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In-Vitro Antimicrobial Activities and Phytochemical Screening of *Calotropis Procera* (Ait) and *Vernonia Amygdalina* (Del.) Extracts Against Some Medically Important Pathogenic Bacteria

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Abstract: The aim of this study is to evaluate phytochemical screening and antibacterial activities of crude extracts obtained from different parts of *Calotropis procera* and *Vernonia amygdalina* against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The stems, roots and leaves of the selected plant species were shade dried and ground to powders and the bioactive components were extracted using ethanol (99.5%), methanol (99.8%), hexane (99.8%) and distilled water. The antibacterial activities of the resulting extracts against the three selected pathogens were evaluated using paper disc method and inhibitory zones were recorded in millimeters at five different concentrations (20, 30, 40, 50 and 60 mg/ml). Agar dilution method is used to determine minimum inhibitory concentrations (MICs) of the plant extracts against selected pathogens. Chloramphenicol and DMSO used as positive and negative controls, respectively. The bioassay results revealed that the crude extracts of ethanol, methanol, hexane and water had antibacterial activities on all three bacterial species at all concentration except at 20 mg/ml of all solvent extracts. Methanol and ethanol extracts had the highest growth inhibitory effects as compared with those of the aqueous and hexane crude extracts. However, the four solvent crude extracts had less antibacterial activities than chloramphenicol. *S. aureus* found to be the most susceptible pathogen to the crude ethanol (99.5%) and methanol (99.8) extracts of the leaves of *Vernonia amygdalina* (22 mg/ml) and ethanol extract of the leaves of *Calotropis procera* (22 mg/ml). Whereas *Pseudomonas auruginosa* was the least susceptible bacterium to crude ethanol extract (99.5%) of the root of *Calotropis procera* at 28 mg/ml and crude water, extract of the root of *Vernonia amygdalina* at 28 mg/ml. The growth inhibitory activities of the crude extracts were found to be significantly different for the four concentrations (30, 40, 50 and 60mg/ml) in both plant parts ($p < 0.05$). Phytochemical screening were done and the following bioactive components are detected such as Tannins, Phenolics, Resins, Amino acids, Flavonoids, Saponins, Reducing sugar, Glycosides, Steroids, Triterpenoids, Anthocyanidins, Sterol and Volatile Oil. In conclusion, this study did not only show the antibacterial activities of *Calotropis procera* and *Vernonia amygdalina*, but also offered a scientific validation for its traditional use against some diseases. There is a need for conducting more studies to identify and characterize the medicinal properties in the tested plant, which may serve as novel compounds for the development of new and more effective antimicrobial drugs.

Keywords: Phytochemical Screening, Paper disc Diffusion, Antibacterial Activities, Minimum Inhibitory Concentration, C. Procera, V. Amaygdalina

1. Introduction

Plants have been used for generations as source of medicine in the treatment of a variety of human illnesses. In Ethiopia, many plants are being used for this purpose by traditional healers without any scientific justification for their therapeutic values. Traditional healers are successfully used plants and plant products as effective therapeutic tools for fighting against diseases and various other health hazards [1]. The local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa [2]. Among non-allopathic system of therapies, traditional medicines propounds in rural and sub-rural area. These therapies has catched eyes worldwide for their efficacies, low price and safer modus operandi [59]. Many research efforts directed towards the provision of empirical proofs to back up the use of plant species in trade and medicinal practices in recent years [3]. Ethiopian medicinal plants are shown to be very effective against some ailments of human and domestic animals [4]. In vitro-antimicrobial potential of some specific plants has been carried out by many workers such as *Thuja orientalis* [60]. There are two common species of *Calotropis: Calotropis gigantea* (Linn) and *Calotropis procera* (Ait) [5]. *Calotropis procera* belongs to the family *Aselepiadaceae*. It is a shrub or small tree 2–4 m tall, with distinctive grey green waxy leaves. According to [6], the stems of this plant species are grey green, smooth, somewhat crooked and covered with a soft, thick, corky bark. The plant often branches at its base. When cut or broken the plant exudes a milky, sticky sap (latex). *C. procera* is used medicinally to treat boils, infected wounds and other skin problems in people and to treat parasitic skin infestations in animals [7]. Antimicrobial evaluation of ethno-medicinal plants from Rajasthan against antibiotic resistant pathogenic bacteria reveals the efficacy of indigenous sources to safe guard from the microbial [61, 62]. *Vernonia amygdalina (aka.* bitter leaf*)* is another medicinal plant commonly found in Ethiopia, [8]. The leaves are dark green colored with a characteristic odor and a bitter taste. *Vernonia amygdalina* is a tropical plant belonging to the family *Compositae* and widely as vegetable and medicinal plant. It is a shrub of about 2 to 5m with a petiolate leaf of about 6 mm in diameter and elliptic shape. The leaves are green with a characteristic odor and bitter taste. It does not produce seeds and has to be propagated through cutting [9]. According to Argheore, almost all parts of *Vernonia amygdalina* are pharmacologically useful in that both the root and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others [10]. Bacterial infections are one of the major attributes of delayed wound healing in chronic wounds and resistance to pathogenic bacteria is one of the deadliest challenges in the modern clinical operative procedures. Current usages of synthetic attributes have led to the series of cidal side effects and dispersive pharmacological lacuna [62].

Calotropis procera and *Vernonia amygdalina* are widely used traditional medicinal plants for the treatment of various ailments. The therapeutic effectiveness of these medicinal plants, however, varies with the geographical location, altitude and climate and soil type from where the plants are growing. The anti-bacterial potentials of *Calotropis procera* and *Vernonia amygdalina* crude extracts obtained using different solvents were not studied *in vitro* in Eastern Hararghe.

The General objective.

The general objective of this study was to determine *in vitro* antimicrobial activity and phytochemical screening of crude extracts of *Calotropis procera* and *Vernonia amygdalina extracted by* using different solvents against *E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*

The Specific objectives

(1) To determine the yield of crude extracts from leaf, root and stem bark extracts from *C. procera* and *V. amygdalina* using aqueous and organic solvents (hexane, methane and ethanol)

(2) To assess the anti-micro*bial activ*ities of the leaf, root and stem bark extracts of *C. procera* and *V. amygdalina* against three test pathogens (*Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus).*

(3) To determine the minimum inhibitory concentrations (MICs) of the extracts against different test pathogens (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus)*

(4) To assess Phytochemical characteristics of the leaf stem bark and root extracts of *Calotropis procera* and *vernonia amygdalina*

2. Materials and Methods

2.1. Experimental Design

The research was designed based on the laboratory chemical analysis in a commercial Chemical relational database (CRD). The treatments were include two plants with three parts of each, extracted by four solvents and three test pathogens in three replications (2x3x4x3x3). DMSO was used as negative control and chloramphenicol used as positive control.

2.2. Sample Collection and Identification of Plant Materials

The leaves, roots and stem barks of *Calotropis procera* (Sodom apple or *Tobiaw*) and *Vernonia amygdalina* (bitter leaf or *grawa*), were randomly collected from Haramaya University and Dire dawa. Healthy leaves and barks from young twigs and roots of *Calotropis procera* (Sodom apple) were collected from Dire Dawa, eastern, Ethiopia. Similarly, healthy leaves and stem barks from young twigs and roots of *Vernonia amygdalina* (bitter leaf) were collected from Haramaya university main campus. The plant materials were identified and authenticated at Haramaya university herbarium with the help of a plant taxonomist.

2.3. Preparation of Crude Extracts from C. Procera and V. Amygdalin

The collected plant parts of *C. procera* and *V. amygdalina* were separately washed thoroughly 3 times with tap water followed by once with sterile distilled water to remove debris and dust particles and cut into smaller sizes using a sterile knife. Then the leaves, roots and stem barks of both plants were dried under shade on a paper towel for two weeks with occasional shifting at room temperature. The resulting dry parts were ground into fine powder with the help of suitable sterile grinder. Then it stored in sterile airtight containers according to [11].

2.3.1. Preparation of Crude Extracts Using Methanol, Ethanol and Hexane

Twenty grams (20.00g) of each of the coarsely powdered plant materials (leaves, roots and stem barks) of *C. procera and V. amygdalina* were suspended in 100ml of different solvents, i.e., methanol (99.8%), ethanol (99.5%) and hexane (99.8%), separately in 250 ml conical flasks. As indicated by Cheesbrough the suspended plant materials were kept on a rotary shaker rotating at 190-220 rpm for 72 hrs at room temperature [12-13]. Muslin cloth was used to filter the plant residue, the filtrate thus obtained was further purified by filtration through Whatman No.1 sterile filter paper, and the resulting filtrates were collected as sources of crude extracts. The filtrates were concentrated under reduced pressure in Rota vapor (STERILIN. Ltd., Stone Staffordshire, England) at 40°C and the gummy residue was further dried in a water bath at 40°C – 50°C for 24 hrs until the solvents were removed [14]. After the evaporation of solvents, the remaining crude extracts were weighed using a balance and the resulting weights recorded. These crude extracts were kept in sample vials with stoppers at 4°C until they were used against the test pathogens [15].

2.3.2. Preparation of Aqueous Extracts

Crude extracts of leaf, stem bark and root of both plants were prepared by adding 100 ml of sterile distilled water to 20.00 g of coarsely powdered plant materials in 250 ml conical flask. The resulting suspension was then shaken at 121 rpm for 24 hrs using a shaker to produce the required infusion. Muslin cloth was then used to filter the plant residue. The filtrate obtained was further purified by filtration through Whatman No.1 sterile filter paper. Then the solution was subjected to hot air water bath evaporation at 35°C, for one week the remaining crude extracts were weighed and diluted with 10 ml of sterile distilled water and then, the crude extracts were preserved in airtight bottles until further use in refrigerator [16].

2.4. Percentage Yield Determination of Crude Extracts

The percentage yield obtained from the plant parts was the amount of the crude extract recovered in mass compared with the initial amount of powdered plant materials used. It is presented in percentage (%) and was determined for each extraction solvent used. The percentage yield was calculated as followes;

$$\%\text{yield(W/W)} = \frac{\text{Extract obtained (in g)}}{\text{Grounded plant sample taken (in g)}} \times 100$$

2.5. Sterility Test of the Plant Extracts, Materials and Source of Test Pathogens

The extracts were tested for sterility by introducing 2 ml of the sterile extract into 10 ml of sterile nutrient broth. This was incubated at 37°C for 24 hrs. The sterile extracts were indicated by absence of turbidity or clearness of the broth after the incubation period. Absence of growth in the extracts after incubation indicated that the extracts were sterile [17]. Then the extracts were assayed for antimicrobial activities.

All materials used was be sterilized. Glasses were washed with detergents, rinsed properly with tap water and dried. They were then sterilized in the oven at 160°C for 2 hrs. Inoculating loops were heated to redness in an open flame. All the media such as 5% Sheep Red Blood Agar, Nutrient Agar and Mueller Hinton Agar, distilled water and McCartney bottles used were sterilized in the autoclave at 121°C and 15 psi for 15 minutes. In addition, the laboratory bench was always swabbed with 70% alcohol before and after each round of experiment [18].

The selected bacterial pathogens were obtained from Ethiopian Public Health Institution (EPHI). Three species of pathogenic bacteria were used in this study. Two species from those that infect the skin (*Staphylococcus aureus* ATCC 29223 and *Pseudomonas aeruginosa* ATCC 27853) and one species (*Escherichia coli* ATCC 25922) from among the enteric bacterial species were used as test organisms in this study.

2.6. Preparation of Culture Media

Red blood agar, Nutrient agar, and Mueller-Hinton agar were prepared according to the manufacturer's instruction. All media were first autoclaved at 121°C and 15 psi for 15 minutes before cultured bacteria.

2.7. Inoculum Preparation and Disk Diffusion Assay

All bacterial cultures were first grown on 5% sheep red blood agar plates at 37°C for 18 hrs prior to inoculation onto the nutrient agar. Few colonies (4–5) of similar morphology of the respective bacterial species were transferred with a sterile inoculating loop to a liquid medium and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained [16].

The 0.5 McFarland turbidity standard was prepared by adding 0.5 ml of a 1.175% (w/v) barium chloride dehydrate (BaCl2 2H20) solution to 99.5 ml of 1% (v/v) sulfuric acid (H2SO4). This mix was considered equivalent to cell density of 1 to 2 x 108 cfu/ml. The turbidity standard is then aliquoted into test tubes identical to those used to prepare the inoculums suspension. McFarland turbidity standard tubes were Seal with Parafilm, to prevent evaporation. Barium sulfate turbidity was compared with identical tubes containing inocula 0.85% NaCl saline solution.

Inoculum of the respective bacterial species were spread on to MHA plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth obtained following incubation. Sterile filter paper discs of 6.0 mm in diameter were cut and soaked with 100 µl of each plant extract at a concentration of 20, 30, 40, 50 and 60 mg/ml. The paper discs were then aseptically placed on Mueller–Hinton Agar (MHA) plates, inoculated with dense inoculums suspension of the test pathogens and the plates were then allowed to stay for 1–2 hrs for pre-diffusion of the extracts at room temperature [19]. Finally, the plates were incubated at 37°C for 18–24 hrs. At the end of the incubation period, the diameter of the zone of inhibition was measured using a sliding caliper [20]. The negative control also received the same amount (100µl) of DMSO. All tests were done in three replications. The inhibition zones were observed after 24 hrs of growth at 37°C.

The antibiotic chloramphenicol, which was purchased at a pharmaceutical store at Harar, Ethiopia, was used as a positive control at a concentration of 0.1 mg/ml, with an equal amount as those of the extracts (100µl), DMSO (100 µl) was used as a negative control [21].

2.8. Determination of Minimum Inhibitory Concentration (MIC)

The agar dilution method was used to determine the MIC for the crude extracts. The ethanol, methanol, hexane and aqueous extracts of the different plant parts (stem bark, root and leaves) of *C. procera* and *V. amygdalina* that showed significant antimicrobial activities in the previous test were selected for determination of MIC [22]. The Mueller-Hinton agar media were first prepared as described in section 2.8 and sterilized by autoclaving. The sterilized media were allowed to cool at 50°C and 18 ml of molten agar was added to test tubes, which contained 2 ml of different concentrations of the crude extracts. The mixture of the media (molten agar and crude extracts) and the test drugs were thoroughly mixed and poured into pre-labeled sterile Petri-dishes on a level surface. Additional Petri-dishes containing only the growth media were prepared in the same way for comparison of the growth of the respective organisms. The concentrations of the extracts used in this test were 22, 24, 26 and 28 mg/ml.

The plates were allowed to dry at room temperature. The suspensions of the respective pathogens whose densities were adjusted to 0.5 McFarland turbidity units (1.5×10^8 CFU/ml) were inoculated onto the series of agar plates using a standard inoculating loop. Three loopfuls of suspension were transferred into each plate. The plates were then incubated at 37°C for 24 hrs. The lowest concentration, which inhibited the growth of the respective organisms, was taken as MIC.

2.9. Phytochemical Screening of the Leaf Stem Bark and Root Extracts of Calotropis Procera and Vernonia Amygdalina

Phytochemical screening was done in order to detect the presence of plant constituents such as Tannins, Phenolics, Resins, Amino acids, Flavonoids, Saponins, Reducing sugar, Glycosides, Steroids, Triterpenoids, Anthocyanidins, Sterol and Volatile Oil using the methods described by Brain, K. R. and Tuner, T. D.[23].

2.9.1. Test for Reducing Sugars

Two grams of the extract was weighed and placed into a test tube. This was diluted using 20 ml of de-ionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40ºC in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethylsulphoroxide (DMSO) as the diluents for the ethanolic, methanol and hexane extracts [24].

2.9.2. Test for Resins

Three grams of the ethanolic, methanolic and hexane extract ware dissolved in 15ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple color, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material [25].

2.9.3. Test for Tannins

Two grams of the aqueous extract was weighed and placed in a test tube. Two drops of 5% ferric chloride solution was then added. The appearance of a dark green color was indicative of the presence of tannins. The same procedure was repeated using the ethanolic, methanolic and hexane extracts [25].

2.9.4. Test for Steroid

One gram of the ethanolic, methanoic and hexane extract was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet color in the supernatant layer were indicative of the presence of steroid glycosides. Same procedure was repeated using the aqueous extract [25].

2.9.5. Test for Flavonoids

Two grams of the ethanolic, methanoic and hexane extract was weighed, placed in a test tube, followed by the addition of 10 ml of DMSO. The mixture was heated, followed by the addition of magnesium metal and 6 drops of concentrated hydrochloric acid. The appearance of red color was indicative of the presence of flavonoids. Same procedure was repeated using aqueous extract [26].

2.9.6. Test for Alkaloids

One gram each of the ethanolic, methanoic and hexane extracts was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragendoff's reagent was added while 2-3 drops of Meyer's reagent were added to the

second test tube. The development of an orange-red precipitate (turbidity) in the first test tube (with Dragendoff's reagent) or white precipitate (turbidity) in the second test tube (with Meyer's reagent) was indicative of the presence of alkaloids. Same procedure was repeated using aqueous extract [25].

2.9.7. Test for Saponins

Five grams of the aqueous extract was weighed and placed in a test tube. This was followed by the addition of 5 ml de-ionised distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins. Same procedure was repeated using DMSO for the ethanolic, methanoloic and hexane extracts [24].

2.9.8. Test for Glycosides

To a volume of 3 ml of the ethanolic, methanoic, hexane and aqueous extracts, 2 ml of chloroform was added. Tetraoxiosulphate VI acid was carefully added to form a lower layer. A reddish brown color at interface indicated the presence of a steroidal ring.

2.9.9. Test for Phenolics

Two drops of 5% ferric chloride were added to 5 ml of the ethanol, mechanic, hexane and aqueous extracts in a test tube. A greenish precipitate was taken as indication of phenolics.

2.9.10. Test for Anthraquinones and Test for Terpenoids

0.5g of the both plant crude extracts were shaked with 10ml of aqueous H_2SO4 and then filtered while hot, the filtrate was shaked with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution was then added. The presence of violet or red colouration in the ammonical (lower) phase was taken as an indication of combined Anthraquinones.

About 0.2g both plant extracts were mixed with 2ml Chloroform and 3ml of concentrated H_2SO4 was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

2.10. Data Analysis

Mean values of zone of inhibitions were analyzed using ANOVA for significant difference with the help of SPSS version 20 statistical software package in Microsoft windows 7 operating system. The data were further subjected to Tukey's HSD analysis. All the experiments were carried out in triplicates. Data were expressed as mean ± standard deviation and statistical significance was calculated. Values corresponding to $P< 0.05$ were considered as statistically significant.

3. Results and Discussion

3.1. Percentage Yield of Crude Extracts

Methanol crude extracts, Hexane crude extracts, Ethanol crude extracts and Aqueous crude extracts of *V. amygdalina* and *C. procera* leaves, stem barks and roots were obtained from the extraction of 20.00 g powders of the plant parts using ethanol, methanol, hexane and aqueous extracting solvents, respectively, as shown in Table 1 and Table 2. The results clearly showed that the percentage yield of the crude extracts of the different plant parts of both plants varied from solvent to solvent. This could be attributed to the difference in polarity and extracting potential of methanol, ethanol, hexane and water. The highest percentage yield was observed in MCE of stem barks of *V. amygdalina.* This finding is in agreement with the results of Kesatebrhan [27]. As Cowan, reported, most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of methanol, ethanol and hexane as extracting solvent than water [28]. When the different parts of the two plants are compared for their yields, the methanol extracts of the leaves of *V. amygdalina* gave maximum yield and the aqueous extracts of the roots of *C. procera* gave minimum yield. This indicates that the bioactive ingredients are not found uniformly throughout the plants and that some plant parts tend to have more bioactive compounds than the others do [29].

Table 1. *The percentage yields of the crude extracts of the leaves, roots, and stem barks of Calotropis procera.*

Plant part	Weight and Percentage Yield of Crude Extracts by Extraction Solvents							
	Ethanol		Methanol		Hexane		Water	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Leaf	1.65	8.25^{cB}	2.42	12.10^{dB}	1.9	5.45^{aB}	0.53	2.65^{aB}
Stem bark	2.09	10.45^{cC}	2.75	13.75^{dC}	1.32	6.60^{bB}	0.59	2.95^{aB}
Root	1.03	5.15^{bA}	1.41	7.05^{cA}	1.12	5.60^{bA}	0.29	1.45^{aA}

Values with different superscripts in the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different in extraction potential.

Table 2. *The percentage yields of the crude extracts of the leaves, roots, and stem barks of Vernonia amygdalina.*

Plant part	Weight and Percentage Yield of Crude Extracts by Extraction Solvents							
	Ethanol		Methanol		Hexane		Water	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Leaf	2.71	13.55^{cB}	3.99	19.95^{dC}	1.25	6.25^{aA}	1.88	9.4^{bA}
Stem bark	2.75	13.75^{dB}	2.41	12.05^{cA}	1.5	7.50^{bB}	0.92	4.60^{aB}
Root	2.42	12.10^{cA}	2.57	12.85^{dB}	1.15	5.75^{bA}	1.01	5.05^{aB}

Values with different superscripts in the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different in extraction potential.

3.2. Antimicrobial Activities of Crude Extracts as Measured by the Paper Disc Method

In this study, the antimicrobial activities of the ethanol, methanol, hexane and aqueous crude extracts of the stems, roots and leaves of *C. procera* and *V. amygdalina* were evaluated using paper disc method. The inhibition zone formed following incubation was measured and the mean diameters were achieved. A total of 24 crude extracts (ethanol, methanol, hexane and aqueous) were prepared from both plants (*V. amygdalina* and *C. procera)* and antibiotics where tested for anti-microbial activities against the test organisms *(E. coli, S. aureus* and *P. aeruginosa)*. The anti-microbial activities of the different extracts of *V. amygdalina* and *C. procera* against the three bacterial species are presented in tables 6 - 11.

Table 3. *Antibacterial activities of crude extracts of the stem bark of C. procera against the test organisms (mean ± SD, n=3).*

Test organisms	Con. mg/ml	Zone of Inhibition(mm)				DMSO	Chloramphenicol (0.1 mg/ml)
	Crude extracts	EsE	MsE	HsE	WsE		
E.coli	20	-	-	-	-	-	
	30	16.66 ± 0.57^{Cd}	14.46 ± 0.50^{Ec}	9.50 ± 0.50^{Ab}	8.96 ± 0.90^{Aa}	-	26.90 ± 0.1^{A}
	40	17.21 ± 0.02^{Dd}	15.10 ± 0.64^{Fc}	10.42 ± 0.00^{Bb}	9.81 ± 0.33^{Ca}	-	
	50	21.33 ± 0.12^{Fd}	16.23 ± 0.32^{Gc}	12.30 ± 0.31^{Cb}	10.32 ± 0.42^{Da}	-	
	60	22.66 ± 0.57^{Gd}	18.80 ± 0.72^{Hc}	16.00 ± 0.28^{Db}	13.16 ± 0.28^{Fa}	-	
P. aeruginosa	20	-	-	-	-	-	
	30	12.70 ± 0.26^{Ac}	9.53 ± 0.50^{Ab}	16.66 ± 0.57^{Ed}	8.66 ± 0.57^{Aa}	-	26.50 ± 0.50^{A}
	40	14.41 ± 0.32^{Bc}	10.23 ± 0.11^{Bb}	18.45 ± 0.14^{Fd}	9.51 ± 0.67^{Ca}	-	
	50	19.12 ± 0.23^{Ec}	12.00 ± 0.00^{Cb}	20.55 ± 0.00^{Gd}	10.01 ± 0.66^{Da}	-	
	60	21.83 ± 0.76^{Fc}	13.50 ± 0.05^{Db}	23.50 ± 0.50^{Hd}	11.33 ± 0.58^{Ea}	-	
S. aureus	20	-	-	-	-	-	
	30	12.83 ± 0.76^{Ab}	14.56 ± 0.28^{Ec}	16.83 ± 0.76^{Ed}	9.00 ± 0.90^{Ba}	-	29.10 ± 10^{B}
	40	14.55 ± 0.12^{Bb}	16.00 ± 0.03^{Gc}	17.00 ± 0.00^{Ed}	11.50 ± 0.43^{Ea}	-	
	50	17.45 ± 0.22^{Db}	19.77 ± 0.93^{Id}	18.60 ± 0.65^{Fc}	13.10 ± 0.55^{Fa}	-	
	60	24.50 ± 0.50^{Hc}	21.00 ± 1.00^{Jb}	21.00 ± 1.00^{Gb}	14.00 ± 1.00^{Ga}	-	

Key: EsE = ethanol stem extract, MsE = methanol stem extract, HsE= Hexane stem extract, WsE= Water stem extract chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ($p<0.05$, 0 = no inhibition zone.

Table 4. *Antibacterial activities of crude extracts of the leaves of C. procera against the test organisms (mean ± SD, n=3).*

Test organisms	Con. mg/ml	Zone of Inhibition(mm)				DMSO	Chloramphenicol (0.1 mg/ml)
	Crude Extracts	ElE	MlE	HlE	WlE		
E. coli	20	-	-	-	-	-	
	30	13.80 ± 0.34^{Ad}	12.50 ± 0.50^{Bb}	8.43 ± 0.40^{Aa}	8.80 ± 0.72^{Da}	-	
	40	14.51 ± 0.06^{Bd}	13.60 ± 0.54^{Dc}	11.42 ± 0.01^{Db}	9.77 ± 0.22^{Ea}	-	26.90 ± 0.1^{A}
	50	15.43 ± 0.13^{Cc}	15.23 ± 0.12^{Fc}	13.60 ± 0.33^{Fb}	11.00 ± 0.32^{Fa}	-	
	60	17.43 ± 0.45^{Fd}	16.83 ± 0.76^{Hc}	15.83 ± 0.76^{Gb}	12.66 ± 0.57^{Ga}	-	
P. aeruginosa	20	-	-	-	-	-	
	30	16.66 ± 0.57^{Ed}	12.93 ± 0.90^{Bc}	10.20 ± 0.34^{Cb}	5.16 ± 0.57^{Aa}	-	
	40	17.41 ± 0.41^{Fd}	14.63 ± 0.14^{Ec}	13.00 ± 0.33^{Eb}	6.55 ± 0.77^{Ba}	-	
	50	18.98 ± 0.53^{Gc}	16.01 ± 0.22^{Gb}	16.00 ± 0.02^{Gb}	7.11 ± 0.26^{Ca}	-	26.50 ± 0.50^{A}
	60	21.50 ± 0.45^{Hd}	18.33 ± 0.57^{Ic}	17.83 ± 0.76^{Hb}	8.66 ± 0.57^{Da}	-	
S. aureus	20	-	-	-	-	-	
	30	14.53 ± 0.50^{Bc}	10.83 ± 0.76^{Ab}	8.50 ± 0.36^{Aa}	8.33 ± 0.57^{Da}	-	
	40	16.15 ± 0.44^{Dc}	11.01 ± 0.05^{Ab}	9.01 ± 0.05^{Ba}	9.40 ± 0.93^{Ea}	-	29.10 ± 0.17^{B}
	50	17.87 ± 0.66^{Fc}	13.07 ± 0.87^{Cb}	11.50 ± 0.76^{Da}	11.10 ± 0.65^{Fa}	-	
	60	19.00 ± 1.00^{Gc}	14.00 ± 0.00^{Db}	12.80 ± 0.72^{Ea}	12.66 ± 0.57^{Ga}	-	

Key: ElE = ethanol leaf extract, MlE =methanol leaf extract, HlE= Hexane leaf extract, WlE= Water leaf extract chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column(upper case) are significantly different ($p<0.05$), 0 = no inhibition zone.

Table 5. *Antibacterial activities of crude root extract of C. procera against the test organisms (mean ± SD, n=3).*

Test organisms	Con. mg/ml	Zone of Inhibition(mm)				DMSO	Chloramphenicol (0.1 mg/ml)
	Crude extracts	ErE	MrE	HrE	WrE		
E. coli	20	-	-	-	-	-	
	30	8.13 ± 0.31^{Ab}	10.50 ± 0.50^{Bc}	8.33 ± 0.41^{Bb}	6.50 ± 0.43^{Aa}	-	
	40	10.11 ± 0.00^{Bb}	13.60 ± 0.54^{Fc}	9.89 ± 0.58^{Db}	6.87 ± 0.44^{Aa}	-	26.90 ± 0.1^{A}
	50	11.33 ± 0.17^{Cb}	15.23 ± 0.12^{Hc}	11.70 ± 0.63^{Fb}	6.99 ± 0.52^{Aa}	-	
	60	13.06 ± 0.11^{Eb}	14.00 ± 0.00^{Gc}	12.83 ± 0.28^{Hb}	7.43 ± 0.40^{Ba}	-	

Test organisms	Con. mg/ml	Zone of Inhibition(mm)				DMSO	Chloramphenicol (0.1 mg/ml)
	Crude extracts	ErE	MrE	HrE	WrE		
P. aeruginosa	20	-	-	-	-	-	
	30	8.26 ± 0.27^{Ac}	9.53 ± 0.28^{Ad}	7.46 ± 0.45^{Ab}	6.73 ± 0.30^{Aa}	-	
	40	10.61 ± 0.74^{Bc}	11.33 ± 0.44^{Cd}	8.10 ± 0.11^{Bb}	6.85 ± 0.97^{Aa}	-	26.50 ± 0.50^{A}
	50	13.68 ± 0.93^{Fd}	13.01 ± 0.25^{Ec}	10.00 ± 0.05^{Db}	7.61 ± 0.76^{Ba}	-	
	60	14.23 ± 0.40^{Gc}	14.00 ± 0.00^{Gc}	11.50 ± 0.50^{Fb}	8.20 ± 0.20^{Ca}	-	
S. aureus	20	-	-	-	-	-	
	30	12.76 ± 0.68^{Dc}	12.40 ± 0.76^{Dc}	7.50 ± 0.45^{Ab}	6.93 ± 0.35^{Aa}	-	
	40	13.16 ± 0.47^{Ec}	14.01 ± 0.11^{Gd}	9.01 ± 0.00^{Cb}	7.40 ± 0.13^{Ba}	-	29.10 ± 0.17^{B}
	50	15.47 ± 0.16^{Hc}	16.34 ± 0.87^{Id}	10.55 ± 0.66^{Eb}	8.08 ± 0.62^{Ca}	-	
	60	16.33 ± 0.57^{Ic}	18.50 ± 0.45^{Jd}	12.23 ± 0.32^{Gb}	8.66 ± 0.41^{Da}	-	

Key: ErE = Ethanol root extract, MrE =Methanol root extract, HrE= Hexane root extract, WrE= Water root extract, chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ($p<0.05$), 0 = no inhibition zone.

Table 6. *Antibacterial activities of crude extracts of the stem bark of V. amygdalina against the test organisms (Mean ± SD, N=3).*

Test organisms	Con. g/ml	Zone of Inhibition(mm)				DMS0	Chloramphenicol (0.1 mg/ml)
	Crude extracts	EsE	MsE	HsE	WsE		
E.coli	20	-	-	-	-	-	
	30	14.56 ± 0.05^{Ed}	13.10 ± 0.45^{Cc}	10.86 ± 0.05^{Bb}	6.26 ± 0.46^{Aa}	-	26.90 ± 0.1^{A}
	40	17.34 ± 0.22^{Gd}	15.50 ± 0.34^{Dc}	11.40 ± 0.22^{Bb}	6.90 ± 0.24^{Ba}	-	
	50	21.23 ± 0.45^{Hd}	18.13 ± 0.32^{Ec}	13.10 ± 0.73^{Cb}	8.09 ± 0.22^{Ca}	-	
	60	24.40 ± 0.36^{Jd}	20.40 ± 0.10^{Fc}	14.70 ± 0.26^{Db}	9.16 ± 0.28^{Da}	-	
P.aeruginosa	20	-	-	-	-	-	
	30	8.00 ± 0.46^{Ab}	8.56 ± 0.45^{Ac}	7.43 ± 0.40^{Aa}	7.13 ± 0.47^{Ba}	-	
	40	8.60 ± 0.64^{Bc}	8.80 ± 0.11^{Ac}	8.10 ± 0.23^{Ab}	7.23 ± 0.00^{Ba}	-	26.50 ± 0.50^{A}
	50	9.20 ± 0.12^{Cd}	9.05 ± 0.87^{Ac}	11.03 ± 0.07^{Bb}	8.32 ± 0.66^{Ca}	-	
	60	10.00 ± 0.30^{Db}	10.60 ± 0.34^{Bc}	13.30 ± 0.51^{Cd}	9.00 ± 0.00^{Da}	-	
S. aureus	20	-	-	-	-	-	
	30	15.56 ± 0.57^{Fb}	16.23 ± 0.25^{Dc}	18.80 ± 0.26^{Ed}	7.33 ± 0.41^{Ba}	-	
	40	17.17 ± 0.27^{Gb}	18.03 ± 0.33^{Ec}	19.88 ± 0.11^{Fd}	8.50 ± 0.14^{Ca}	-	
	50	20.77 ± 0.19^{Hc}	20.00 ± 0.23^{Fb}	20.56 ± 0.60^{Gc}	9.43 ± 0.55^{Da}	-	29.10 ± 0.17^{B}
	60	23.76 ± 0.25^{Ic}	21.50 ± 0.45^{Gb}	21.86 ± 0.32^{H}	10.00 ± 0.00^{Ea}	-	

EsE = Ethanol stem extract, Ms =Methanol stem extract, HsE= Hexane stem extract, WsE= Water stem extract chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column (upper case) are significantly different ($p<0.05$) 0 = no inhibition zone.

Table 7. *Antibacterial activities of crude extract of the leaves of V. amygdalina against the test organisms (Mean ± SD, n=3).*

Test organisms	Con. mg/ml	Zone of nhibition(mm)				DMSO	Chloramphenicol (0.1 mg/ml)
	Crude extracts	ElE	MlE	HlE	WlE		
E.coli	20	-	-	-	-	-	
	30	9.20 ± 0.34^{Ab}	9.23 ± 0.40^{Ab}	10.43 ± 0.37^{Ac}	6.00 ± 0.00^{Ea}	-	
	40	11.67 ± 0.54^{Cc}	11.14 ± 0.00^{Bb}	11.00 ± 0.55^{Bb}	6.83 ± 0.84^{Fa}	-	26.90 ± 0.1^{A}
	50	14.66 ± 0.43^{Dd}	13.43 ± 0.11^{Dc}	11.29 ± 0.43^{Bb}	7.05 ± 0.90^{Fa}	-	
	60	16.10 ± 0.37^{Ed}	14.53 ± 0.47^{Fc}	12.00 ± 0.00^{Db}	8.43 ± 0.40^{Ga}	-	
P.aeruginosa	20	-	-	-	-	-	
	30	10.43 ± 0.40^{Bb}	13.50 ± 0.30^{Dc}	10.63 ± 0.23^{Ab}	1.30 ± 0.10^{Aa}	-	
	40	12.45 ± 0.11^{Cc}	14.90 ± 0.14^{Fd}	11.00 ± 0.53^{Bb}	2.00 ± 0.21^{Ba}	-	26.50 ± 0.50^{A}
	50	14.32 ± 0.18^{Dc}	15.60 ± 0.85^{Gd}	11.80 ± 0.06^{Cb}	2.81 ± 0.22^{Ca}	-	
	60	16.93 ± 0.25^{Fc}	16.43 ± 0.40^{Hc}	12.43 ± 0.40^{Db}	3.63 ± 0.37^{Da}	-	
S. aureus	20	-	-	-	-	-	
	30	12.66 ± 0.41^{Cc}	12.36 ± 0.35^{Cc}	10.20 ± 0.26^{Ab}	8.43 ± 0.20^{Ga}	-	
	40	14.18 ± 0.11^{Db}	14.00 ± 0.34^{Eb}	14.76 ± 0.33^{Ec}	11.60 ± 0.54^{Ha}	-	29.10 ± 0.17^{B}
	50	17.00 ± 0.13^{Gc}	16.00 ± 0.00^{Hb}	16.26 ± 0.50^{Fc}	14.63 ± 0.00^{Ia}	-	
	60	18.40 ± 0.40^{Hb}	18.36 ± 0.32^{Ib}	18.33 ± 0.30^{Gb}	16.36 ± 0.40^{Ja}	-	

Key: ElE = ethanol leaf extract, MlE =methanol leaf extract, HlE= Hexane leaf extract, WlE= Water leaf extract chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscript on the same raw(lower case) and values with different superscript on the same column(upper case) are significantly different($p<0.05$)

Table 8. *Antibacterial activities of crude extracts of the roots of V. amygdalina against the test organisms (Mean ± SD, n=3).*

Test organisms	Con. mg/ml	Zone of Inhibition(mm)				DMSO	Chloramphenicol 0.1mg/ml
	Crude extracts	ErE	MrE	HrE	WrE		
E. coli	20	-	-	-	-	-	
	30	10.46 ± 0.45^{Cd}	11.43 ± 0.80^{Bc}	8.70 ± 0.26^{Ab}	7.50 ± 0.30^{Aa}	-	26.90 ± 0.1^{A}
	40	12.00 ± 0.22^{Dc}	12.62 ± 0.99^{Cd}	8.90 ± 0.65^{Ab}	7.95 ± 0.74^{Aa}	-	
	50	13.12 ± 0.22^{Ec}	13.30 ± 0.11^{Dc}	9.69 ± 0.33^{Bb}	7.98 ± 0.60^{Aa}	-	
	60	14.80 ± 0.26^{Fc}	14.30 ± 0.51^{Ec}	10.86 ± 0.41^{Cb}	8.00 ± 0.00^{Ba}	-	
P. aeruginosa	20	-	-	-	-	-	
	30	8.40 ± 0.36^{Bb}	12.90 ± 0.36^{Cc}	12.43 ± 0.40^{Dc}	7.16 ± 0.28^{Aa}	-	
	40	10.23 ± 0.11^{Cb}	15.53 ± 0.16^{Fd}	14.00 ± 0.88^{Ec}	7.90 ± 0.31^{Aa}	-	26.50 ± 0.50^{A}
	50	12.12 ± 0.90^{Db}	18.60 ± 0.55^{Hd}	17.83 ± 0.05^{Fc}	8.84 ± 0.82^{Ca}	-	
	60	14.66 ± 0.35^{Fb}	20.00 ± 0.43^{Ic}	19.76 ± 0.25^{Gc}	10.43 ± 0.37^{Ea}	-	
S. aureus	20	-	-	-	-	-	
	30	6.63 ± 0.47^{Aa}	8.93 ± 0.30^{Ac}	8.66 ± 0.20^{Ac}	7.86 ± 0.23^{Ab}	-	
	40	8.00 ± 0.51^{Ba}	11.00 ± 0.09^{Bc}	8.98 ± 0.93^{Ab}	8.70 ± 0.04^{Cb}	-	29.10 ± 0.17^{B}
	50	10.00 ± 0.14^{Cc}	14.03 ± 0.01^{Fb}	9.26 ± 0.40^{Aa}	9.53 ± 0.40^{Da}	-	
	60	12.43 ± 0.40^{Db}	16.06 ± 0.11^{Gc}	10.43 ± 0.40^{Ca}	10.40 ± 0.40^{Ea}	-	

Key: ErE = ethanol root extract, MrE =methanol root extract, HrE= Hexane root extract, WrE= water root extract chloramephenicol = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column(upper case) are significantly different($p<0.05$)

As indicated in all tables (3-8) above, the plant extracts obtained using organic solvents showed better results than aqueous extracts. This observation clearly indicated the existence of non-polar residues in the extracts, which had higher antibacterial activities. [28] had also reported that most of the antibiotic compounds already identified in plants were aromatic or saturated organic molecules, which can easily solubilize in organic solvents. Similar results also showed that the alcoholic extracts had the best antimicrobial activity [30]. The antibacterial activities observed could be due to the presence of secondary metabolites [31]. Some other reporters had also reported that various parts of *C. procera* and *V. amygdalina* (root, leaf, flower and stem bark) showed antimicrobial activities [32-34]. The difference in antimicrobial properties of plant extracts is attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light, soil type and water), incorrect preparation and dosage (concentration) [35, 36].

Several investigators had also reported that plants contain antibacterial substances [37, 38]. The present study also showed that there was variation in the degree of antibacterial activities of the extracts due to that high level of phytochemicals present in organic solvent extracts than in aqueous extracts [18]. Similarly, a number of studies have also reported the antimicrobial efficacy of ethanol, methanol and hexane extracts of other plants [31, 39- 42].

3.3. Minimum Inhibitory Concentration (MIC) of the Crude Extracts

The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the extracts that showed significant antimicrobial activities in the previous tests. MIC was determined for extracts that showed significant growth inhibition zone at 30 mg/ml. The test was performed using the Agar dilution method. In agar dilution, the extract solution at 30 mg/ml was serially diluted to get 28 mg/ml, 26 mg/ml, 24 mg/ml and 22 mg/ml concentrations. Then, each of the three test pathogens were added to the dilute ethanol, methanol, hexane and water extracts of concentrations ranging from 22 mg/ml up to 28 mg/ml. The results are shown in table 12 and 13.

The reason for this slight discrepancy may be attributable to a possible difference in the characteristics of bacteria strains and difference in solvent extractions used. In addition to this, the high MICs of the extracts could be due to high resistance rate of the test organisms [32]. The same reasons also reported by [43]. The MIC of ethanol leaf and stem extracts of C. *procera* were the lowest of all the solvents' extract, implying that ethanol extracts were the most potent (at lower concentration) and that ethanol was the best extracting solvent.

Table 9. *The minimum inhibitory concentration (MIC) of crude extracts of leaves, stem barks and roots of C. procera against the selected bacterial test organisms in mg/ml.*

Test organisms	plant parts	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
E. coli	Leaf	24^{aB}	26^{bB}	26^{bB}	28^{cA}
	Stem bark	26^{aC}	26^{aB}	26^{aB}	28^{bA}
	Root	-	-	-	-
S. aureus	Leaf	22^{aA}	24^{bA}	24^{bA}	28^{cA}
	Stem bark	22^{aA}	24^{bA}	24^{bA}	28^{cA}

Test organisms	plant parts	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
	Root	28^{aD}		28^{aD}	-
P. aeruginosa	Leaf	26^{aC}	26^{aB}	26^{aB}	28^{bA}
	Stem bark	24^{aB}	26^{aB}	26^{aB}	28^{bA}
	Root	28^{aD}	-	-	-

Values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different at $p<0.05$, - = no growth

Table 10. *The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of V. amygdalina against the selected bacterial test organisms in mg/ml.*

Test organisms	plant part	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
	Leaf	24^{aB}	24^{bB}	24^{bB}	26^{bA}
E. coli	Stem bark	24^{aC}	24^{aB}	26^{aB}	28^{cB}
	Root	28^{aC}	28^{aC}	28^{aC}	28^{aB}
	Leaf	22^{aA}	24^{aA}	26^{bB}	26^{bA}
S. aureus	Stem bark	24^{aB}	24^{aB}	24^{bB}	28^{cB}
	Root	26^{aC}	28^{bC}	28^{bC}	28^{bB}
	Leaf	26^{aB}	26^{aB}	26^{aB}	28^{bA}
P. aeruginosa	Stem bark	26^{aB}	26^{aB}	26^{aA}	28^{bB}
	Root	28^{cB}	28^{bC}	28^{bC}	28^{bB}

Values with different superscript on the same raw (lower case) and values with different superscript on the same column (upper case) are significantly different at $p<0.05$.

Generally, the MIC values were recorded for the leaf ethanol extract against *E. coli*, which confirms that, the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. MIC is important because populations of bacteria exposed to an insufficient concentration of the extract can develop resistance to antibacterial agents. The high activity of antimicrobial agents at low concentrations is very essential for chemotherapeutic purposes because of their low toxicity to patients administered with such agents [22].

Table 11. *Phytochemical characteristics of the leaf stem bark and root extracts of Calotropis procera.*

Phytochemicals	Ethanol extraction			Methanol extraction			Hexane extraction			Water extraction		
	Leave	stem bark	Root	Leave	stem bark	Root	Leave	stem bark	Root	Leave	stem bark	Root
Alkaloids	+	++	-	+	+	_	-	+	-	+	-	-
Tannins	++	+	+	++	++	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	-	-	-	+	+	-
Flavonoids	+++	++	+	+++	+	+	+	-	-	-	+	-
Saponins	++	++	_	+++	+	+	+	+	-	+	+	+
Reducing sugar	_	_	_	_	+	-	++	++	+	-	-	+
Glycosides	-	-	+	+++	++	+	+	++	+	+	+	+
Steroids	+	+	+	++	++	++	+	_	_	+	_	_
Triterpenoids	+	+	+	-	-	-	+	+	-	-	+	-
Anthocyanidins	+	+	+	+	+	+	-	-	-	+	-	-
Sterol	+	+	-	+	+	+	-	-	-	+	+	-
Phenolics	++	++	+	+	+	+	-	-	-	+	+	-
Resins	++	++	+	+	_	_	+	+	-	-	+	-
Volatile Oil	_	_	_	_	_	_	_	_	_	_	_	_

Keys: + + = abundantly present, += present in low concentration, - = absent (not detected)

Table 11 above shows Phytochemical compounds found in the leaf stem bark and root different solvent extracts of *Calotropis procera*. A lot of researchers were reported that tannins bind the cell wall of bacteria, preventing growth and protease activity and can also be toxic to filamentous fungi, yeasts and ruminal bacteria [44]. Cardiac glycosides, which have been reported to have antimicrobial properties [45, 46], were found in all the extracts. Saponins were detected in all the extracts. They are effective in the treatment of syphilis and certain skin diseases [44, 47]. Flavonoids are known for their anti-allergic effect as well as a wide variety of activity against Gram-positive and Gram-negative bacteria, fungi and viruses [48]. The properties of the phytochemical ingredients (Table 11) could have attributed to the results of the antibacterial activities observed in the present study.

Table 12. *Phytochemical characteristics of the leaf stem bark and root extracts of Vernonia amygdalina.*

Phytochemicals	Ethanol extraction			Methanol extraction			Hexane extraction			Water extraction		
	Leave	stem bark	Root	Leave	stem bark	Root	Leave	stem bark	Root	Leave	stem bark	Root
Alkaloids	+	++	-	+	+	_	-	+	-	+	-	-
Tannins	++	+	+	++	++	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	-	-	-	+	+	-
Flavonoids	+++	++	+	+++	+	+	+	-	-	-	+	-
Saponins	++	++	_	+++	+	+	+	+	-	+	+	+
Carbohydrates	_	_	_	_	+	-	++	++	+	-	-	+
Glycosides	-	-	+	+++	++	+	+	++	+	+	+	+
Steroids	+	+	+	++	++	++	+	_	_	+	_	_
Triterpenoids	+	+	+	-	-	-	+	+	-	-	+	-
Anthocyanidins	+	+	+	+	+	+	-	-	-	+	-	-
Sterol	+	+	-	+	+	+	-	-	-	+	+	-
Phenolics	++	++	+	+	+	+	-	-	-	+	+	-
Resins	++	++	+	+	_	_	+	+	-	-	+	-
Volatile Oil	_	_	_	_	_	_	_	_	_	_	_	_

Keys: + + = abundantly present, += present in low concentration, - = absent (not detected)

As indicated on the above table (Table 12) the preliminary phytochemical screening revealed the presence of these compounds in the extracts of V. amygdalina by using different extraction solvents. Ethanol and Methanol Leaf and stem bark showed the highest compound extracts compared with hexane and water root extract. One of the factor that affect microbial susceptibility is the concentration of the active component; the more the concentration, the higher the activity of the chemical substance [49, 50]. It is reported that, some compounds as an indication of the potential medicinal value of the plants in which they appear. Flavonoids constituent exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cystostatic and antioxidant properties, anticancer activities reported that tannins are known to react with protein to provide the typical tannins effect which is important for the treatment of ulcer [52- 55]. Tannins have been found to form irreversible complex with proline-rich protein resulting in the inhibition of cell protein synthesis [56]. Herbs that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery [57]. This observation therefore supports the use of Vernonia amygdalina in herbal cure remedies. Reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that Vernonia amygdalina has potential as a source of important bioactive molecule for the treatment and prevention of different disease causing bacteria [58]. There is also saponins and alkaloids, steroidal compounds present in Vernonia amygdalina extracts. They are important and interest due to their relationship with various anabolic hormones including sex hormones.

4. Conclusion

The findings of this study revealed that *Vernonia amygdalina* and *Calotropis procera* collected from Haramaya university main campus and Dire Dawa district respectively exhibited significant antimicrobial effect by the crude extracts against the three bacterial strains (*E. coli*, *S. aureus*, and *P. auruginosa*) which is an indication for the presence of antimicrobial agents in it. The antimicrobial effect of the crude extract of each solvent was found to be concentration dependent against the tested pathogens. The four solvents employed for the extraction process i.e., water, and organic solvents (ethanol, methanol and hexane) have showed different extraction efficiency, which could be due to their difference in polarity. The result of this work indicated that ethanol, hexane and methanol are better solvents than water for the extraction of the active ingredients of these plants.

Based on the results of the present study, it could say that the plant extracts contain chemical constituents of pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened using additional solvents, could yield drugs of pharmaceutical significance. The results of the study also support the folklore claim along with the development of new antimicrobial drugs from both the plant parts.

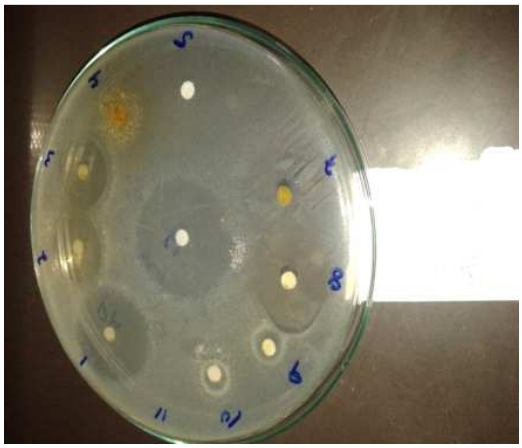
Appendix

Some of laboratory and field activities

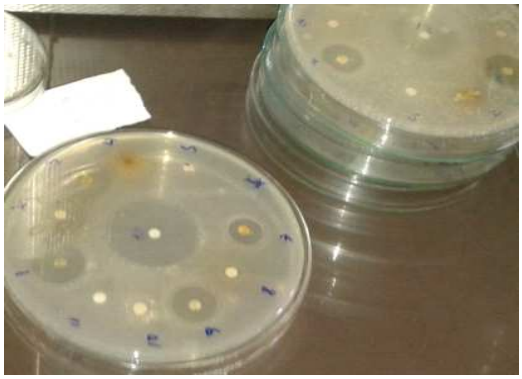
Figure 1. *C. procera.*

Figure 2. *V. amygdalina.*

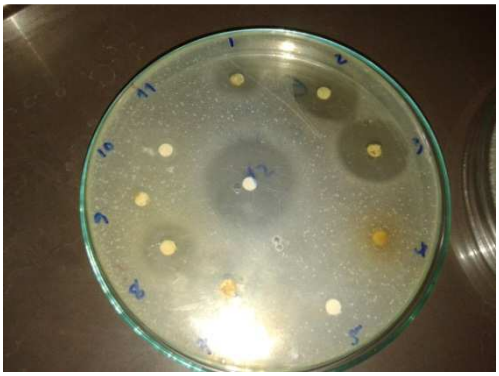
Figure3. *Crude extracts.*



(a)

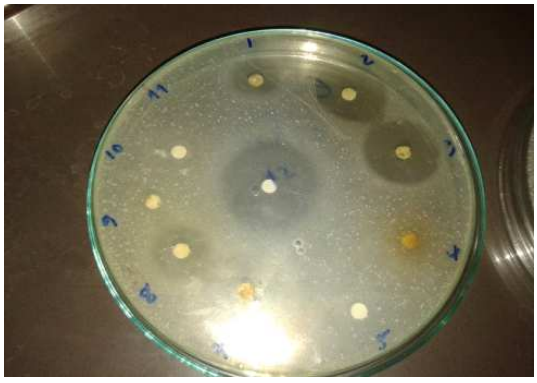


(b)

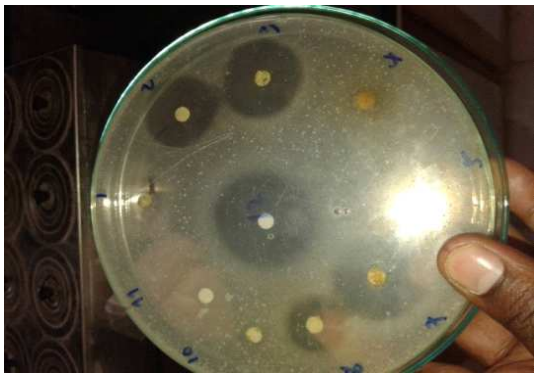


(c)

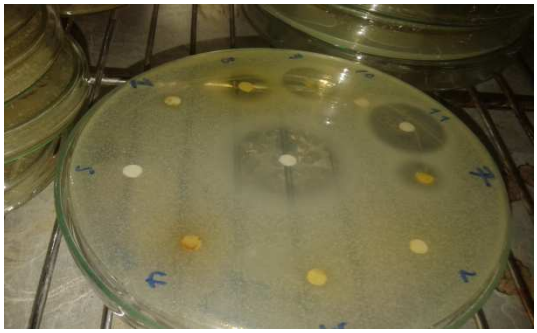
Figure 4. *Antimicrobial activities of leave of C. procera and V. amygdalina (a) against E.coli;(b) against P. aeruginosa; (c) against S. aureus*



(a)



(b)

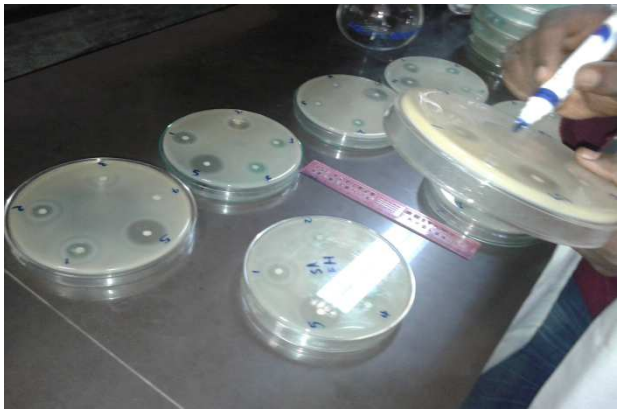


(c)

Figure 5. *Antimicrobial activities of stem bark of C. procera and V. amygdalina (a)against E.coli;(b) against P. aeruginosa; (c) against S.*

(a)

(b)



(c)

Figure 6. *Antimicrobial activities of root of C. procera and V. amygdalina (a)against E.coli;(b) against P. aeruginosa; (c) against S.*

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