Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online) DOI: 10.7176/ALST
Vol.72, 2019

In Vitro Antibacterial Activity of Rumex nervosus and Clematis simensis Plants Against Some Bacterial Human Pathogens

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Abstract
Due to quick growth of resistance and high cost of new generation antibiotics, lots of efforts were made to discover new antimicrobial agents from various sources. So, current study was assessed antibacterial activity of ethanol, methanol, acetone, diethyl ether and hexane leave extracts of *Rumex nervosus* and *Clematis simensis by* used paper disc diffusion and broth dilution procedures against six human pathogenic bacterial strains. The pathogenic bacteria were *Shigella dysenteriae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae* were susceptible to ethanol, methanol and acetone extracts of the leaves of *Rumex nervosus* followed by *Clematis simensis,* but hexane extract didn't displayed any activity. The extreme inhibition zone of 16.3±0.57mm was detected against *E coli* by ethanol extract of *Rumex nervosus* and MIC of 3.125mg/ml against *Escherichia coli* and *Shigella dysenteriae* by methanol extract. *The methanol extract of Clematis simensis formed a marked inhibition of 13.1±0.37mm against Escherichia coli* and ethanol extract of *Clematis simensis* displayed activity against *Shigella dysenteriae* 14.4±0.45mm and MIC of 6.25mg/ml against *Salmonella typhi.* Four dissimilar antibiotics like Ciprofloxin, Tetracyclin, Kanamycin and Chloramphenicol were used as standard for tested antibacterial activity against six different human pathogens. The activities were recognized the presence of some secondary metabolites existed in the tested floras which have related with antibacterial activities.
Keywords: Antibacterial activity*, Clematis simensis,* Human pathogens*, Rumex nervosus*
DOI: 10.7176/ALST/72-02
Publication date:March 31st 2019

1. Introduction

Traditional medicine is a popular form therapy in developing countries and its use broadly recognized in numerous literatures. The improving emergence of antimicrobial resistance deteriorates the impact (Mulu *et al.,* 2006; Olivier *et al.,* 2010). It has been shown that risk of negative clinical consequences, mortality, and high treatment costs with drug-resistant bacteria is generally higher compared to patients infected with the same non-resistant bacteria (WHO 2003). Improved prevalence of resistant bacteria, together with lack and high cost of new generation drugs has escalated infection-related morbidity and mortality particularly in developing countries like Ethiopia (Mulu *et al.,* 2006; Borkotoky *et al.,* 2013). This proliferation endorsed to undifferentiating use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters and ongoing epidemics of HIV infection (Dean and Burchard 1996; Gonzalez *et al*., 1996). However, the progress of new antibiotics should continue as the primary significance to retain the usefulness of antimicrobial treatment (Marchese and Shito 2001). The potential of floras are bases for modern medicine to achieve new values (Evans *et al.,* 2002).

In recent years, pharmacological enterprises consumed a lot of time and money in developing natural products extracted from plants, to harvest extra cost real medicines that are reasonable to the population (Doughari 2006). Today, many commercially confirmed drugs used in modern medicine were firstly used in crude form in traditional or folk healing performs, or for other purposes that suggested potentially useful biological activity. The therapeutic floras around the world contain various compounds with antibacterial activity (Marjorie 1999). So, orderly screening them may result in the detection of novel real antimicrobial compounds (Costa et *al.*, 2008). The screening of plant extracts and plant products for antimicrobial activity has shown that floras represent a potential source of new anti-infective agents (Amani *et al.,* 1998; Costa *et al.,* 2008). Many researches have carried out to screen natural products for antimicrobial property (Nair and Chanda 2006). Therapeutic floras possess immune modulatory and antioxidant properties, leading to antibacterial activities. They have versatile immune modulatory activity by stimulating both non-specific and specific immunity (Pandey and Chowdhry 2006).

Rumex nervosus mostly originated high altitude areas (above 1000m) and continue about 200 species. The leaves of this plant are edibles in Ethiopia. In Ethiopia, the leaves and stem of this herb are used for purifying the body by women traditionally as substituent of olive tree, to do this, the leaves are put on fire then they cover the patient body with that hot leaves and blanket so that the vapors and smoke surround all the body (Madhu *et al .,* 2014). *Rumex* species contains anthrax derivatives like chrysophanol, physician, emodin, aloe-emodin, rhein; which are the main biologically active compounds responsible for anti-cancer, cytotoxic, genotoxic and mutagenicity properties (Wegiera *et al.,* 2012). Traditionally in Ethiopia, the leaves, stems and roots of *Rumex*

nervosus were used as traditional medicines, for the eye disease, taeniacapitis, hemorrhoids, infected wounds, arthritis, eczema, abscess and gynecological disorders.

Clematis simensis is woody climber that escalates up to 10m or more, occasionally with long branches lying on the ground. The stem is pubescent; leaves are pinnate while the leaflets are ovate. The superior of the leaves have disseminated hairs while the inferior one is to Mentos. The inflorescence was various flowered, the flowers being pale yellow to white in color (Edwards *et al.,* 2000). Traditionally in Ethiopian the plants leaves were used for dress wounds and also for the treatment of eczema, *tinea capitis* and tropical ulcers *and also the* seeds of this plant were used for rheumatic pain while the sap was used as a febrifuge and against bloat in animals. A recent study reported that the leaves of *C. simensis* used in combination with another plant from the same family (Addis *et al.,* 2001; Gedif *et al.,* 2001). Traditionally plants used for the treatment of gonorrhea, syphilis and sore throat. The leaves have also been used for the treatment of leprosy, fever and various skin diseases and headaches (Iwu, 1993; Kakwaro, 1976). *The* extracts leaves of *C. simensis* by aqueous and methanol are exhibit activity against certain bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungi *Candida albicans* (Desta *et al.,* 1993; Cos *et al.,* 2002).

In Ethiopia, medicinal plants are still the most important and occasionally the only bases of therapeutics for nearly 80% of human and more than 90% in livestock population. Estimated floras of 6,500 to 7,000 species of higher plants are originated in Ethiopia and about 12% are endemic to the country (Tadeg *et al.,* 2005). Despite their vital role in providing for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is irreversible loss and declining to deterioration due the oral passage of herbal heritage from generation to generation rather than in writings (Mesfin *et al.,* 2009). Ecological degradation, farming growths, cultivation of marginal lands and suburbanization are also posing a significant threat to the future wellbeing of human and animal populations that have relied on these resources to fight several ailments for generations (Lulekal *et al.,* 2008: Devi *et al.,* 2009).

2. Materials and Methodology

2.1 Location of the study area

The study was conducted on selected medicinal plants composed from Sinana and Agarfa districts of Bale zone, Oromia Regional State, South Eastern Ethiopia. Sinana district was found at 430 km southeast of Addis Ababa. The area was situated at $7^{O}7'$ N and $40^{O}10'$ E and 2,400 masl. The mean average rainfall of the area was 353 mm. For the same period, average annual maximum temperature was 21.2^{o}C and minimum temperature was 9.4^{o}C. The dominant soil type was pellic vertisol and slightly acidic (pH = 6). Agricultural production system of the study area was mixed farming. Agarfa district was located at 464 km south east of Addis Ababa. The area was situated at $6^{O}11'$ N and $40^{O}3'$ E and 2,350 masl. The mean average rainfall of the area was 880 mm and bimodal. The average annual maximum temperature was 24.75^{o}C and minimum temperature was 7.1 ^{o}C. The dominant soil type was clay soil and slightly acidic (pH = 5.8). Agricultural production system of the study area was mixed farming.

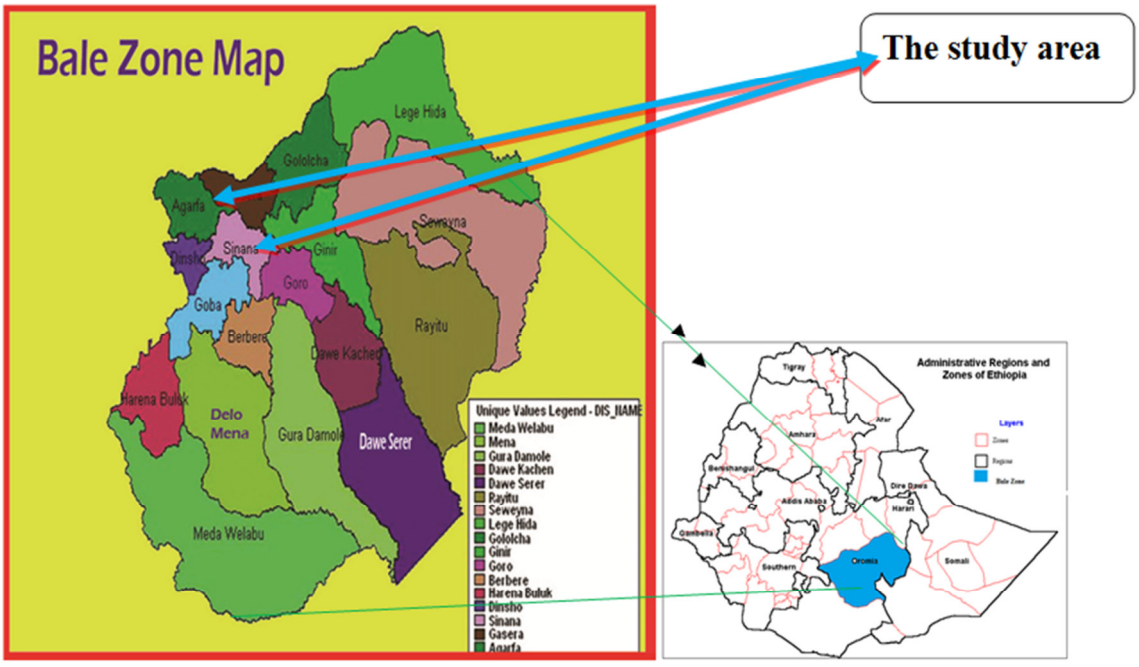

Figure 1 Map of study area

2.2. Collection and identification of plant materials

Two medicinal plants *Rumex nervosus* and *Clematis simensis* were collected from Bale Zone, Sinana and Agarfa district Oromia region, Ethiopia. The taxonomic situation of the plants was identified and authenticated by plant experts from National Herbarium in Addis Ababa University. Leaves from the study plants were taken in a large quantity and repeatedly washed under tap water to remove any debris and were air dried under shade for fifteen days.

2.3. Preparation of plant's crude extracts

The preparation of crude extracts of plants under this study was conducted followed the methods used by Tadeg and coll. (2005) used different solvents. Five hundred grams of leaves from each plant was taken for extraction procedure and ground in a mortar and pestle separately under aseptic condition. Twenty grams of each powdered plant material were extracted with apparatus with 250 ml of ethanol, methanol, diethyl ether, hexane and acetone separately by maceration for 48 h with frequent agitation on orbital shaker for continuous two days and the resulted liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation used Rota vapor (BU¨CHI Rota-vapor R-205, Switzerland) at 40 ˚C. The resulted dehydrated mass was then crushed, packed into a glass vial until used. Finally, the gram yield of dried residue of each plant extracts were calculated. The concentrated extracts were stored at 4°C for the next antimicrobial study. Dried residues were dissolved in 100 % dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100 mg/ml, which was kept at 4 °C until used.

2.4. Preparation of tested microorganisms

The tested microorganisms included Escherichia coli, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. These microorganisms were suspended in nutrient broth and subcultured into fresh nutrient agar medium and kept at 4°C until used. The inoculated preparation was standardized by inoculated bacterial strains from the exponential phase and standardized with 0.5 McFarland turbidity standard prepared by added a 0.5 mL aliquot of 1.175% w/v $BaCl_2.2H_2O$, added to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v).

2.5. Antimicrobial Assay

2.5.1. Antibacterial sensitivity tested used disc diffusion method

The antibiotic susceptibility tested, stock concentrations of (100mg/ ml) plant crude extracts were prepared in DMSO. A circular antibiotic assay disc of 6 mm diameter was prepared from the Whatman filter paper No.3 and sterilized by autoclave for 15 min at 121° C. The sterile discs were impregnated with 50µl of the reconstructed extract and were dried completely at 37 °C overnight. A sterile cotton swab was dipped into a homogenous suspension of tested microorganism with adjusted 0.5 McFarland turbidity standards. The tested pathogenic microorganisms were swabbed gently by cotton swab onto Muller Hinton Agar (MHA) and were then allowed to dry for half an hour. The discs were aseptically placed over plates of Muller Hinton Agar (MHA) (Haniyeh *et al*., 2010). The plates were incubated in an upright position at 37 °C for 24 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 12 mm was considered as had low antibacterial activity. Diameters between 12 and 16 mm was considered moderately active, and these with >16mm was considered highly active (Indu *et al*., 2006). The tested microorganisms were tested for their sensitivity against the standard antibiotics, Ciprofloxacin (35 µg), Chloramphenicol (30 µg) Tetracycline (30 µg) and Kanamycin (20µg) by the disc diffusion method (Bauer *et al*., 1966).

2.5.2. Minimum Inhibitory Concentration (MIC) assay methods

The minimum inhibitory concentration (MIC) was determined by compared the various concentrations of plant extracts which have different inhibitory effect and selected the lowest concentration of extract showed inhibition (Agatemor, 2009). The minimum inhibitory concentration (MIC) was determined for extracts that showed inhibition zone of $\geq$ 7 mm diameter and for extract that inhibited the growth of all tested bacteria at concentration of 200 mg/ml. The tested was performed by used standard tube dilution (serial dilution) method used nutrient broth as diluents. Accordingly, the plant extract was prepared by double serial dilution from 200 mg/ml to obtain 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 in order to get 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml concentration of extract respectively using 50% DMSO. 1 ml of each extracts was dissolved in sterile test tubes which contained 9 ml of nutrient broth. Then, 0.1ml of the tested microorganism was inoculated to the each tube. One tube was used as the control (broth + extract). The tubes were incubated at 37°C for 24 h and the existence of growth was assessed by compared the optical density (OD) of each well before and after incubation. When the difference of OD value (after incubation-before incubation) of the test (broth + extract + organism) was greater than that of the control (broth + extract) at each concentration, it was considered as presence of

turbidity or growth of bacteria. The lowest concentration, at which there was no turbidity, was also regarded as MIC value of the extract.

2.6 Data Analysis

Data on mean inhibition zone formed by each plant extract and MIC on various bacteria were entered in to Microsoft excels spreadsheet and SPSS (Statistical Package Software for Social Science version 16). Values were given as mean± SD.

3. Results

3.1 Antibacterial activity of the plant extracts

The crude extracts study plant such as *Rumex nervosus* and *Clematis simensis* were tested for antibacterial activity on six human pathogens. The solvents that were used in this study produced an overall yield of plant crude extracts that were ranging from 0.6 to 2.4 gm from different plants (Table.1).

In-vitro antimicrobial activity of *crude extracts of plants under this study was evaluated against human pathogenic bacteria of Escherichia coli, Salmonella typhi, Shigella* dysenteriae*, Pseudomonas aeruginosa, Staphylococcus* aureus and *Klebsiella pneumoniae.* The results obtained in the present study revealed that the tested two medicinal plants (Rumex nervosus and Clematis simensis) extracts possess a potential antibacterial activity.

Table 1: The yield of plant crude extracts by using different solvents

Plant species	Parts used (gm)	Extraction type	Yield in grams (Mean in mm)
Rumex nervosus	(20gm) Leaves	Methanol	1.6
		Ethanol	1.35
		Diethyl ether	0.6
		Acetone	1.6
		Hexane	1
Clematis simensis	(20 gm) Leaves	Methanol	2
		Ethanol	2.4
		Diethyl ether	1.3
		Acetone	2.1
		Hexane	1.2

3.1.1. The antibacterial activity of *Rumex nervosus* crude extracts

The antibacterial activity of *Rumex nervosus* crude extracts was assayed by disc diffusion method. The methanol and ethanol leaves extract of *Rumex nervosus* showed considerably a higher mean antibacterial activity as compared to other solvents. The highest antibacterial activity was exhibited on *Escherichia coli* (16.3±0.57 mm) by ethanol extract, followed by *Shigella dysenteriae* (12.5±0.5 mm) and a moderate inhibition of *Klebsiella pneumoniae* (10±1.0 mm) and the least activity against *Salmonella typhi* (6.1±0.76 mm). The methanol extracts showed a strong inhibitory activity against *S. typhi* (14.8±0.76 mm), followed by *Shigella dysenteriae* with a zone of inhibition 11±0.57mm and a moderate inhibition against *Staphylococcus aureus* (9.8±0.28 mm) and *P. aeruginosa* (8.8±0.76mm). With methanol, a minimum zone of inhibition of *Rumex nervosus* (6.5±0.5 mm) was exhibited by *E. coli*.

Acetone extracts of *Rumex nervosus* were exhibited a maximum zone of inhibition against *Salmonella typhi* (11.9±0.35 mm) followed by *Staphylococcus aureus* (10.5±0.5mm) and minimum activity against *Pseudomonas aeruginosa* (5.4±0.5 mm). Diethyl ether extracts showed inhibitory activity against only three pathogens. The maximum inhibition was detected on *Salmonella typhi* (6.2±0.68 mm) followed by *Klebsiella pneumoniae* (7.9± 0.17mm) and least activity against *E. coli* (4.8±0.76mm). Hexane extract didn't show any antibacterial activity against tested pathogenic bacteria. (Table .2)

Table 2: The effect of the different extracts of the leaves of *Rumex nervosus* against tested pathogenic bacteria (Zones of inhibition in mm; Mean± SD mm)

Test organisms	Mean **Inhibition zone** of leaves extract of * *R.nervosus in mm* (Mean± SD mm)				
	Methanol	Ethanol	Diethyl Ether	Acetone	Hexane
Escherichia coli	6.5±0.5	16.3±0.57	4.8±0.76	-	-
Salmonella typhi	14.8±0.76	6.1±0.76	-	11.9±0.35	-
Shigella dysenteriae	11±0.57	12.5±0.5	6.2±0.68	7.3±0.57	-
Staphylococcus aureus	9.8±0.28	8.6±0.52	-	10.5±0.5	-
Pseudomonas aeruginosa	8.8±0.76	6.1±0.36	-	5.4±0.5	-
Klebsiella pneumoniae	8.8±0.28	10±1.0	7.9±0.17	5.8±0.28	-

-= implies no inhibition zone detected*; ** = a crude extract at concentration of 100mg/ml was used for assay.

3.1.2. The antibacterial activity of *Clematis simensis* crude extracts

The methanol extract of C.simensis formed a marked inhibition zone of 13.1±0.37mm in diameter against *E. coli*, followed by *K. pneumoniae* (10.9±0.3mm) and *S. typhi* (9.7±0.64 mm). The methanolic extracts exhibited the least inhibitory activity against *S. dysenteriae* and *S. aureus* with mean inhibition zone of 7.2±0.46mm and 7.7±0.45mm respectively. The prominent zone of inhibition from the ethanol extract of *C.simensis* against *Shigella dysenteriae* was 14.4±0.45mm followed by *K. pneumoniae* (13.9±0.35mm), *S. typhi (*12.9±0.51mm) and 11.5±0.51mm against *E. coli*. Moderate inhibitory activity was noticed against *S. aureus* (10±0.15mm) followed by 12.1±0.3mm against *P. aeruginosa* and a moderate activity of 8±0.2mm against *S. dysenteriae* and 7.9±0.35mm against *K. pneumoniae* and minimum inhibitory activity against *E. coli* with a zone size of 5.6±0.52mm. Acetone extract of *C.simensis* inhibited *S. aureus* with a highest zone of inhibition 11.9±0.25 mm and minimal inhibition was 6.8±0.2 mm and 5.7±0.32mm against *S. dysenteriae* and *P. aeruginosa*. No good antibacterial activity was excreted by the Hexane extracts (Table 3).

Table 3: The effect of the different extracts of the leaves of *Clematis simensis* tested pathogenic bacteria (Zones of inhibition; Mean± SD mm)

Test Organisms	Mean **Inhibition zone** of leaves extract* *Clematis simensis* (Mean± SD mm)				
	Methanol	Ethanol	D/ Ether	Acetone	Hexane
Escherichia coli	13.1±0.37	11.5±0.51	5.6±0.52	-	-
Salmonella typhi	9.7±0.64	12.9±0.36	6.2±0.62	-	3.7±0.26
Shigella dysenteriae	7.2±0.46	14.4±0.45	8±0.2	6.8 ±0.2	-
Staphylococcus aureus	7.7±0.45	10±0.15	-	11.9±0.25	-
Pseudomonas aeruginosa	-	8.5±0.55	12.1±0.32	5.7±0.32	-
Klebsiella pneumoniae	10.9±0.3	13.9±0.35	7.9±0.35	6±0.2	-

-= implies no inhibition zone detected*; *= a crude extract of at concentration of 100mg/ml was used for assay.

3.1.3 Inhibitory Zones of test pathogens with Standard Antibiotics (Positive control)

Four dissimilar antibiotics, Ciprofloxin, Tetracyclin, Kanamycin and Chloramphenical were used as standard and as positive control for the testing of antibacterial activity of six different human pathogens. Ciprofloxin displayed maximum zone of inhibition ranging from 20-35 against all pathogens; Kanamycin exhibited average zone of inhibition 20mm, Tetracycline exhibited ranging from 8-20mm and Chlomphenicol showed least inhibition against all test pathogens.

Table 4: The inhibition zone of antibiotics against human pathogens

Test organisms	Zone of inhibition in mm			
	Ciprofloxin	Kanamycin	Tetracycline	Chlromphenicol
Escherichia coli	30	20	15	10
Salmonella typhi	35	20	15	10
Shigella dysenteriae	32	20	13	10
Staphylococcus aureus	31	20	10	5
Pseudomonas aeruginosa	30	15	8	5
Klebsiella pneumonia	20	15	20	11

3.2. Minimum Inhibitory Concentration of Plant Extracts (MIC)

The Minimum Inhibitory Concentration assay was employed to evaluate the effectiveness of the plant extracts to inhibit the growth of bacterial tested microorganisms. The extracts of the two medicinal plants were exposed to the concentrations ranged from 0.78 mg/ml to 100mg/ml. In the antibacterial activity tested, five different solvents were used for their *in vitro* antibacterial tested among which only best three solvents methanol, ethanol and acetone had selected for MIC test.

3.2.1. Minimum Inhibitory Concentration (MIC) of *Rumex nervosus* leaf extracts against tested pathogenic bacteria (in mg/ml)

The methanol extract of *Rumex nervosus exhibited the lowest MIC at 3.12mg/ml against E. coli and S. dysenteriae followed by S. typhi and Pseudomonas aeruginosa at a concentration of 6.25mg/ml.* The ethanol extract exhibited MIC at 3.12 mg/ml concentration against *S. dysenteriae* and *K. pneumoniae* and at concentration of 6.25 mg/ml against *E. coli.* The ethanol extract also displayed its MIC at concentration of 12.5 mg/ml against *S. typhi* and *S. aureus* .The MIC of acetone extract of *Rumex nervosus* was 6.25 mg/ml against the *E. coli* and *S. typhi* followed by *S. dysenteriae* at 25 mg/ml and *S. aureus* at 50mg/ml (Table.5).

3.2.2. Minimum Inhibitory Concentration (MIC) of *Clematis simensis* leaf extracts against tested pathogenic bacteria in mg/ml

The methanol extract of *Clematis simensis showed* MIC activity at 6.25 mg/ml concentration against *E. coli and S. typhi* followed by *S. dysenteriae* and *K. pneumoniae* at 12.5 mg/ml concentration. The ethanol extracts showed

strong MIC activity at 1.56 mg/ml against *S. dysenteriae* and *against S. typhi at 6.25 mg/ml concentration followed by S. aureus* and *Pseudomonas aeruginosa at 12.5 mg/ml. The acetone extract of Clematis simensis exhibited a MIC at12.5 mg/ml against S. dysenteriae followed by S. aureus at 25 mg/ml and at 50 mg/ml against P. aerugenosa and K. pneumoniae (Table 6).*

Table 5: Minimum Inhibitory Concentration (MIC) of *Rumex nervosus* leaf extracts against bacterial tested microorganism in mg/ml

Rumex nervosus	Conc. mg/ ml	*Escherichia coli*	*Salmonella typhi*	*Shigella dysenteriae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Methanol	1.56	-	-	-	-	-	-
	3.12	**	-	**	-	-	-
	6.25	+	**	+	-	**	-
	12.5	+	+	+	-	+	-
	25	+	+	+	**	+	-
	50	+	+	+	+	+	-
Ethanol	1.56	-	-	-	-	-	-
	3.12	-	-	**	-	-	**
	6.25	**	-	+	-	-	+
	12.5	+	**	+	**	-	+
	25	+	+	+	+		+
	50	+	+	+	+	**	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	**	**	-	-	-	-
	12.5	+	+	-	-	-	-
	25	+	+	**	-	-	-
	50	+	+	+	**	-	-

** = Minimum Inhibitory concentration, + = Positive inhibition observed, - = No activities

Table 6: Minimum Inhibitory Concentration (MIC) of *Clematis simensis* leaf extracts against bacterial tested microorganism in mg/ml

Clematis simensis	Con mg/ ml	*Escherichia coli*	*Salmonella typhi*	*Shigella dysenteriae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Methanol	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	**	**	-	-	-	-
	12.5	+	+	**	-	-	**
	25	+	+	+	-	-	+
	50	+	+	+	**	-	+
Ethanol	1.56	-	-	**	-	-	-
	3.12	-	-	+	-	-	-
	6.25	-	**	+	-	-	-
	12.5	**	+	+	-	-	**
	25	+	+	+	**	**	+
	50	+	+	+	+	+	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	12.5	-	-	**	-	-	-
	25	-	-	+	**	-	-
	50	-	-	+	+	**	**

** = Minimum Inhibitory concentration, + = Positive inhibition observed, - = No activities (bacterial growth observed)

4. Discussions

Ethno botanical investigations have been found to offer significant evidences in the identification and development of traditionally used therapeutic florae into modern drugs. Involvement of the field has also reflected in the current study. The first step towards this goal was the in vitro antibacterial activity assay (Samy

and Ignacimuthu, 2000). Many reports were available on the antiviral, antibacterial, antifungal, anthelminthic, and anti-inflammatory properties of plants (Palombo and Semple, 2001; Kumarasamy *et al*., 2002).

In the present study, *Rumex nervosus* and *Clematis simensis was extracted by* used different solvents such as methanol, diethyl ether, ethanol, acetone and hexane. The results of current study were an indication of such understandings. The yield of the extract that was obtained by different solvents considerably differs in two of the medicinal plants (Table 1).

In the present study, among the solvents used to extract the biologically active substances from two medicinal plants, ethanol and methanol were the best solvents, followed by acetone and least by diethyl ether and hexane (Table 2 to 5). This specified that the extraction of medicinal plants with different solvents may produce different *in vitro* inhibitory result which based on the potential of the solvents used to extract the biologically active constituents (George *et al*., 2010). The methanol and ethanol leaf extracts of *Rumex nervosus* showed significant antibacterial activity against most of bacterial human pathogens evaluated in the present study. The highest antibacterial activity exhibited was against *E. coli* (16.3±0.57 mm) by ethanol extract, followed by *S. dysentriae* (12.5±0.5 mm) and a moderate inhibition against *K. pneumoniae* (10±1.0mm). In the present study, the methanol extract exhibited the second with inhibition zone of 14.8±0.76 mm against *S. typhi*, followed by *S. dysentriae* with a zone of inhibition of 11±0.57mm. A different study reported that the antibacterial activity of the methanolic extracts of *Rumex nervosus* leaves against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans* and *Candida albicans*, with zones of inhibition of 38, 36, 15, 38 and 32 mm, respectively (Mariam *et al*.,1993).

Pavithra and co-workers (2011) reported that the methanol extracts of *Mollugo cerviana* inhibited the growth of *S. aureus* and *E. coli* with zones of 7.33±0.57 mm and 11±1mm, respectively while chloroform extracts were ineffective against these bacterial strains. Current study showed that the methanolic extract of *Rumex nervosus* to have a strong inhibitory activity against tested pathogens which were in concordance with other studies. The decrease of antibacterial activity of *Rumex nervosus* against tested pathogens in the current study may be attributed to the difference in the initial plant extract used and extraction method used the difference in the strains of tested pathogens or due to unexplained reasons.

The acetone extracts of *Rumex nervosus* exhibited the maximum zone of inhibition against *S. typhi* (11.9±0.35mm) followed by *Staphylococcus aureus* (10.5±0.5mm) and minimum activity against *P. aeruginosa* (5.4±0.5mm). Related investigations have reported where acetone extracts showed a marked inhibitory effect on the growth of pathogenic bacteria (Abdullahi *et al.,* 2010). The methanol and Ethanol extract of *Rumex nervosus* exhibited the lowest MIC at 3.21mg/ml concentration against *Escherichia coli* and *Shigella dysentriae* and *K. pneumoniae.* The result of the present study showed that the plant extracts of *Clematis simensis* exhibited antibacterial activity against some of the common pathogenic bacteria. The prominent zone of inhibition from the ethanol extract of *Clematis simensis* against *S. dysenteriae* was 14.4±0.45 mm and against *K. pneumoniae* was 13.9±0.35mm followed by *Salmonella typhi* 12.9±0.51 mm. Previous study showed that ethanolic extract of *Clematis simensis* exhibited a highest zone of inhibition (28.33 mm) against *S. aureus* with MIC 12.5µg/ml (Mariam *et al*., 1993) a result higher than the size of inhibition zone in current study. The results of this study showed that the extracts from *Clematis simensis* was found to have significant antibacterial activity against both the selected Gram positive and Gram negative bacteria.

The methanol extract of *Clematis simensis* produced a pronounced inhibition zone of 13.1±0.37mm against *E. coli*, followed by *K. pneumoniae* with a zone of inhibition of 10.9±0.3 mm and *S. typhi* 9.7±0.64mm. In current study , the result clearly showed that this plant was effective against *E.coli*. The possible explanation for this difference in inhibitory activity might be the ecological difference on their distribution plants which might have contributed to variations in the concentration of the active ingredients. The methanol extract of *Clematis simensis* showed MIC activity at 6.25 mg/ml concentration against *E. coli* and *S. typhi* which was supported by work of (Mariam *et al*.,1993) where the minimum inhibitory concentration (MIC) of isolated compounds from *Clematis simensis* against *S. aureus, E. faecalis, E. coli* and *P. aeruginosa* was found to be varied from 16 µg/ml to more than 250 µg/ml. Ethanol extract showed a very minimal MIC of 1.56 mg/ml against *S. dysenteriae* and *S. typhi* which was strongly supported by the results of Tegenu Gelana (2011) where the Acetone and ethyl acetate extracts of the leaves of *Z. scabra* showed best activity against *S. aureus* exhibited an MIC of 1.56mg/ml and 0.781mg/ml respectively. The least inhibition zone was observed for hexane extract against *Salmonella typhi* according to Tsuchiya and coll. (1996).

5. Conclusion

From the above results it could be determined that the crude extracts of the two plants especially the ethanol and methanol revealed the fact that they have higher potential to produce broad spectral antibacterial activity with minimal concentration against a wide range of human pathogens. The extracts were good in inhibited *Escherichia coli, Salmonella typhi, Shigella dysenteriae, P. aeruginosa* and in some instances *K. pneumoniae.* The results of this study provided an insight into the antimicrobial properties of the extracts of *Clematis simensis*

and *Rumex nervosus*. As well as it formed an opportunity for selection of bioactive extracts for initial fractionation and further studies of these two medicinal plants in the antibacterial assays. This *in vitro* study demonstrated that these two folklore medicinal plants have good potential. This study gives a suggestion of the efficacy of the plants acquired from the traditional healers. The results of study initiate basis for further studies of the powerful plants so as to segregate the compounds responsible for the antimicrobial activity. Numerous modern drugs were extracted from traditional therapeutic floras through the use of plant material succeeding the ethno botanical leads from indigenous cures used by traditional remedial systems.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors would like to thanks the Madda Walabu University for providing the facilities to work and Ethiopian Public Health Institute (EPHI) for support standard pathogen isolation and National Herbarium in Addis Ababa University for taxonomic plants identified and authenticated.

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