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In vitro Antimicrobial Activities of Crude Extracts of *Vernonia amygdalina* and *Croton macrostachyus* against Some Bacterial and Fungal Test Pathogens

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ABSTRACT

The aim of the present study was to investigate the antibacterial and antifungal activities of crude extracts of *Vernonia amygdalina* and *Croton macrostachyus* against four bacterial pathogenic species (i.e. *Staphylococcus aureus, Streptococcus agalactiae, Salmonella typhi, Escherichia coli*) and two fungal pathogenic species (i.e. *Aspergillus flavus* and *Aspergillus niger*). Antimicrobial activities of the resulting extracts were determined by measuring zone of inhibitions on cultures of six selected pathogens using the disc diffusion method. The minimum inhibitory concentration (MIC) of the two plant extracts against the same pathogens was determined using the broth dilution method. Chloroamphenicol was used as positive control for for bacterial pathogen and Tilt was also used as positive controls fungal pathogens. Meanwhile Dimethyl Sulfoxide was taken as a negative control for both. Highest percentage yield was recorded for methanol extract of *V. amygdalina* (14.89±0.25%) and the lowest yield was observed for the water extract of *V. amygdalina* (4.58±0.27). All the methanolic and ethanolic crude extracts had the highest growth inhibitory effects as compared with the water crude extracts on all bacterial pathogens. The methanol crude extract of *C. macrostachyus* had highest zone of inhibition (21±0.29 mm) against *S. aureus*, with a minimum inhibitory concentration of 25mg/ml. Similarly, the ethanol crude extract of *C. macrostachyus* had shown highest antifungal activity against *A. niger* with inhibition zone of 15.5±0.29. The results indicate the potential of these medicinal plants in treating some bacterial and fungal infection. Thus, further detailed studies are recommended to be carried out to characterize their bioactive compounds for development of new effective antimicrobial drugs.

Keywords: Antibacterial activities, Antifungal activities, Broth dilution, Disc diffusion, Minimum inhibitory concentration.

INTRODUCTION

Medicinal plants are used in both developing and developed countries as a source of drugs or as a source of herbal extracts for various therapeutic purposes [1]. Use of plant derived natural compounds as part of herbal preparations and as alternative sources of medicine continues to play major role in the well being of the people all over the world. Majority of people in many parts of the world nowadays uses medicinal plants in treating various diseases. The applications of such medicinal plants may, however, vary from the administration of different parts of the plants to the utilization of extracts and decoction from the whole plants. Nowadays, due emphasis has been given towards medicinal plants and has been trying to identify compounds which are biologically active from the extracts of renown medicinal plants [2].

In developing countries, 80% of the population relies on traditional medicinal plants for primary health care systems [3]. Nevertheless, much work has not been done in investigating the efficacy of the medicinal plants in treating the claimed diseases. From the existing 250,000 species of higher plants described, only 5-15% has been scientifically studied for their curative value and potential as a source of drugs [2, 4]. Similarly, in Ethiopia where traditional medicine has been used for many centuries and is integrated with the way of life in all communities. There are about 7000 species of higher plants in Ethiopia, of which 12% belong only to the country [5]. In folk medicine of Ethiopia, the source of around 95% of traditional medicine preparations are emanate from medicinal plants [6] as supported by the deep indigenous knowledge on the use of medicinal plants for therapy of various human and animal disease. Furthermore, the importance of medicinal plants and their derivatives becomes enormous at a time when resistance of pathogenic microorganism against antibiotics has become a challenge throughout the world. The occurrence of multiple resistances in human pathogenic microorganism is becoming increasing, mainly because drugs which are commercially sold are used in the treatment randomly without targeting the specific infectious diseases [7]. Due to fast spreading drug and multidrug resistant of bacterial

and fungal pathogens the need for new drugs with less toxic effects is a highly demanding public interest. Therefore, intensive research activities are required to find out alternative solutions against drug resistant microorganisms. This needs evaluation of crude extracts and active constituents of traditionally used medicinal plants against human bacterial and fungal pathogens.

Ethnobotanical investigations in Mecha *wereda*, west Gojjam zone of Ethiopia, have shown that *Vernonia amygdalina* and *Croton macrostachyus* are commonly and extensively used in the folk medicine to treat many kinds of diseases such as malaria, gastrointestinal infections, wound, dermatitis, chigger, toothache, ringworm, cardiovascular diseases, dandruff and other bacterial and fungal diseases [8]. With this background information, the present study aimed at elucidating the *in vitro* antimicrobial (antibacterial and antifungal) activities of the two widely used medicinal plants (*Vernonia amygdalina* and *Croton macrostachyus*) on selected test pathogenic bacterial and fungal species, namely: *Staphylococcus aureus, Streptococcus agalactiae, Salmonella typhi, Escherichia coli, Aspergillus flavus* and *Aspergillus niger* by using different solvent extraction methods

MATERIALS AND METHODS

Plant materials collection and Identification

The plants, leaves parts of *Vernonia amygdalina* were randomly collect from main campus of Haramaya University, and leaves of *Croton macrostachyus* were collected from Damota, which is a part of Haramaya district about 5 Kilometers away from main campus of Haramaya University. The voucher specimens were identified by comparing them with specimens kept at the Herbarium of Haramaya University with the help of technical assistant. The experiments were, however, conducted in the laboratory of the School of Plant Sciences and Crop Protection and the General Laboratory of the Department of Biology at Haramaya University.

Preparation of Plant for Solvent Extraction

After collection, the plants were separately washed with tap water to remove unnecessary particles. Then the washed plant parts were shade dried at room temperature for five days. The dried leaves were separately ground using an electric grinder into fine powder (using 2 mm mesh size of sieve). Then they were stored in sterile airtight containers, finally kept in a refrigerator at 4°C until use [9].

Preparation of Ssolvent Extracts

Preparation of Water Extracts

Fifty grams of the ground material (*Vernonia amygdalina and Croton macrostachyus*) were soaked in 250 ml distilled water in a 1000 ml conical flask and allowed to stand for 24 hrs in a rotary shaker at 120 rpm. After 24 hrs, amuslin cloth was employed in filtering the suspension followed by Whatman No. 1 filter paper. After that, the filtrates were evaporated at 50°C with a water bath to obtain the solid crude extracts. They were kept in a refrigerator at 4°C for further anti-microbial activities [10].

Preparation of Ethanol and Methanol Extracts

Fifty grams of air dried powdered plant materials were placed in 250 ml of absolute ethanol and 99.8% methanol kept in a conical flask and was shaken in a rotary shaker at 121 rpm for 24 hrs to help thorough mixing and enough maceration of the plant parts. After 24 hrs, the suspension was filtered separately with Whatman No. 1 filter paper. The resulting filtrate was concentrated using a vacuum rotary evaporator at 40°C to remove the solvent. After the evaporation of solvents, the remaining crude extracts were weighed and recorded. Finally the crude extracts were kept in a refrigerator at 4°C until tested for anti-microbial activities [11].

Study Design

This study involved a laboratory based experimental design. The antimicrobial activities of the plant extracts against the test pathogens were determined using disk diffusion method. The minimum inhibitory concentrations (MIC) were determined for extracts against the selected pathogens using the broth dilution method. The treatments included two plants with one part each extracted by three solvents and each extract applied on six test pathogens in three replications (2x1x3x6x3). Chloroamphenicol and Tilt were used as positive controls for bacterial and fungal pathogens, respectively, whereas DMSO was used as a negative control for both.

Preparation of Culture Media and Sterilization

Potato Dextrose Agar, Nutrient broth, potato dextrose broth and Mueller-Hinton agar were prepared according to the manufacturer's instructions. All media were first autoclaved at 121 ^{o}C and 15 psi for 15 minutes before use.

Test Pathogens

The bacterial test pathogens were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa. These included *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 12386, *Salmonella typh*i ATCC 13311, *Escherichia coli* ATCC 25922. The fungal pathogens such as *Aspergillus niger* and *Aspergillus flavus* was obtained from infected ground nut in plant pathology laboratory of the School of Plant Sciences, Haramaya University. The cultured bacterial strains were also sub-cultured using Muller Hinton Agar (MHA) (and was allowed to incubate at 37°C for 18-24 h) while fungal cultures were sub-cultured and maintained using Potato Dextrose Agar (PDA) and incubated at 27 ^{o}C for 5-7 days [12].

Preparation of Iinoculums

Three to five well isolated bacterial colonies were selected from the plates and transferred into test tubes containing sterile normal saline solution and agitated with a vortex mixer to get a suspension accustomed to 0.5 McFarland turbidity standard of 1.5 x 10^{8} CFU/ml by using UV-Visible spectrophotometer at 625 nm [13]. Similarly, sterilized distilled water was used to extract spore suspensions of *A. niger* and *A. flavus* from PDA medium and adjusted to 10^{6}spores/ml using a hemacytometer [14].

Yields of Crude Extracts

After having extracted the plant part with appropriate solvents the filtrates were transferred in to container and concentrated using a vacuum rotary evaporator. After the evaporation of solvents, the crude extracts were weighed and recorded. All extracts were prepared in three replicates. The percentage yield of the crude extracts was determined using the following formula.

$$\text{Percentage yield } = \frac{x1 - x2}{y}\ x\ 100$$

Where,
$x1$ = Weight of container with crude extract,
$x2$ = Empty container and
y = the weight of the soaked powder [15].

Determination of Antimicrobial Activity

Determination of Antibacterial Activity

In testing the antibacterial activities of the different solvent crude extracts of the plants, disc diffusion method was used according to Kirby-Bauer [16]. Whatman No. 1 filter paper and puncher were used to prepare diffusion discs of approximately 6 mm diameter and put in a beaker and sterilized by oven at 180 °C for 30 minutes. A stock solution

of 500 mg of each extract per ml of 99% DMSO was prepared. 20µl of each plant extract was then put onto a sterile filter paper disc and allowed to dry for 30 minutes. Five milliliter of a culture suspension of bacteria with a concentration of 1.5×10^8 CFU/ml was added to 250 ml of MHA medium agar (maintained at 45-50 ^{o}C in a molten state) [16]. The medium was then transferred into petriplates and let solidified. The impregnated discs were then put with forceps aseptically on the surface of the pre-inoculated MHA medium. In case of negative control (DMSO), paper discs were prepared with similar procedure but without plant extract. Meanwhile, paper discs impregnated with Chloroamphenicol (at a concentration of 0.1 mg/ ml) were used as positive control. The antibiotic was reconstituted by dissolving 50 mg of powder in 500 ml DMSO to get a concentration of 0.1 mg/ml [17]. The plates were then left at room temperature for 30-60 minutes for proper diffusion and incubated at 37^{0}C for 24 hours. Plates were observed for zone of inhibitions (zones showing no microbial growth) around the discs after the incubation period and then the resulting diameters of zones of inhibition were measured using a ruler. Then zones of inhibition of the tested bacterial test pathogens were recorded by subtracting diameter of disc (6 mm) from final zone of inhibition. All plates were prepared in three replicates.

Determination of Antifungal Activity

The antifungal activities of the crude extracts against *A. flavus* and *A. niger* were similarly conducted using disc diffusion method. Sterile filter paper discs (6 mm in diameter) were impregnated with 50 µl of each plant extract (at a concentration of 1000 mg/ml) and allowed to dry for 30 minutes. Five milliliters of a culture suspension with a concentration of 10^6 spores/ml was added to 250 ml of PDA medium which had been previously cooled down to 45°C-50°C [14]. The medium was then transferred into petriplates and allowed to solidify. The impregnated discs were placed with sterile forceps aseptically on the surface of the pre-inoculated PDA medium. Paper discs that were handled in similar fashion but without plant extract (DMSO) served as negative control and paper discs impregnated with commercial fungicide, Tilt (at the concentration of 0.1 mg/ml), served as a positive control. The plates were incubated at 27^{0}C for 4 days to determine zones of inhibition of tested fungal pathogen by subtracting diameter of disc (6 mm) from final zone of inhibition. All plates were prepared in three replicates.

Determination of Minimum Inhibitory Concentration (MIC)

MIC against Bacterial Test Pathogens

The solvent extracts of the selected plants that showed antimicrobial activity with disc diffusion were selected for determination of MIC using broth dilution method. Two fold serial dilution was used to dilute the initial concentration of the plant extract (200mg/ml) by transferring 1ml from the plant extract (stock) solution into 1ml of sterile nutrient broth kept in a vial and mixing the contents thoroughly and then serially diluting in 3 other vials. The serial dilutions gave concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml [18]. After obtaining different concentrations of the extract, each concentration was inoculated with 0.02 ml of the standardized bacterial cell suspension and incubated at 37ºC for 24 hours. The growth of the bacterial test pathogens was assessed by comparing turbidity or cloudiness of broth containing test tubes with the negative control. The lowest concentration, at which there was no turbidity, was considered as the Minimum Inhibitory Concentration (MIC) value of the extract.

MIC against Fungal Test Pathogens

The MIC of the crude extracts against fungal test pathogens was similarly determined by the broth dilution method. The initial concentration of the plant extract (1000mg/ml) was diluted using double fold serial dilution by transferring 2ml from plant extract (stock) solution into 2ml of sterile potato dextrose broth and mixing the contents in a vial and then serially diluting in 3 other vials. The serial dilutions gave concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml. Each vial was inoculated with 0.02ml of the standardized fungal spore suspension and incubated at 27ºC for 72 hours [18]. The growth of the fungal test pathogens was assessed by comparing turbidity or cloudiness of broth containing test tubes with the negative control. The lowest concentration, at which there was no turbidity, was considered as the Minimum Inhibitory Concentration (MIC) value of the extract.

Data Analysis

All the experiments were carried out in triplicates. Data were analyzed using computer software SPSS, version 20. All results of the antimicrobial activity studies were expressed as mean values of zone of inhibition ± standard error of mean. Statistical tests were also done using analysis of variance (one way ANOVA) post-hoc test at $p<0.05$ coupled with least significant difference (LSD) to compare results. All data were analyzed at 95% degree of confidence ($\alpha = 0.05$) and $p<0.05$ values were considered to indicate statistically significant difference.

RESULTS AND DISCUSSION

Antimicrobial Activities of the Crude Extracts

A total of 6 crude extracts (ethanol, methanol and water extracts) prepared from all selected plants and the antibiotics used as control were tested for anti-microbial activities against the test organisms (Table 1-2).

Antimicrobial Activities of *C. macrostachyus* Against Test Pathogens

The leave extracts of *C. macrostachyus* were tested for their antimicrobial properties against the test pathogens. As can be seen in Table 2, the diameters of the zones of inhibition ranged from 4.0 -21.50 mm for methanol and from 5.76 -17.43mm for ethanol crude extracts of leave of *C. macrostachyus.* Water-based extracts had antimicrobial activity only against *A. niger* with zone of inhibition 13.38±0.198 mm. The diameters of zones of inhibition observed due to the antibiotics on the tested bacteria and fungus ranged from 18.67 - 47.67 mm and 29.5 -38.5 mm for Chloroamphenicol and Tilt respectively. The negative control used here, DMSO, showed no inhibition against all the bacterial and fungal test pathogen.

The ethanol extract of *C. macrostachyus* showed maximum zone of inhibition against *S. aurous* (17.43±0.23 mm) and minimum zone of inhibition against *E.coli* (5.76±0.151 mm). Methanol crude extracts of *C. macrostachyus* showed maximum zone of inhibition against *S. aureus* (21.50±0.29 mm) and minimum zone of inhibition against *E.coli* (4.0±0.00 mm).

Table 1 also shows that the ethanol extracts of *C. macrostachyus* show that the zones of inhibition for *S.agalactae* and S. *aureus* were significantly higher ($p<0.05$) than those observed against *E. coli* and *S. typhi*. However, the ethanol extracts of *C. macrostachyus* didn't show significant antibacterial activities against *E. coli* and *S. typhi*. Water extract of *Croton macrostachyus* didn't show significant antibacterial activities against all bacterial pathogen. Similarly, the ethanol extracts of *C. macrostachyus* show that the zones of inhibition against *A. niger* were significantly higher than ($p<0.05$) methanol and water extracts of the same plant at the same concentration.

Table 1: Antimicrobial activities of crude extracts of *C. macrostachyus* and standard antibiotics against test pathogens (mean ± SEM, n = 3)

	Zone of Inhibition (mm)							
Test pathogen	**EtCE**		**MtCE**		**WCE**		**Chl**	**Tilt**
	500mg/ml	**1000mg/ml**	**500mg/ml**	**1000mg/ ml**	**500mg/ml**	**1000mg/ml**	**0.1mg/ml**	**0.1mg/ml**
S.agalactiae	16.43±0.23[Bb]	ND	19.78±0.17[Ba]	ND	0.00±0.00	ND	33.0±1.53	ND
E.coli	5.76±0.15[Ca]	ND	4.0±0.0[Db]	ND	0.00±0.00	ND	18.67±1.45	ND
S. typhi	6.17±0.61[Cb]	ND	9.5±0.23[Ca]	ND	0.00±0.00	ND	36.0±1.15	ND
S. aureus	17.43±0.23[Ab]	ND	21.50±0.29[Aa]	ND	0.00±0.00	ND	47.67±1.33	ND
A. niger	ND	15.5±0.29[a]	ND	9.56±0.23[c]	ND	13.38±0.198[b]	ND	38.5±0.29
A. flavus	ND	0.00±0.00	ND	0.00±0.00	ND	0.00±0.00	ND	29.5±0.29

Key: EtCE = Ethanol crude extract, MtCE = Methanol crude extract, WCE = Water crude extract, Chl = Chloramphenicol, n = number of experimental replicates, SEM = standard error of the mean, ND = Not determined, means with the same lower case superscript letters in the same row are not significantly different; means with the same upper case superscript letters in the same column are not significantly different (p<0.05)

Antimicrobial Activities of *V. amygdalina* against Test Pathogens

The *in vitro* antimicrobial activities of the ethanol, methanol and water crude extracts of the leaves of *V. amygdalina* at concentrations of 500 mg/ml were evaluated against the bacterial test pathogen. As indicated in Table 3, the zones of inhibitions of the ethanol, methanol and water crude extracts of *V. amygdalina* were in the range of 17.67 - 7.83 mm, 14.0 - 5.0 mm and 13.0 – 4.24 mm, respectively. Among the crude extracts, the ethanol leaf extract of *V. amygdalina* showed maximum zone of inhibition against *S. aureus* (17.67±0.67 mm) and minimum zone of inhibition against *E. coli* (7.83±0.73 mm). The methanol leaf extract of *V. amygdalina* showed maximum zone of inhibition against *S. typhi* (14.0±1.15mm) and minimum zone of inhibition *E. coli* (5.0±0.58 mm).

From the two fungal species, *A. niger* was the most sensitive organisms to ethanol and water crude extract of *V. amygdalina* with the zone of inhibition of 14.26±0.37and 13.0±0.57 respectively at concentrations of 1000 mg/ml while the methanol extracts of *V. amygdalina* showed no activity against *A. niger*. *A. flavus* was resistant to the extracts obtained using all three types of crude extracts as shown in Table 2.

Table 2: Antimicrobial activities of crude extracts of *V. amygdalina* and standard antibiotics against test pathogens (mean ± SEM, n = 3)

	Zone of Inhibition (mm)							
Test pathogen	**EtCE**		**MtCE**		**WCE**		**Chl**	**Tilt**
	500mg/ml	**1000mg/ml**	**500mg/ml**	**1000mg/ml**	**500mg/ml**	**1000mg/ml**	**0.1mg/ml**	**0.1mg/ml**
S.agalactiae	14.3±0.33[Ba]	ND	9.0±0.58[Bb]	ND	4.24±0.145[Bc]	ND	33.0±1.53	ND
E.coli	7.83±0.73[Ca]	ND	5.0±0.58[Cb]	ND	0.00±0.00[Cc]	ND	18.67±1.45	ND
S. typhi	8.33±0.67[Cb]	ND	14.0±1.15[Aa]	ND	0.00±0.00[Cc]	ND	36.0±1.15	ND
S. aureus	17.67±0.67[Aa]	ND	6.67±0.67[Cb]	ND	4.67±0.33[Bc]	ND	47.67±1.33	ND
A. niger	ND	14.26±0.37[a]	ND	0.00±0.00	ND	13.0±0.57[b]	ND	38.5±0.29
A. flavus	ND	0.00±0.00	ND	0.00±0.00	ND	0.00±0.00	ND	29.5±0.29

Key: EtCE = Ethanol crude extract, MtCE = Methanol crude extract, WCE = Water crude extract, Chl = Chloramphenicol, n = number of experimental replicates, SEM = standard error of the mean, ND = Not determined, means with the same lower case superscript letters in the same row are not significantly different; means with the same upper case superscript letters in the same column are not significantly different (p<0.05)

Minimum Inhibitory Concentration (MIC) of the Crude Extracts

The minimum inhibitory concentration (MIC) assay was used to determine the efficacy of the extracts that showed antimicrobial activities in the previous tests. The data revealed that the MIC of ethanol, water and methanol crude extracts of plants against the tested bacterial pathogens with the interval of from 25 to 100 mg/ml. At 25 mg/ml, methanol extracts (against *S. aureus* and *S. agalactiae*) and ethanol extract of *C. macrostachyu* (against *S. aureus)* and ethanol extract of *V. amygdalina* (against *S. aureus)* shared the least MIC. For fungal pathogen MIC values also determine using broth dilution method. The data revealed that the MIC of ethanol, water and methanol crude extracts of plant against the tested fungal pathogen ranged from 250 to 500 mg/ml (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of plant extracts against bacterial and fungal test pathogens

Test pathogen	**EECM**	**MECM**	**WECM**	**EEVA**	**MEVA**	**WEVA**
S. agalactiae	50	25	_	50	100	100
E. coli	100	100	_	100	100	_
S. typhi	100	50	_	100	50	_
S. aureus	25	25	_	25	50	100
A. niger	250	500	500	500	_	500

Key: - =not tested, EECM= Ethanol extract of *C. macrostachyus*, MECM = Methanol extract of *C. macrostachyus*, WECM = Water extract of *C. macrostachyus*, EEVA = Ethanol extract of *V. amygdalina*, MEVE = Methanol extract of *V. amygdalina,* WEVA = Water extract of *V. amygdalina,* MIC = Minimum Inhibitory Concentration.

DISCUSSION

The study revealed that ethanol and methanol extract of *C. macrostachyus* significantly inhibited the growth all test bacteria. However, their inhibition potential and pattern is not the same which could be due to the difference in bacterial strain and the type of solvent used in plant part extraction. The ethanol extract of *C. macrostachyus* showed maximum antibacterial activity against *S. aurous* and lowest activity against *E.coli*. Methanol crude extracts of *C. macrostachyus* showed antibacterial activity against *S. aureus* and lowest activity against *E.coli*. The difference in their antibacterial activities of extracts against *E.coli* and *S. aureus* could be due to the difference in the composition of outer membrane of both organisms. Gram-negative bacteria contain high permeable barrier for numerous antibiotic molecules which could also be similar for these extracts. Furthermore, the periplasmic space of these bacteria comprises enzymes, which have the capability to break down foreign molecules [19] and appeared to be less vulnerable to plant extracts than gram positive bacteria. However, Gram-positive bacteria do not posses such outer membrane and cell wall structure [20]. Hence, this in agreement with the result obtained in the present study in which most of the extracts showed lower activity against *S. typhi* and *E. coli* than that of *S. aureus* and *S.agalactiae*.

In this study, methanol and ethanol extract of *C.macrostachyus* leaves showed maximum inhibition zone against *S. aurous* and minimum inhibition zones against *E. coli*. A study elsewhere by Wagaw *et al.* [19] found that ethanol and methanol extract of *C.macrostachyus* leave showed maximum inhibition zone against *S. aurous* and minimum inhibition zones against *E. coli*. A study conducted by Jackie *et al.* [21] evaluated that the methanol extracts showed active inhibition against *E. coli* (9.0±1.1mm) and very low activity against *S. typhi* (2.3 ± 1.87mm). However, this report has great variation with the present study, in which methanol extract of *C.macrostachyus* showed 4.0±0.00 and 9.50±0.23 mm inhibition zone against *E. coli* and *S. typhi* respectively. The possible reason for discrepancy observed between the present study and other studies similar could be the difference in concentration of methanol used. In the studies elsewhere, 80% methanol was used whereas in the present study 99.8% methanol was used for extraction. Hence, this could have been influenced the result since they have differences in the polarity and concentrations of methanol [22]. The other difference could be emanated from the plant part used in the experiment. In the previous studies, steam bark was used while in the present study leave was used. So this plant parts may have difference in distribution of the active ingredient.

The water extracts of *C.macrostachyus* showed no activity against all the tested bacterial pathogen. In contrary to this study, Getachew [23] reported that water extracts of *C. macrostachyus* leaves were found to have antibacterial activity against *S. aureus* and *E. coli* with the zone of inhibition of 18.67 ± 1.75 and 22.00 ± 2.10 mm respectively. The difference of the results encountered might be related to factors like the site of plant material collection, the season and time of harvest, type of soil, storage conditions, and method of preparation. The present study revealed that the nature of the micro-organism had effect on the antimicrobial activities of the plant extracts. The data generally suggested that *A. niger* was more sensitive than *A. flavus* to all types of crude extracts obtained from *C. macrostachyus*. The ethanol, methanol and water crude extract of *C. macrostachyus* resulted in inhibition zones of 15.5±0.29, 9.56±0.23 and 13.38±0.198 mm respectively, on *A. niger* while no zone of inhibition was detectable on *A. flavus* at the same concentration of all three types of crude extracts.

Crude extracts obtained using ethanol and methanol extracts of *V. amygdalina* showed higher inhibition zone than water crude extracts. This could be due to the higher volatility nature of ethanol and methanol which have higher tendency to extract more active compounds from the plant parts than water [24]. The ethanol leaf extract of *V. amygdalina* showed maximum zone of inhibition against *S. aureus* and minimum zone of inhibition against *E. coli*. A study conducted by Enyi-Idoh *et al.* [25] also showed that the ethanol extract of *V. amygdalina* leave had maximum zone of inhibition (16 mm) at 500mg/ml, a finding which is in agreement with the present study. This may be due to ability of ethanol to extract bioactive compounds like tannis, saponnins, flavonoid, alkaloid, anthraquinone, phenol and steroid which have higher concentrations in *V. amygdalina* [26]. In the study of Alo *et al*. [27] the ethanol, methanol and water extract of *V. amygdalina* antibacterial activities against *E. coli, S. typhi* and *K. pneumonia* was studied using disc diffusion method. And it was found that, the ethanol extract showed inhibition against *S. typhi* and *E. coli* with inhibition zone diameter of 13 mm and 23 mm respectively. The methanol extract of *V. amygdalina* leave also inhibited the growth of *S. typhi* and *E. coli* with the zone of inhibition of 20 mm and 23 mm respectively which in agreement with the present study.

The water leaf extract of *V. amygdalina* showed zone of inhibition against *S. aureus* and *S.agalactiae* but did not showed zone of inhibition against *E.coli* and *S. typhi*. The study conducted by Adetunji *et a*l. [28] antibacterial activity for water crude extract of leaf of *V. amygdalina* was analyzed against three bacteria strains (*P. aeruginosa, S. aureus* and *E. coli*). The maximum antibacterial activities were observed against *S. aureus* at a concentration of 200mg/ml, which is much in agreement with the present study. Alo *et al*. [27] also reported water extract of *V. amygdalina* could not inhibit the growth of *S. typhi*. In the study conducted by Uzoigwe and Agwa [29] the ethanol and water crude extract of leaf and steam of *Vernonia amygdalina* was studied for their antimicrobial activities against urinary tract pathogens *(Klebsiella sp, Staphylococcus sp. E. coli,)* using agar well diffusion method. The study revealed that, ethanol leaf extracts were found to have higher zones of inhibition than the stem extracts but these extracts were merely effective against *Klebsiella* sp while *E. coli* and *Staphylococcus* sp showed no vulnerability to both leaf and stem extracts. However, in the present studies reported that, antimicrobial activity of ethanol crude extract of *V. amygdalina* leaf on standard strains of *E. coli* ATCC25922 and *S. aureus* ATCC 25923 has effective activity with inhibition zone 7.83 mm and 17.67 mm respectively. The discrepancies with the present result study could be as a result of *S. aureus* and *E. coli* standard strain differences [29]. Uzoigwe and Agwa [29], also showed that the water extract of both leaf and stem extracts were not foud to be effective against any test isolates (*Klebsiella sp, Staphylococcus sp. E. coli*) even at higher concentrations (20 mg/ml).This work was similar with the present study, in which water extract of *V. amygdalina* leaf showed no inhibition activity against *E. coli.*

Generally, the plant extracts obtained using organic solvents (ethanol and methanol) showed better results than water extracts. This could be due to their capability to efficiently degrade the cell wall which has non-polar character. Moreover, alcohols were found to be easier to pass the cellular membrane to extract the intracellular contents from the plant material and the existence of non-polar residues in the extracts, which had higher antibacterial activities. The present results also showed that different plant assayed possess different levels of antimicrobial activities. Among the extracts of the two different types of plants tested against the four bacterial pathogens, the leave extracts of *C. macrostachyu* showed higher range of antibacterial activities than the leave extracts of *V. amygdalina*. When comparing tested bacteria, *S. aureus* and *S.agalactiae* was highly susceptible bacterial strain, while *E. coli* and *S. typhi* were less sensitive. Similarly, *A. flavus* was resistant fungal pathogens for all crude extract of selected plants. The possible reason for the difference in the activity of these plant extracts could be the difference in type of plant species or bioactive compounds which could not be found uniformly among different plants, nature of the micro-organism and potency of extracting solvent.

The methanol extract *C. macrostachyus* was found to have lower MIC value of 25mg/ml than ethanol extract of *C. macrostachyus* (50 mg/ml) against *S. agalactiae.* In one study, methanol extracts of leaves and roots of *C. macrostachyus* were active against *E. coli* having MIC of 250 mg/m [30]. The relatively low MIC values recorded for the *C. macrostachyus* extracts against the test pathogens verify the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. The higher MIC observed for *E. coli* and *S. typhi* in this study could be due to increased

resistance to some of the bioactive ingredients in the plant. MIC is important because populations of bacteria and fungus exposed to an insufficient concentration of the extract can develop resistance to antimicrobial agents. The high activity of antimicrobial agents at low concentrations is very crucial in search of new drugs for chemotherapeutic as their toxicity is believed to be low to patients administered with such agents.

CONCLUSION

The findings of this study revealed that*, V. amygdalina* and *C. macrostachyu* exhibited significant antimicrobial effect by the crude extracts against the four bacterial pathogen (*E. coli, S. aureus*, *S. agalactiae* and *S. typhi*) and two fungal pathogen (*A. niger* and *A. flavus*) which is an indication for the presence of antimicrobial agents in it. The three solvents employed for the extraction process i.e., water, methanol and ethanol have showed different power in their extraction efficiency which could be due to their difference in polarity. The traditional practitioners use mainly water as the solvent but in this study found that the plant extracts by alcohol (ethanol and methanol) provided more consistent antimicrobial activity compared to those extracted by water. Generally, the result of the present study confirmed the traditional use of these plants against various microbial infections by the people in Ethiopia.

Conflict of Interest

The author declares no conflict of competing interest.

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