aeruginosa</i>	14.0±0.0	12.7±0.58	14.7±0.58	12.7±0.58	NI		11.0±0.0	11.7±0.58	15.7±0.58	11.7±0.58	NI	
Bacterial Species	Ostegia integrifolia Benth											
<i>E.coli</i>	9.83±0.29	11.7±0.58	13.7±0.58	24.1±1.00	NI							
<i>K. pneumoniae</i>	9.83±0.29	22.3±0.58	14.0±0.0	25.7±0.58	NI							
<i>S.aureus</i>	13.7±0.58	11.7±0.58	14.3±0.58	26.3±0.58	NI							
<i>P. aeruginosa</i>	12.0±0.0	14.3±0.29	12.8±0.0	21.7±0.58	NI							

Abbreviations: NI, No Inhibition; G, Gentamycin; DMSO, Dimethylsulfoxide.

Table 5 Antifungal Activity Results of Plant Extracts Against Fungal Species (200 mg mL⁻¹)

Fungal Species	Diameter of Inhibition Zone (mm) (Mean ± S.D)									
	<i>Dodonea angustifolia</i> L.f.					<i>Echinops kebericho</i> Mesfin				
<i>C. albicans</i>	Hexane	Chloroform	Methanol	C	DMSO	Hexane	Chloroform	Methanol	C	DMSO
	10.7 ± 0.58	13.7 ± 0.58	11.7 ± 1.15	13.7±0.58	NI	13.0±0.58	13.7±0.58	13.0±1.00	15.0±0.0	NI
	<i>Croton marostachyus</i> Del.					<i>Calpurnia aurea</i> (Ait.)Benth				
	Hexane	Chloroform	Methanol	C	DMSO	Hexane	Chloroform	Methanol	C	DMSO
	12.0±1.00	28±0.29	21.0±1.00	28.3±3.10	NI	12.3±0.58	23.3± 1.15	14.3±1.15	23.3± 1.15	NI
	<i>Ostegia integrifolia</i> Benth									
	Hexane	Chloroform	Methanol	C	DMSO					
18.0±0.0	23.3±0.29	13.7±0.58	20.7±0.58	NI						

Abbreviation: C, Contamazole.

higher and comparable antibacterial activity of chloroform extracts might be due to the possible extraction of active secondary metabolites from plants by chloroform following like dissolve like principle.³⁹

Therefore, the antimicrobial screening results also support the traditional usage of these plants for the treatments of infectious diseases in the study areas, and similar research findings were reported in Pakistan,³⁸ in different part of Ethiopia such as Tigray,⁴⁰ Gozamin *Wereda*,⁴¹ Ankober district,¹⁹ Ada'a district,²⁰ and around Fitcha district.²¹

This study was limited to the crude extractions of four plants and their antimicrobial activity against three bacterial and one fungal reference strains. Moreover, the isolation of active compounds from the extracts was not done which is actually responsible for the antimicrobial activities.

Conclusions

Medicinal plants from the Euphorbiaceae, Lamiaceae, and Sapindaceae families have been used to treat a variety of diseases in the study area. The phytochemical screening test revealed the presence of secondary metabolites and confirms the traditional uses of the studied plants for the treatments of diseases. The study indicated that chloroform extracts showed comparable antimicrobial activities with the standard drugs, and the result obtained here is an indicator for the potential sources of the studied medicinal plants as an antimicrobial agent; and thus, further *in vitro* and *in vivo* antimicrobial activity studies of chloroform extracts against *K. pneumonia* bacterial and *C.albicans* fungal strains were recommended. Isolation of active compounds from chloroform extracts was also recommended.

Data Sharing Statement

All required data are included in this paper.

Ethics Approval and Consent to Participate

Ethics approval for this study was obtained from Salale University. Verbal consent was obtained from all study participants before the data collection started and privacy was secured. Since all study participants are above age 18 years old and highly secured information is not needed the verbal consent was obtained, and the ethics committee of Salale University approved this procedure.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors had declared no conflicts of interest for this work.

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