International Journal of Livestock Research, Vol. 15 (3) Mar' 2025
http://www.ijlr.org
eISSN: 2277-1964
NAAS Score:4.31

Original Research

In Vitro Evaluation of Antibacterial Activities of Selected Ethno-Medicinal Plants from West Shewa Zone

Bacha Abata Chala[1] Wesenu Berhanu Enoro[2*] and Kebede Abdissa [3]

[1]Horo Guduru Wolega Agricultural and Livestock Office, Shambu, ETHIOPIA
[2]Sebeta sub-city Agricultural Office, Sebeta, ETHIOPIA
[3]Ambo University, Ambo, ETHIOPIA

*Corresponding Author: wesenu1621@gmail.com

How to cite this paper
Chala, B. A., Enoro, W. B., & Abdissa, K. (2025). In Vitro Evaluation of Antibacterial Activities of Selected Ethno-Medicinal Plants from West Shewa Zone. *International Journal of Livestock Research*, 15 (3), 20-31.

Received : Jul 11, 2024
Accepted : Feb 22, 2025
Published : Mar 31, 2025

Abstract

An in vitro experimental study was conducted in Ambo University, Mamo Mezemir Campus from November, 2020 to August, 2021 to evaluate antibacterial effect of Leaves of Withania sominifera, steam of Clausena anisata, leaves of Carica papaya L, fruit of Solanum incanum, roots of R. nepalensis and leaves of Nicotiana tabacum against Bacterial species (Escherichia coli/ATCC 25322, Staphylococcus aureus/ATCC 13076, Entrococcus feacalis/ATCC 29212 and Pseudomonas aeruginousa/ATCC 27853). The antimicrobial activities of the extract against test organisms were tested by using agar well diffusion assay, and the MIC and MBC values were determined by agar dilution assay. The results revealed that the methanol extracts of Nicotiana tabacum, Carica papaya, Rumex nepalensis, Solinum incanum and Withania somnifera had the most antibacterial activity against Staphylococcus aereus, Escherichia coli, Enterococcus faecalis, and Pseudomonus aeruginousa. Still, the Methanol extract of Clausena anisata, had no activity against E. coli, E. faecalis and Staphylococcus aeruginousa. The methanol extract of Rumex nepalensis had more activity than other extracts against Staphylococcus aereus, Escherichia coli, Enterococcus faecalis, and Staphylococcus aeruginousa, with MIC ranging from 6.25mg/ml to 25mg/ml and MBCs ranging from 12.5mg/ml to 50mg/ml. Moderate antibacterial activity results have also been recorded from leaves of Withania somnifera and stem of Clausena ansita extract against Staphylococcus aureus (18.83±0.76mm) and (13±0), respectively. The methanolic extract of Carica papaya exhibited maximum activity against Staphylococcus aureus (25.67±0.58 mm) and least towards Escherichia coli (7.67±0.58mm). The main components of plant-derived phytochemicals revealed from present studies are flavonoids, glycosides, tannins, saponins, phenolics, and Phlobatanins. However, Phytosterols were found only in Solinum incanum extracts. Generally, the current experimental study showed that all extracts have antibacterial activities against all tested standard organisms, which play a vital role in substituting resistant drugs, as evidenced by a high zone of inhibition. However, further antibacterial activity tests need to be performed using other methods for those plants that did not show any antibacterial activity in this study.

Keywords: Antibacterial Activity, Crude Extract, Minimum Inhibitory Concentration, Phytochemicals, West Shoa Zone.

Introduction

Plants are naturally God gifted for the synthesis of medicinal compounds and greatly help in the discovery in the area of chemical diversity because of the unknown availability either as a standardized extract or as a pure compound (Hassan and Ulla, 2019). Various medicinal plants have proved their significance in curing diseases, including bacteriological infections and some life-threatening serious diseases (Tyagi *et al.,* 2016). Medicinal plants also play a major role and constitute the backbone of TM (Traditional System of Medicine) practices. Hence, developing countries should endeavor to develop and utilize local medications that are most appropriate to their local circumstances, especially for Primary Health Care (PHC), to cut down on the huge cost associated with incessant drug importation (Tadele, 2017).

Traditional medicine is the sum of total knowledge and practices used in diagnosis, prevention and elimination of physical, mental, or societal imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing (WHO, 2017). Nowadays, eighty percent of the world's population depends on traditional medicines as an essential source of their primary health care (Batiha *et al.,* 2020).

In Ethiopia, there are more than 80 ethnic groups, each having their indigenous knowledge on the use of traditional medicine. About 80% of the population uses traditional medicine for primary health care (Bultum *et al.,* 2019). Ethiopia is considered the home of some of the most diverse plant species in Africa that serve as sources of many traditional medicinal plants. It has been reported that approximately 800 species of medicinal plants grown in Ethiopia are used for treating about 300 medical conditions (Gebrehiwet and Gebremichael, 2019).

There are various forms of medicinal plants, including trees, shrubs, climbers, and herbs; of these, herbal medicinal plants are dominantly used for different human and animal treatments in Ethiopia. These plants are collected mainly from riverbanks, cultivated areas, bushlands, forests, woodlands, and grasslands, among others (Admasu and Yohannes, 2019). Availability of some ethno-veterinary medicinal plants was affected by season; many of the plants were available all the time, some were available seasonally, and the rest were difficult to get. According to the informants, both pounding and powdering as a strategy permit to preservation of the plant materials that are not available all seasons (Guluma *et al.,* 2017).

Although synthetic antimicrobial agents have already been approved in many countries, the usage of natural compounds that are derived from microbial, animals, or plants attracts the attention of many researchers (Moloney, 2016). These compounds have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens (Rossiter *et al.,* 2017). Among all the available options, plant-derived compounds have displayed more potential applications in fighting bacterial infections. The extensive existence of these compounds has demonstrated beneficial advantages in terms of antioxidant, antibacterial, and antifungal activities. They can restore the clinical application of older antibiotics by increasing their potency and, as a consequence, avoid the development of resistance (Barbieri *et al.,* 2017).

Staphylococcus aureus, *Escherichia coli*, and *Streptococcus pneumoniae* have well-established histories of pathogenicity and have been implicated in various human and animal infections with varying degrees of severity. Although antibiotics have been developed against these pathogenic organisms, the twin problems of drug toxicity and antibiotic resistance have made the continuous search for newer antibiotics against these pathogens a necessity (Price *et al.,* 2017). Effective treatment of a disease requires the development of new pharmaceuticals or some capability source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem (Manandhar *et al.,* 2019).

The fresh *extracts* of *C. papaya* leaves and bark of *Cl. anisata* showing good antibacterial activity. These extracts directly can be used as natural alternative preventives to control various food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications (Lawal *et al.,* 2015; Lonkala and Reddy, 2019). *N. tabacum* is a well-known medicinal plant that is used for human and veterinary disease treatment (Ameya *et al.,* 2017). *R. nepelensis*, has the potential to act as a rich source of drug against life threatening diseases due to its remarkable biological activities (Shaikh *et al.,* 2018). *W. somnifera* and *S. incanum* possessed good antibacterial activity (Eshetu *et al*., 2015, Kumari and Gupta, 2015).

Emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. The occurrence of the evolution of resistance has caused the existing antibacterial drugs to become less effective or even ineffective. However, commonly used medicinal plants of our community could be an excellent source of drugs which play a vital role to substitute resist drugs. Therefore, the main significance of this study was to check the in vitro antibacterial activities of *Nicotiana tabacum, Carica papaya linn., Clausena anisata, Rumex nepelensis, Solinum incanum* and *Withania somnifera* to carry out further qualitative investigation of secondary metabolites found in these medicinal plants. Those plants were highly effective against those resistant bacteria's since they are new remedies in the areas. The objectives of this study were:

- To evaluate antibacterial activities of *Nicotiana tabacum, Carica papaya L., Clausena anisata, Rumex nepelensis, Solinum incanum* and *Withania somnifera* in West Shewa zone of Oromia Regional State, Ethiopia.
- To screen the antibacterial activity against standard pathogenic bacteria (*E. coli, E. faecalis, S. aureus and P. aeruginosa)* of *Nicotiana tabacum, Carica papaya., Clausena anisata, Rumex nepelensis, Solinum incanum* and *Withania somnifera in vitro* based on Minimum Inhibitory Concentration (MIC).
- To determine minimum bactericidal concentration of *Nicotiana tabacum, Carica papaya L., Clausena anisata, Rumex nepelensis, Solinum incanum* and *Withania somnifera.*
- To compare the efficacy of the antibacterial activity of extracts of *Nicotiana tabacum, Carica papaya L., Clausena anisata, Rumex nepelensis, Solinum incanum* and *Withania somnifera* with standard antibiotics against the test organisms.

Materials and Methods

Plant Collection Area

The plants were collected from Toke Kutaye District, West Shoa Zone of Oromia Regional State of Ethiopia, and located 126 km away from Addis Ababa (Fig. 1). According to (21), stated in the figure 1 below the area is located on 8°58′N 37°46′E, latitude and longitude respectively. The topography of the zone is flat, which makes the area an ideal place for agriculture. The mean annual temperature and rain fall range from10–29°C and 1000mm respectively. The crop-livestock mixed farming system is a common practice. The soil type of the area is black soil (Vertisol). It is slightly acidic with PH range of 5.5-6.

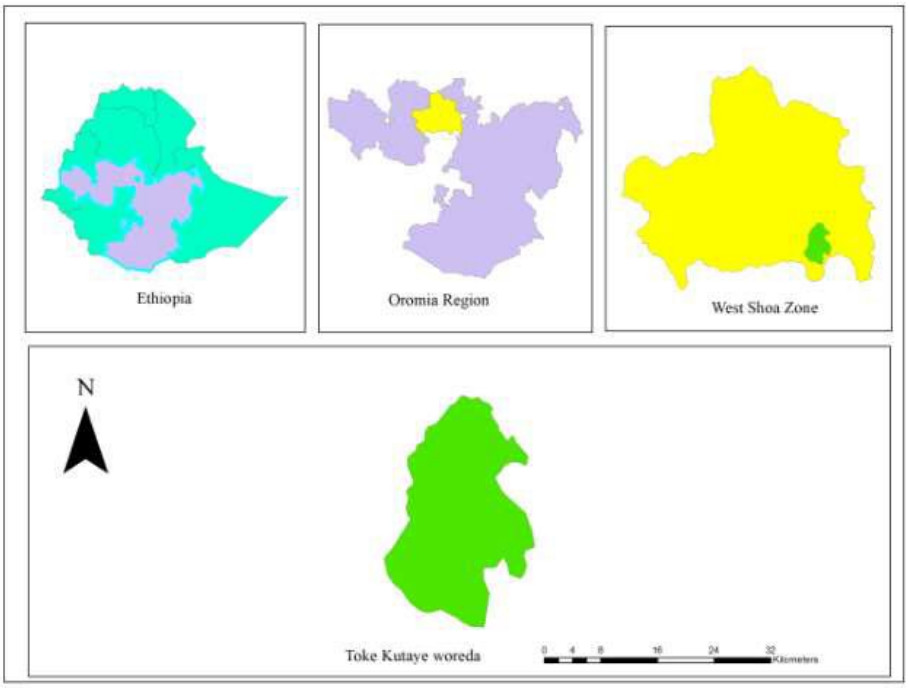

Figure 1: Map of Experimental Area (Source: Adopted from Arc GIS Computation and Ethiopian Map, 2019)

Study Design

An *in vitro* experimental study was conducted in Ambo University, Mamo Mezemir Campus from November, 2020 to August, 2021 to evaluate antibacterial effect and phytochemical screening of Leaves of *W. somnifera* (L), steam of *Cl. ansita,* leaves of *C. papaya,* fruit of *S. incanum,* roots of *R. nepalensis* and leaves of *N. tabacum* against *E. coli, S. aureus, E. feacalis and P. aeruginousa*. Methanol solvents were used for plant extraction. Average value of zone of bacterial growth inhibition by each plant extracted and MIC test were evaluated.

Plant Collection and Processing

Medicinal plants were collected based on frequencies of their indigenous use and repeated claims by traditional healers. Scientific name of the selected plants is confirmed by Mr Fikadu Lebeta from the department of plant science and Ambo University Mega Project. Six medicinal plants which were known to be used by traditional healers for the treatment of diarrhea, wounds, and other infections have been identified for evaluation of biological activities (Table 1). These plants were collected from Toke Kutaye woreda for effective evaluation for antibacterial. Different parts of selected medicinal plants collected from the field were carefully cleaned with tap water, dried under shade, mechanically ground with a mortar and Pestle, and coarsely powdered using a grinder machine. The powder was collected and stored at room temperature until extraction procedures were undergone. The powdered specimens were then subjected to extraction using a solvent (methanol) to extract bioactive compounds.

Table 1: Medicinal plants/herbs selected for the study.

No.	Scientific name	Parts	Local name
1	*Withania sominifera*	Leaf	Awashanga
2	*Carica papaya*	Leaf	Papayaa
3	*Clausena anisate*	Steam	Ulumaa
4	*Nicotiana tabacum*	Leaf	Tamboo
5	*Solanum incanum*	Fruit	Hiddii
6	*Rumex nepalensis Spreng*	Root	Shultii

Plant Extractions

Plant extracts were prepared by maceration. A total of 250g of the pounded materials were soaked in 1000 ml of methanol (99.90%) (1:4 ratios as enough to cover the extracted powder), followed by shaking with Shaker rotator (Ltd, China) filtered. This was repeated three times (with frequent agitation for 72 hours) to allow the solvents extract substantial quantities of the chemical constituents from the pounded plant materials. The mixture was filtered using vacuum pipe and the filtrate was passed through sterile filter papers (Whatman No. 1-3, Whatman Ltd. England). The fractions obtained were concentrated under vacuum on rotary evaporator and dried by Oven at a temperature of 40 C^o. The crude extracts were stored at 4C^o until used. Finally, the extracted plant reconstituted into solvents 5% of Dimethyl Sulfoxide (Sisco Reaserch Laboratories Pvt, Ltd, India) to do serial dilution for antibacterial activities.

In Vitro Evaluation of Antibacterial Activities

Test microorganisms

Standard bacterial (*Escherichia coli* ATCC 25322, *Staphylococcus aureus*/ATCC 13076, *Entrococcus feacalis*/ATCC 29212 *and Pseudomonas aeruginosa*/ATCC 27853) strains, were used to check the effectiveness of medicinal plants. These bacteria were obtained from Ethiopian Public Health Institute and selected for this study because they are the main bacteria for the cause of diarrhea, wounds, and drug resistance development.

In Vitro Antibacterial Activity of Extracts

Antimicrobial assay of extracts of different plants were performed by agar well diffusion method in Mueller Hinton Agar plates (Sisco Reaserch Laboratories Pvt, Ltd, India). The solidified medium was streaked entirely on the surface with culture of test organism which was adjusted to 0.5 McFarland turbidity using sterile swab stick. Then

the well or holes of equidistant diameter was made on surface of the seeded plates using with sterile cork-borer of 6mm. According to the concept of Burman (22) 100µl of each extract prepared at (50, 100, 200, 400 mg/ml) concentration and dissolvent solution was poured into respective wells by using of micropipette and then standard drug (Gentamicin 10µg (Merseyside, UK), Penicillin 10unit (Merseyside, UK), and Ciprofloxacin 5µg (Merseyside, UK) distributed on the agar surface. Gentamicin, Penicillin, and Ciprofloxacin antibiotics were used as positive control against the test organisms whereas 5% DMSO (solvent) served as a negative control. The plates left at room temperature for 1hrs to let the extracts and controls diffuse in the agar medium and incubate at 370C for 24 hours. After 24 hours of incubation the antibacterial activity was evaluated by measuring the diameter of the zone of inhibition including the well size (23). Finally, antimicrobial activity of each extract against test organism was determined based on the CLSI's (2020) by measuring their zones of inhibition in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration of the extract preventing growth of the microorganisms (24). Minimum Inhibitory Concentration of each plant extracted was tested by Broth dilution method according to the procedure described by Enejiyon (25) Nutrient broth (2 ml) was added to 0.5 ml of varying concentrations of extracts (6.25mg/ml, 12.5mg/ml, 25mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml) in a test tube, followed by addition of loopful of the test microorganisms. The same procedure was used for all the test organisms at varying concentrations and a test tube containing nutrient broth and loopful of organisms was used as a control. All the test tubes were inoculated at 37°C for 24 hours after which the test tubes were observed for turbidity. The lowest concentration of the extract that showed no observed turbidity was recorded as MIC for the extract.

Minimum Bactericidal Concentration (MBC) Determination

The minimal bactericidal concentration (MBC) was defined as the lowest concentration of an antibiotic killing the majority (99.9%) of bacterial inoculums. Following a broth dilution MIC test, from each tube that shows no growth, 50µl of suspension were removed and distributed on nutrient agar plates. After incubation (37oC) the concentration at which no visible growth was seen noted as the MBC (26).

Phytochemical Screening

Preliminary phytochemical screening was performed on methanol extract Leaves of *W. somnifera* (L), *C. papaya*, *N. tabacum*, steam of *Cl. ansita*, fruit of *S. incanum*, and roots of *R. nepalensis* to determine the presence of Saponins, Phenols, phlobotannins, Glycosides, Tannins, Flavonoids and Phytosteroles.

Phenols: Approximately, two ml of extracts was dissolved in equal amount of 3% FeCl**2**. Formation of deep blue color indicates the presence of phenol (27).

Phlobotannins: About two to five drops of extract was boiled along with 1% HCl. Formation of red precipitate indicates the presence of phlobotannins (27).

Glycosides: Two ml of chloroform and 2 ml of acetic anhydride was added to 2 ml of extract. Formation of violet to blue to green reddish brown ring indicates the presence of glycosides (26).

Saponins: Three ml of extract was mixed with six ml of distilled water in a test tube. The test tube was shaken vigorously for about 5 minutes and it was allowed to stand for 10 to 25 minutes and observed for froth. It indicates the presence of saponins (27).

Flavonoids: One ml of concentrated hydrochloric acid was added to the extract; then warmed on water bath for 15 minutes and stay for an hour. A strong red or violet color indicates presence of flavonoids (28).

Phytosterols: The Extracts was treated with 5ml of concentrated chloroform and filtered. After filtration, it was treated with two drops of acetic anhydride, boiled, and cooled. Then 3ml of concentrated H2SO4 was added. Formation of brown ring at the junction indicates the presence of phytosterols (28).

Tannins: Done ferric chloride test;-the extract (0.05g) was dissolved in five ml of distilled water. 2 to 5 drops of 5% ferric chloride solution are then added. A dark green color indicates the presence of tannins (28).

Data Analysis

All data were recorded in Microsoft excel 2007 and analyzed using SPSS software version 20. Antimicrobial efficacy of the plant extracts and the minimum inhibitory concentration were articulated with mean ± standard error of the mean of the zone of growth inhibition and one way analysis of variance (ANOVA) test was used to compare means between groups. A p - value of less than 0.05 ($P<0.05$) was taken as significant at 95% confidence interval.

Results

Antibacterial Activities of Plant Extracts

The current attempt was made due to resistance development in bacteria to available drugs. The selected medicinal plant species (Table 1) commonly reported by local healers were evaluated for their effectivities *in vitro* against 4 bacteria species: *S. aureus, P. aeruginosa, E. faecalis* and *E. coli*. Different zones of inhibition were recorded from each plant extract and from different concentrations for each species of bacterium.

The methanol extracts of the plant species listed in Table (2 and 3) were tested using different concentrations (50, 100, 200, and 400 mg/ml) against four bacterial species: *S. aureus, P. aeruginosa, E. faecalis* and *E. coli*. The all extracts showed dose-dependent potential activity and affect the tested pathogens. Of all the plants tested, *R. nepalensis* showed a wide spectrum of antibacterial activities against the investigated bacterial species. Regarding the dosage, 400 mg/ml showed best antibacterial activities in all plant species. Better antibacterial activities with maximum zone of inhibition (31.50 mm) in *R. nepalensis* were recorded in methanol crude extracts at a concentration of 400 mg/ml against *S. aureus* followed by *S. incanum* (31.33 mm) at the same dose (Table 3). Methanol extracts of *Cl. ansita* did not show inhibit growth of any bacterial species except *S. aureus (*13±0*)*, while *N. tabacum* show antibacterial activities against all standard bacteria used in this study (Tables 2). The methanol extracted fraction of *W. sominifera* and *Cl. ansita* showed 18.83 ± 0.76 mm and 13 ± 0 mm zone of inhibition with *S. aureus* respectively, which is comparatively less than that *R. nepalisis, S. incanum, C. papaya* and *N. tabacum* extracted.

Table 2: Mean zone inhibition (mm) of different bacteria at various concentrations of the test extracts of the experimental plants.

Organism	**Extracts in mg**		**Extracts**		
		S. aureus	*E. faecalis*	*P. aerogenosa*	*E. coli*
C. papaya	400	25.67±0.58	13±0	NA	7.67±0.58
	200	21.8±0.29	11.33±0.58	NA	NA
	100	15.67±0.5 8	9±0	NA	NA
	50	7.5±0.5	NA	NA	NA
Cl. ansita	400	13±0	NA	NA	NA
	200	12±0	NA	NA	NA
	100	10.33±0.58	NA	NA	NA
	50	7.83±0.29	NA	NA	NA
W. sominifera	400	18.83±0.76	9.33±0.58	7.93±0,12	NA
	200	15±0	NA	NA	NA
	100	13.33±0.58	NA	NA	NA
	50	11.67±0.58	NA	NA	NA
N. tabacum	400	22.6±0.53	12.33±0.58	13±1	13±0
	200	20.67±0.58	10.33±0.58	11.67±0.58	7.53±0.50
	100	14.67±0.58	NA	8.33±1.15	0±0
	50	10.83±0.76	NA	0±0	0±0

NA- No activities

Table 3: Mean zone inhibition (mm) of different bacteria at various concentrations of the test extracts of the experimental plants.

Organism	Extracts in mg	Extracts			
		S. aureus	*E. faecalis*	*P. aerogenosa*	*E. coli*
S. incanum	400	31.33±1.53 1	14.30±0.61	NA	15±1
	200	24.67±0.58	9.67±0.58	NA	7.33±0.58
	100	23.00±0.00	8±1	NA	NA
	50	16.00±0.00	NA	NA	NA
R. nepalensis	400	31.50±0.50	29.17±0.76	22.5±1.32	21.33±0.58
	200	23.13±0.81	23.60±0.53	19.17±1.04	16.00±1.00
	100	19.33±0.58	22±1	16.20±0.72	14.97±1.00
	50	18.50±0.50	9.17±0.76	14±0.87	12.33±0.58
Ciprofloxacin	5µg	18	18	15	25
Penicillin	10uni	25	22	-	-
Gentamicin	10µg	18	22	18	20
5%DMSO		NA	NA	NA	NA

NA- No activities; the values are mean ± SEM (n=3); significant at P<0.05; a compared to Gentamicin, Penicillin, and Ciprofloxacin; the negative control has shown no antibacterial activity.

Minimum Inhibitory Concentration (MIC)

The MIC for crude extracts of each plant for the respective test bacterium was done using the agar dilution method. The minimum inhibitory concentration values of active plant extracts shown in Table 4, ranged from 6.25mg/ml to 400mg/ml. All the crude extracts of evaluated *C. papaya*, *Cl. ansita*, *N. tabacum* and those from the *W. sominifera* had considerable antibacterial activity with MIC (50mg/ml) and MBC (100mg/ml) value, while *S. incanum* showed MIC at 12.5mg/ml against standard strains of *S. aureus*. The lowest value between 6.25 and 25 Mg/ml of MIC was recorded from methanol extracts of *R. nepalensis* roots, against all the test bacteria. For *N. tabacum*, the MICs were between 12.5 and 400 in all the test bacteria. *Cl. ansita* showed MIC at 50mg/ml concentration to *S. aureus*, but exhibit the growth of all bacteria employed in this study. Methanol extracts of *W. sominifera* showed MIC at 400mg/ml for *E. faecalis* and *P. aerogenosa* whereas *S. incanum* confirmed MIC 100mg/ml for *E. faecalis* and 200mg/ml for *E. coli*. The methanolic *extract C. papayas* reveal MI*C* at 100mg/ml and 400mg/ml for *E. faecalis* and *E. coli* respectively.

Table 4: Minimum inhibitory concentration and Minimum bactericidal concentration of Methanol extract of selected plants against selected microbial strains.

Organism	C. *papaya*		Cl. *ansita*		Extrats W. *sominifera*		N. *tabacum*		*S. incanum*		*R. nepalensis*	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
S. aureus	50	100	50	100	50	100	50	100	12.5	25	6.25	12.5
E. faecalis	100	200	-	-	400	-	400	-	100	200	6.25	12.5
P. aerogenosa	-	-	-	-	400	-	100	200	-	-	25	50
E. coli	400	-	-	-	-	-	200	400	200	400	25	50

Phytochemical Screening

All methanol extracts of selected plants were showed antibacterial activities against tested organism. Phytochemical analyses of methanolic extracts of selected experimental plants are summarized in table 5, Tannins, Phenols, Saponins, and Glycoside were found in all tested extracts. However, Phytosterols were found only in *S. incanum* extracts.

Table 5: Qualitative phytochemical screening results of plant extracts.

No.	Extracts	Solvents	Secondary Phenols	Metabolite Tannins	Saponins	Falvinoids	Glycosides	Phytosterols	Phlobatanins
1	*C. papaya*	Meth	+	+	+	+	+	-	+
2	*Cl. ansita*	Meth	+	+	+	-	+	-	+
3	*W.somnifera*	Meth	+	+	+	+	+	-	-
4	*N. tabacum*	Meth	+	+	+	+	+	-	+
5	*S. incanum*	Meth	+	+	+	+	+	+	-
6	*R. nepalensis*	Meth	+	+	+	+	+	-	+

Keys: (+)-presence; (-)-Absence

Discussion

Infectious diseases are the cause of major deaths worldwide (Pathak, 2019). Due to a high incidence of antibiotic resistance, evaluating the antibacterial effect of herbal medicines as potent agents of treating animal as well as human health problems is imperative (Divya *et al.*, 2014). In the present study, the antibacterial activity of the extract and isolated compounds of studied plants were examined at a concentration of 50mg/ml-400mg/ml against four pathogenic bacterial strains: two Gram-positive bacteria, *S. aureus* and *E. faecalis* and two Gram-negative bacteria, *E. coli* and *P. aeruginosa* (table 4).

For most of the test extracts, the highest concentration (400 mg/ml) exhibited a significantly higher ($P<0.05$) zone of inhibition as compared to the respective lowest concentration (50 mg/ml). The highest activity was recorded with the crude extract of *R. nepalensis at* 400 mg/ml concentration against 4 pathogens, *E. coli (*21.33±0.58*), S. aureus (*31.50±0.50*), E. faecalis* (29.17±0.76mm) and *p. aerogenosa (*22.5±1.32mm*)* followed by the extracts of *S. incanum*, *C. papaya*, *N. tabacum* and Cl. *ansita* against a standard strain of *S. aureus.* This suggests that the extract of this plant has a broad spectrum of antibacterial activities. However, the methanol extracts of *Cl. ansita* stem showed no activities against all bacteria used in this study except *S. aureus (*13±0*);* this is less recorded even against *S. aureus* when compare with present studied plants (table 4).

The medicinal plants investigated in the present study showed good antimicrobial potential and were found to be most effective against *S. aureus*. *S. aureus* and *E. faecalis* is most sensitive to *R. nepelensis* and *S. incanum* (table 4B). The possible reason may be due to the cell membrane and cell wall structural sensitivity of Gram positive bacteria (Reygaert, 2018). However, *N. tabacum* is more effect to *P. aerogenosa* and *E. coli* than *E. faecalis.* This is may be, because each of the class of phytochemicals reported in this study contains several compounds that can affect a diversity of microorganisms in many ways (Cushnie *et al.,* 2014).

Moderate antibacterial activity result has also been recorded from leaves of *W. sominifera* extract against *S. aureus (*18.83±0.76mm*),* but not effective against *E. coli*. The methanolic extract of *C. papaya* exhibited maximum activity against *S. aureus* (25.67±0.5 8mm) in contrary to study reported by Ajiboye and Olawoyin, (2020) with acetone extract 17.2±0.3mm at 400 mg/ml and least towards *E. coli* (7.67±0.58mm) which, have comparable agreement to studies that reported antibacterial activity of methanolic extract of *C. papaya* on *E. coli* (5.33±0.57) at 400mg/ml by Unaeze *et al.,* (2018). This indicates that solvent used and may influence antimicrobial activity of the extracted compounds. The methanol extract of fruit *S. incanum* at 100mg/ml concentration against *S. aureus* is 23mm which is nearest recorded with ethanol extract 26mm reported by Indhumathi and Mohandass, (2014). The difference in result may be due to solvent used and different geographical area from where the plant was obtained.

Root of *R. nepalenses* extract with methanol on *E. coli* achieved 16mm at 200mg/ml while, study reported by Waqas *et al.,* (2016) showed 15mm zone of inhibition at 300mg/ml that indicate both leaf and root of *R. nepalenses* have good antibacterial activities against *E. coli*. The methanol extract of *C. papaya* and *S. incanum* were not effective against *P. aerogenosa*. This may be due to solvent and part of plant used. *Solinum incanum* exhibited much higher antibacterial activity to *S. aureus* in lesser concentration comparative to *Cl. ansita.* The antibacterial properties of the active plants may be due to the presence of different bioactive chemical agents in the extracts which, are known

to act by a different mechanism to exert antibacterial action.

The current observation on the inhibitory activity of *R. nepalensis, S. incanum, C. papaya, N. tabacum* against *S. aureus* was more pronounced than a standard antibiotic used (Ciprofloxacin (18mm) and Gentamicin (18mm), while *C. papaya* against *S. aureus* (25.67±0.58) and *R. nepelensis* against *E. coli* (21.33±0.58) showed comparable activities with Pencillin (25mm) and Gentamicin (20mm) respectively. The methanolic extraction of leaves of *W. sominifera* showed less activity with *P. aerogenosa* 7.93±0.12mm and with *E. faecalis* 9.33±0.58mm zone of inhibition as compared with the standard antibiotics such as gentamicin and ciprofloxacin, while they have comparable inhibitory activities against *S. aureus* (18.83±0.76mm) shown in (Table 4A and 4B). However, *Cl. ansita* was observed to be significantly lower than that of the standard antibacterial drug used as positive control in this study. This could be due to the fact that the sample extracts used in the test were crude preparation which may not necessarily contain enough of the active chemical.

The Minimum inhibitory concentration assay was employed to evaluate the effectiveness of the extracts to inhibit the growth of the tested bacteria. The plant extracts with high activity against a particular organism usually give low MIC value while the extracts with low activity give high MIC value (Doughari, *et al.,* 2007). In agreement with this general assertion, in the present study, the MIC value of the extracts agreed with their corresponding antibacterial activities. All of tested plants showed MIC value ranged from 6.25mg/ml to 50mg/ml against *S. aureus*. The MIC value of *C. papaya and N. tabacum* ranged from 50 mg/ml to 400.00 mg/ml against *S. aureus, E. faecalis* and *E. coli*. However, the methanol extract of *Rumex nepalensis* had most activity than other extracts against *S. aereus, E. coli, E. faecalis* and *P. aeruginousa,* with MIC ranged from 6.25mg/ml to 25mg/ml and MBC ranged from 12.5mg/ml to 50mg/ml (table 5). The obtained result shows that root of this plant have potential antimicrobial activity that makes it a good candidate for further exploration regarding drug discovery.

The Minimum inhibitor concentration of methanolic extract of *C. papaya* and *S. incanum* was 100mg/ml against *E. faecalis*, whereas *P. aeruginosa* were insensitive to all concentrations of this extract in contrast to *S. aureus* that was affected by all concentrations. Among from organism tested *P. aerogenosa* is less susceptible to the extract than the other in opposite to *S. aureus*, however, negative results do not indicate that the bioactive constituents are absent or that the plant is inactive. This is may be due to active compounds present in insufficient quantities in the crude extracts or might be due to the difference in morphological constituents between these microorganisms.

The main components of plant-derived phytochemicals revealed from present studies are flavonoids, glycosides, tannins, saponins, phenolics and Phlobatanins. However, Phytosterols were found only in *S. incanum* extracts. Previous studies indicated that the flavonoids, glycosides, tannins, saponins and phenolics were positively tested in the leaves of *Nicotiana tabacum* L (Oeung *et al.*, 2017), leaves of *Withania sominifera* (Saleem *et al.,* 2020), roots of *Rumex nepelensis* (Gonfa *et al.,* 2021), leaves of *Carica papaya* (Awah *et al.,* 2017) and stem of *Clausena ansita* (Lawal *et al.,* 2015); which is similar to our result demonstrating the presence of flavonoids, glycosides, tannins, saponins and phenolics. The presences of those phytochemicals in different extracts were an indication of that extracts have antibacterial activity and can be used as alternative medicine.

Conclusion

Resistance to antimicrobial agents has become a major source of morbidity and mortality worldwide. The current experimental study showed that all extracts have antibacterial activities against all tested standard organism which, play a vital role to substitute resist drugs as evidenced by high zone of inhibition. The lowest value of MIC was recorded from extracts of *R. nepalensis* roots, against all the test bacteria. The obtained result shows that root of this plant have potential antimicrobial activity that makes it a good candidate for further exploration regarding drug discovery. These agents can act alone or in combination with antibiotics to enhance the antibacterial activity against a wide range of bacteria. However, the methanol extracts of *Cl. ansita* stem showed less activities against all bacteria used in this study except *S. aureus*. The main components of plant-derived phytochemicals revealed from present studies are flavonoids, glycosides, tannins, saponins, phenolics and Phlobatanins. The presences of these phytochemicals in different extracts were an indication of that extracts have antibacterial activity and made this plant extract very interesting from bioactive point of view. Further antibacterial activity tests are needed to be performed using other methods for that plant did not show any antibacterial activity in this study. Further analysis and fractionation should be conducted on plant components showing antimicrobial activity to isolating bioactive compounds in its purest form and used for new drug development programs. It is also recommended that the

extracted plants that did not show antibacterial activity tests should be performed against other bacterial species that were not tested. *In-vivo* studies should also be conducted to confirm the safety and efficacy of this plant's secondary metabolites.

Acknowledgement

The authors will like to thank to all contributors during plant collection and Ambo University for allowing us to do the collected data in Microbiology laboratory. In addition, we would like to thank Ambo University Mega project, traditional healers, the Ethiopian Public Health Institution and Veterinary laboratory where the study was conducted.

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Hassan, A., & Ullah, H. (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. *Journal of chemistry*, *2019*(1), 5264943.
2. Tyagi, R., Sharma, G., Jasuja, N. D., & Menghani, E. (2016). Indian medicinal plants as an effective antimicrobial agent. *J Crit Rev*, *3*(2), 69-71.
3. Tadele, A. (2017). Ethiopian herbal medicine research article profile. *Ethiopian Public Health Institute*, 1-343.
4. World Health Organization. (2017). Planning for cost-effective traditional medicines in the new century: A discussion paper. *Centre for Health Development*.
5. Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2).
6. Bultum, L. E., Woyessa, A. M., & Lee, D. (2019). ETM-DB: integrated Ethiopian traditional herbal medicine and phytochemicals database. *BMC complementary and alternative medicine*, *19*, 1-11.
7. Tesfahuneygn, G., & Gebreegziabher, G. (2019). Medicinal plants used in traditional medicine by Ethiopians: a review article. *J Respir Med Lung Dis*, *4*(1), 1-3.
8. Moges, A., & Moges, Y. (2019). Ethiopian common medicinal plants: their parts and uses in traditional medicine-ecology and quality control. *Plant science-structure, anatomy and physiology in plants cultured in vivo and in vitro*, *21*, 78-101.
9. Kitata, G., Abdeta, D., & Amante, M. (2017). Ethnoknowledge of plants used in veterinary practices in Midakegn district, west showa of Oromia region, Ethiopia. *Population (Tadeg et al., 2005; Giday et al., 2009)*, *26*, 7.
10. Moloney, M. G. (2016). Natural products as a source for novel antibiotics. *Trends in pharmacological sciences*, *37*(8), 689-701.
1. 11Rossiter, S. E., Fletcher, M. H., & Wuest, W. M. (2017). Natural products as platforms to overcome antibiotic resistance. *Chemical reviews*, *117*(19), 12415-12474.
11. Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research*, *196*, 44-68.
12. Price, L. B., Hungate, B. A., Koch, B. J., Davis, G. S., & Liu, C. M. (2017). Colonizing opportunistic pathogens (COPs): The beasts in all of us. *PLoS pathogens*, *13*(8), e1006369.
13. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of tropical medicine*, *2019*(1), 1895340.

14. Lawal, I. O., Grierson, D. S., & Afolayan, A. J. (2015). The antibacterial activity of Clausena anisata hook, a South African medicinal plant. *African Journal of Traditional, Complementary and Alternative Medicines*, *12*(1), 23-27.
2. 16Lonkala, S., & Reddy, A. R. N. (2019). Antibacterial activity of Carica papaya leaves and Allium sativum cloves alone and in combination against multiple strains. *Pharmacognosy Journal*, *11*(3).
3. 17. Ameya, G., Manilal, A., & Merdekios, B. (2017). In vitro antibacterial activity and phytochemical analysis of Nicotiana tabacum L. extracted in different organic solvents. *The Open Microbiology Journal*, *11*, 352.
4. *18*Shaikh, S., Shriram, V., Srivastav, A., Barve, P., & Kumar, V. (2018). A critical review on Nepal Dock (Rumex nepalensis): A tropical herb with immense medicinal importance. *Asian Pacific Journal of Tropical Medicine*, *11*(7), 405-414.
5. 19. Eshetu, G. R., Dejene, T. A., Telila, L. B., & Bekele, D. F. (2015). Ethnoveterinary medicinal plants: preparation and application methods by traditional healers in selected districts of southern Ethiopia. *Veterinary world*, *8*(5), 674.
6. 20. Kumari, M., & Gupta, R. Á. (2015). In vitro antibacterial effect of Withania somnifera root extract on Escherichia coli. *Veterinary world*, *8*(1), 57.
7. 21. Nigussie, D., Mulugeta, W., Molla, A., Bishaw, Z., & Biradar, C. (2019). GIS-based multi-criteria land suitability mapping for scaling faba bean varieties in Ethiopia. *African Crop Science Journal*, *27*(4), 687-708.
8. 22. Burman, S., Bhattacharya, K., Mukherjee, D., & Chandra, G. (2018). Antibacterial efficacy of leaf extracts of Combretum album Pers. against some pathogenic bacteria. *BMC complementary and alternative medicine*, *18*, 1-8.
9. 23. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in microbiology*, *9*, 1639.
10. 24Sbhatu, D. B., & Abraha, H. B. (2020). Preliminary antimicrobial profile of Solanum incanum L.: A common medicinal plant. *Evidence-based Complementary and Alternative Medicine*, *2020*(1), 3647065.
11. 25. Enejiyon, S., Abdulrahman, A. H., Adedeji, A., Abdulsalam, R., & Oyedum, M. (2020). Antibacterial activities of the extracts of Allium sativum (Garlic) and Allium cepa (Onion) against selected pathogenic bacteria. *Tanzania Journal of Science*, *46*(3), 914-922.
12. 26Usman, H., & Osuji, J. C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of Newbouldia laevis. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(4), 476-480.
13. 27. Karthikeyan, G., & Vidya, A. K. (2019). Phytochemical analysis, antioxidant and antibacterial activity of pomegranate peel. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*, *5*(1), 218.
14. 28. Teresa, B (2018): Extraction and Qualitative Phytomanischemical Screening of Medicinal Plants, *Int. J. Applied. Pharmaceut. Bio. Res*, **2**(6):15-23.
15. 29. Pathak, M. (2019). Antimicrobial Activity of Lagenaria siceraria Fruit Extract Against Pathogenic Bacterial Strains. *EC Pharmacology and Toxicology*, *7*, 825-830.
16. 30. Divya, N., Thenmozhi, S., Sureshkumar, B. T., & Selvan, M. (2014). Antibacterial activity of medicinal plant against wound infected pathogens. *International journal of pharmaceutical sciences and research*, *5*(11), 4942.
17. 31. Reygaert, W (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, **4**(3): 482–501. DOI: 10.3934/microbiol.2018.3.48
18. 32. Cushnie, T. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International journal of antimicrobial agents*, *44*(5), 377-386.
19. *33.* Ajiboye, A. E., & Olawoyin, R. A. (2020). Antibacterial activities and phytochemical screening of crude extract of Carica papaya leaf against selected pathogens. *Global Journal of Pure and Applied Sciences*, *26*(2), 165-170.
20. 34. UNAEZE, B. C., OCHIABUTO, O. M. T. B., EJIKE, E. C., OBI, M. C., & NWANKPA, S. N. (2018). Antimicrobial activities of Carica papaya leaf against diarrhoea causing agents. *International Journal of Advanced Engineering Research and Science*, *5*(8), 264253.
21. 35. Indhumathi, T., & Mohandass, S. J. I. J. C. M. A. S. (2014). Efficacy of ethanolic extract of Solanum incanum fruit extract for its antimicrobial activity.
22. 36. Waqas, M., Afridi, S., Ahmad, N., Sohail, A., Hayat, A., Ramzan, R., & Niaz, M. (2016). Anti-microbial activity of Rumex Nepalensis and Urtica Diocia. *Int J Sci Res*, *5*(3), 1563-1566.
23. 37. Doughari, J. H., Elmahmood, A. M., & Manzara, S. (2007). Studies on the antibacterial activity of root extracts of Carica papaya L. *African Journal of Microbiology Research*, *1*(3), 037-041.
24. 38. Oeung, S., Nov, V., Ung, H., Roum, K., Yin, V., Keo, S., & Chea, S. (2017). Phytochemical analysis of

different extracts of leaves of Nicotiana tabacum L. of Cambodia. *Asian Journal of Pharmacognosy*, *1*(3), 18-26.

25. 39. Saleem, S., Muhammad, G., Hussain, M. A., Altaf, M., & Bukhari, S. N. A. (2020). Withania somnifera L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian journal of basic medical sciences*, *23*(12), 1501.
26. 40. Gonfa, Y. H., Beshah, F., Tadesse, M. G., Bachheti, A., & Bachheti, R. K. (2021). Phytochemical investigation and potential pharmacologically active compounds of Rumex nepalensis: an appraisal. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*, 1-11.
27. 41. Awah, N. S., Agu, K. C., IIkedinma, J. C., Uzoechi, A. N., Eneite, H. C., Victor-Aduloju, A. T., ... & Ilikannu, S. O. (2017). Antibacterial activities of the aqueous and ethanolic extracts of the male and female Carica papaya leaves on some pathogenic bacteria. *Bioeng Biosci*, *5*(2), 25-29.
28. 42. Lawal, I. O., Grierson, D. S., & Afolayan, A. J. (2015). The antibacterial activity of Clausena anisata hook, a South African medicinal plant. *African Journal of Traditional, Complementary and Alternative Medicines*, *12*(1), 23-27.

****