IN-VITRO ANTIMICROBIAL ACTIVITIES OF SODOM APPLE (*CALOTROPIS PROCERA)* AND BITTER LEAF (*VERNONIA AMYGDALINA)* EXTRACTS AGAINST SOME MEDICALLY IMPORTANT PATHOGENIC BACTERIA

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In-vitro Antimicrobial Activities of Sodom Apple (*Calotropis procera)* and Bitter Leaf (*Vernonia amygdalina)* Extracts Against Some Medically Important Pathogenic Bacteria

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By

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APPROVAL SHEET

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As thesis research advisors, we hereby certify that we have read and evaluated the Thesis Entitled: ***In vitro*** **Antimicrobial Activities of Sodom Apple (*Calotropis procera)* and Bitter Leaf (*Vernonia amygdalina)* Extracts against Some Medically Important Pathogenic Bacteria** prepared under our guidance by Yirgashewa Asfere. We recommend that it be submitted as fulfilling the thesis requirement.

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As members of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Yirgashewa Asfere and examined the candidate. We recommend that the Thesis be accepted as fulfilling the thesis requirement for the *degree of Master of Science in Biotechnology*.

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DEDICATION

This thesis manuscript is dedicated to my brother Zenebe Asfere who had significant contribution in the execution of this work.

STATEMENT OF THE AUTHOR

I hereby confirm that this thesis is my own original work and the use of all material from other sources has been properly and fully acknowledged. This thesis has been submitted in partial fulfillment of the requirements of M.Sc. degree in Biotechnology at Haramaya University and is deposited at the university library to be made available to borrowers under rules of the library. I certainly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH OF THE AUTHOR

The author was born in Aseko district, East Arsi Zone, Oromia Region, from his father Asfere Wuhibe and his mother Aberash Seyoum in November 1988. He attended his elementary school education at Aseko Primary School. He also attended his junior and secondary school education at Adama General Secondary and Hawas Preparatory School. After completing his high school education in 2006, he joined Debre Berehan University in October 2007 and graduated with Bachelor of Education Degree in Biology in July 2010.

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LIST OF ACRONOMYS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CFU	Colony Forming Unit
EPHI	Ethiopian Public Health Institution
HSD	Honest Significant Difference
GAS	Group A *Streptococcus*
M.a.s.l	Meters Above Sea Level
MHA	Mueller–Hinton Agar
MIC	Minimum Inhibitory Concentration
SPSS	Statistical Package for Social Sciences
psi	Pressure Per Square Inch
SD	Standard Deviation
SSTI	Skin and Soft Tissue Infections
MCE	Methanol Crude Extract
ECE	Ethanol Crude Extract
ACE	Aqueous Crude Extract
HCE	Hexane Crude Extract

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ABSTRACT

Plants have been used for generations as source of medicine in the treatment of a variety of human illnesses. In Ethiopia, many plants are used for this purpose by traditional healers without any scientific justification for their therapeutic values. Thus, this study was aimed at assessing the 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 the paper disc method and the inhibitory zones were recorded in millimeters. Minimum inhibitory concentrations (MICs) of the plant extracts against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were assessed using the agar dilution method. Chloramphenicol and sterile distilled water were used as positive and negative controls, respectively. The bioassay studies of the crude extracts were undertaken at five different concentrations (20, 30, 40, 50 and 60 mg/ml). The results revealed that the crude extracts of ethanol, methanol, hexane and water had antibacterial activities on all three bacterial species 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 chloramephenicol. S. aureus was 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 ($p < 0.05$).

In conclusion, this study did not only show the antibacterial activities of Calotropis procera and Vernonia amygdalina, but also offered a scientific foundation for its traditional use against some diseases.

KEY WORDS*: Paper disc diffusion, Antibacterial activities, Crude extract, Percentage yield, Minimum inhibitory concentration*

1. INTRODUCTION

The human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and its long journey from ancient time to modern age. It has successfully used plants and plant products as effective therapeutic tools for fighting against diseases and various other health hazards (Ghani, 2003). The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al.*, 2002). Herbal medicine is the oldest form of healthcare known to humankind and over 50% of all modern clinical drugs are of natural products origin; and at present natural products still play important roles in drug development in the pharmaceutical industry (Preethi *et al.*, 2010)

Many research efforts have been directed towards the provision of empirical proofs to back up the use of plant species in trade and medicinal practices in recent years (Ojo *et al*., 2005). Several researchers have examined the effects of plants used traditionally or by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated. Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care. Consequently, in the past few years, a number of studies have been conducted in different countries to prove such applications (Uba *et al*., 2005; Oyi *et al*., 2007; Salihu and Garba, 2008). Medical practitioners whether allopaths (medical doctors), homeopaths, naturopaths, herbalists or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants (Akujobi *et al*., 2004). Plants are common clinical phenomena (Abebe *et al*., 2003; Nwafor, 2004); and some medicinal plants may produce adverse long-term effects such as hepatotoxicity (Steenkamp *et al.*, 2006). Ethiopian medicinal plants are shown to be very effective against some ailments of human and domestic animals (Endashaw, 2007).

There are two common species of *Calotropis: Calotropis gigantean* (Linn) and *Calotropis procera* (Ait) (Yelne *et al.*, 2000). *Calotropis procera* belongs to the family Asdepiadaceae and is used medicinally to treat boils, infected wounds and other skin problems in people and to treat parasitic skin infestations in animals (Himalaya, 2002). It also yields ash for making gunpowder. The latex is processed and used in treating vertigo, baldness, hair fall, toothaches, intermittent fevers, rheumatoid/joints swellings and paralysis (Vohra, 2004). The pungent latex extracted from the leaf and flowers of *C. procera* is processed and used in the commercial preparation of eye tonic (Vohra, 2004). Traditionally, the leaves of the plant are warmed and tied around any body organ in pain. It is practically useful in backache and joint pains; warm leaves also relieve from stomach ache if tied around. Inhalation of burnt leaf cures headache. The traditional folk healers use the milky latex of *C. procera* for several ailments. Leaf latex if applied on fresh cut skin, stops bleeding immediately.

Recent investigation has found that the alkaloids calotropin, calotaxein and uskerin are stimulant to heart (Ashwari, 2009; Kuta, 2006). Some researchers have also reported that the stem-bark of *C. procera* is a promising antifungal agent, which could be used against dermatophytes. They further suggested that *C. procera* could be a potential source of chemotherapeutic agents and thus could be used for the treatment of tinea diseases. The leaf and fruit extracts of *C. procera* when boiled together can be used in the extraction of guinea worm by immersion of the infected limbs, for either several hours or three consecutive days. The dry leaves in northern Nigeria are used as a remedy for asthma, cough, etc. (Noatay, 2005).

Vernonia amygdalina is another medicinal plant commonly found in Ethiopia, which is called bitter leaf (Ibrahim *et al*., 2000) in English because of its bitter taste. African common names include *grawa* (Amharic), *ewuro* (Yoruba), *etidot* (Ibibio), *onugbu* (Igbo), *ityuna* (Tiv), *oriwo* (Edo), *chusar-doki* (Hausa), *mululuza* (Luganda), *labwori* (Acholi), and *olusia* (Luo), *ndolé* (Cameroon) (Kokwaro, 2009). The leaves are dark green coloured with a characteristic odour and a bitter taste. *Vernonia amygdalina* is a tropical plant belonging to the family Compositae and is used 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 odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting (Egedigwe, 2010).

Vernonia, consisting of about 200 species, grows under a range of ecological zones in Africa, produces a lager mass of forage, and is drought tolerant. *Vernonia amygdalina* is indigenous to tropical Africa and is found wild or cultivated all over sub-Saharan Africa (Bosch *et al.*, 2005).

According to Argheore *et al.* (2000), 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. Particularly, the aqueous extracts of the leaves have been shown to be effective for the treatment of a variety of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus among others due to their broad spectrum of activities (Argheore *et al.,* 2000). Antihelmintic and antimalarial properties (Abosi and Raserika, 2003) as well as antitumourigenic properties (Izevbigie *et al.*, 2004); have also been reported from extracts of the plant. Other studies have demonstrated hypoglycemic and hypolipidaemic effects of the leaf extracts in experimental animals (Nwanjo, 2005). The leaves and barks in Ethiopian local medicine are used as purgatives, against menstrual pain and in wound dressing (Uhegbu and Ogbuchi, 2004). *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 the amount of crude extract that could be obtained from the leaves, roots and stem barks of *Calotropis procera* and *Vernonia amygdalina using different solvents and to evaluate their in vitro* anti-microbial activities against *E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*

The Specific objectives

- 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).*
- to determine the yield of potential anti-microbial substances from leaf, root and stem bark extracts of *C. procera* and *V. amygdalina* using aqueous and organic solvents (hexane, methane and ethanol)
- to determine the minimum inhibitory concentrations (MICs) of the extracts against test Pathogens (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus)*

2. LITERATURE REVIEW

2.1. Medicinal Plants

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India, and the Near east (Mohsenzadeh, 2007). Natural products perform various functions and many of them have interesting and useful biological activities. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (Philip *et al*., 2009).

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000–500,000 plant species only a small percentages has been investigated. Phytochemicals and the fractionation submitted to biological and pharmacological screening is even small (Ramor and Ponnampulam, 2008). Historically pharmacological screening of natural compounds or synthetic origin has been the source of enumerable therapeutic agents (Semere, 2006). Random screening as tool in discovering new biological active molecules has been most productive in the area of antibiotics (Penna *et al*., 2001). Even now, contrary to common believes drugs from higher plants continue to occupy an important position in modern medicine (Kokoska *et al*., 2002).

Medicinal plants represent a rich source of antimicrobial agents on a global basis; at least 130 drugs, all single chemical entities extracted from higher plants are modified further synthetically and currently in use, but some of them are not being made synthetically for economic reasons (Ramor and Ponnampulam, 2008). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Borchardt *et al*., 2008). Numerous biologically active plants have been discovered by evaluation of ethno pharmacological data and these plants may offer the local population immediate and accessible therapeutic products (Bruck *et al*., 2004).

Medicinal plants are important sources of traditional medicines for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the over grown populations of the planet (Celikel and Kavas, 2008). Several herbs were known to possess medicinal value including antimicrobial properties (Jaturapronchai, 2003).

The extensive use of synthetic drugs causes increased side effects, which are more serious problems than the disease itself (Babu *et al.*, 2010).

In addition to the increasing demand for new drugs, over use and misuse of antibiotics have contributed to the development and spread of antibiotic resistant microorganisms (Elgayyar *et al*., 2001). Due to this factor, researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infection (Philip *et al*., 2009). For centuries, most of the population in Ethiopia, as elsewhere in many other developing countries have relied on a system of traditional medicine which consists of both empirco rational and magic religious elements and a combination of both (Dawit *et al*., 2005).

The search for new antimicrobial agents from medicinal plants is even more urgent in the context of developing countries like Ethiopia where bacterial and fungal infectious diseases are not only rampant but also the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Abebe *et al*., 2003). Considering the high cost of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified (Ashebir and Ashenafi, 1999). It is believed that plants, which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenodis, alkaloids, and polyphenols, are generally superior in their antimicrobial activities (Cowan, 1999). This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of a natural product.

In Ethiopia about 80% of human population and 90% of livestock rely on traditional medicine (Mesfin , 2006) due to the socio cultural appeal, the cultural acceptability of healers and local pharmacopoeias, accessibility and effectiveness of medicinal plant against a number of health problems (Teferi and Hahn, 2003). Traditional medicinal practitioners used different type of medicine including plants, animals and minerals. The plant parts that are used in the preparation of medicine include roots, stems, barks, leaves, flowers, and seeds (Mohsenzadeh, 2007). The traditional drugs are also prescribed in formulated form, additives are usually incorporated and more than one drug is used in a single dosage form (Rojas *et al*., 2007). These drugs are administered using different routes.

The major ones being topical, oral, respiratory and anal (Tena, 2008). Herbal medicines are parts of the entire system of traditional medicine (Taylor, 2003). An integration of traditional medicinal plants with modern medicine has been also practiced in countries such as Egypt, Ghana, India, China and Sudan (Penna *et al*., 2001).

The indigenous traditional knowledge of medicinal plants of various ethnic communities has been transmitted orally from generation to generation (Velickovic *et al*., 2003). But now a day due to the advert of modern technology, transformation of traditional culture, migration from rural to urban areas, industrialization, rapid loss of natural habitat and changes in life style are on the ways of increasing the disappearance of this indigenous traditional knowledge from the surface of the earth (Semere, 2006).

Thousands of plant products with inhibitive effect on microorganisms have shown *in vitro* activity and have been used for centuries by various cultures in the treatment of different diseases (Ates and Erogrul, 2000). However, these plants are used at a very high concentration with serious side effects on the patients. Therefore, this requires the evaluation of the concentration against the standard antibiotics that have been ready on a market (Balagojevick *et al*., 2006).

Herbs, shrubs, higher plants and climbers remain as an important and reliable source of potentially useful chemical compounds for direct use as drugs and to synthesize proto types for synthetic analogous in terms of drug efficacy (Dewanjee *et al*., 2007). Several studies have been undertaken in Ethiopia regarding medicinal plants that have been screened for their antimicrobial activities. In Ethiopia, different plant parts have been used as a source of traditional medicine to solve different health problems and human sufferings. Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of Ethiopian people (Haile, 2005).Due to cultural acceptability of healers, efficacy against certain types of diseases, physical accessibility and economic affordability as compared to modern medicine, there is large magnitude of use and interest in traditional medicinal plants in Ethiopia. Herbal remedies are parts of the entire system of traditional medicine (Dawit, 2001).

Medical plants are currently used in the production of modern drugs, as source of direct therapeutic agents, as raw material for the manufacturing of complex semi synthetic compounds and as taxonomic markers in the search of new compounds (Suppakul *et al*., 2003).

The majority of Ethiopian rural areas have been dependent on the traditional medicinal plants as their only source of health care due to the lack of adequate clinics, hospitals, volunteers health care professionals, absence of vehicular roads and expensiveness of modern medicines (Ermias, 2005). Veterinary traditional medicines are locally available and cheaper than the standard treatments. As a result of this, livestock holders can prepare and use homemade remedies with minimum expense (Fekadu, 2001). In addition, due to relatively few veterinarians and shortage of other facilities, traditional medicinal plants are the only choice to treat many ailments in developing countries like Ethiopia (Tena, 2008).

2.2. Current Importance of Medicinal Plants

Scientists are in search of new phytochemicals that could be used as useful antimicrobial agents for the treatment of infectious diseases (WHO, 2010). Currently, out of 80% of pharmaceuticals derived from plants, very few of them are used as antimicrobial agents. However, plants are rich in a wide variety of secondary metabolites that have antimicrobial properties (Ramor and Ponnampulam, 2008). The challenge of purification and structure elucidation, pharmacological and toxicological characterization, formulation and other preclinical work required to move a lead compound to drug candidate status (Kumaraswamy *et al*., 2008). Because of continued emergence of resistant microorganisms, these considerations are especially critical to realization of major advances in the therapies available for the treatment of infectious disease (Haile, 2005).

The capacity of higher plants to produce inhibitors of microbial virulence factors have been reported (Balagojevick *et al*., 2006) but not widely exploited as source of new drugs. Presumably, these inhibitors function to protect the plant from microbial pathogens encountered in the environment, and thus would be expected to have potential utilities as antimicrobial drugs or antibiotics (Stephen and Horace, 2000). In the context of countries like Ethiopia, the prohibitively expensive cost of efficacious antibiotics and the emergence of single and multiple antibiotic resistance of bacterial disease call for the search of alternative agents with possible antibacterial effect from natural products.

One possible means to manage this problem is the search for pharmacologically active agents by screening traditionally claimed medicinal plants for their possible antibacterial effects (Ahmed *et al*., 2007). Now days, because of the steadily increasing number of multi–drug resistant strains of bacteria and an alarming increase in the incidence of new and re–emerging infectious diseases, there is a continuous and urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action (Semere, 2006).

2.3. Taxonomy and Distribution of *Calotropis procera* and *Vernonia amygdalina*

These plants are growing in different part of Ethiopia and they contain their own botanical description and taxonomy based on their distribution area and species.

2.3.1. Botanical description of *Calotropis procera*

Calotropis procera is a shrub or small tree 2–4 m tall (rarely up to 6 m tall), with distinctive grey green waxy leaves. According to Grace (2009), 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). Leaves are arranged in opposite pairs along the stems and are sessile, glaucous, and ovate to obovate, 5–20 cm long and 4–10 cm wide, with six prominent veins on the underside and a short, pointed tip (apex). Leaf bases are cordite (heart-shaped) and partially clasp the stem. The plant is not deciduous. Petioles are 3–4 mm long and 4–5 mm wide. The inflorescence is dense, multi-flowered, umbellate cymes. Flower buds are globular. The open corolla is white and pink/purple, and 2–3 cm in diameter. Each flower has five petals and flowers are grouped in umbels. The fruit is described by the Roman Jewish historian Josephus, who saw it growing near Sodom. “ As well as the ashes growing in their fruits; which fruits have a color as if they were fit to be eaten, but if you pluck them with your hands, they dissolve into smoke and ashes." (Grace, 2006)

Most reproduce sexually through the formation of seeds. However, suckers can be produced from the roots (Weed Identification undated). While most seeds fall close to the parent plants, they have a silky pappus that enables wind-dispersal over several hundred meters (Staples and Herbst, 2005).

2.3.1.1. Classification of *Calotropis procera*

Calotropis is a genus of flowering plant, which is commonly known as milk seed because of the latex they produce. Calotropis species are considered common weed in some part of the world. The classification of calotropis procera species is shown in table 1.

Table 1: Scientific classification of *Calotropis procera* (Tobiaw, ghindaqimbo)

Taxonomic Rank	Taxon
Kingdom	Plantae
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Spermatophyta Magnoliopsida*
Subclass	*Asteridae*
Order	*Gentianales*
Family	*Asdepiadaceae*
Genus	*Calotropis*
Species	*Procera*

2.3.1.2. The geographical distribution of *Calotropis procera*

Calotropi sprocera is Native to northern Africa (i.e. Algeria, Egypt, Libya, Morocco, Eritrea, Ethiopia, Somalia, Sudan, Kenya, Tanzania, Uganda, Cameroon, Equatorial Guinea, Gambia, Ghana, Guinea-Bissau, Mauritania, Nigeria, Senegal and Sierra Leone), the Arabian Peninsula (i.e. Saudi Arabia, Oman and Yemen), the Middle East (i.e. Iran, Israel, Jordan) and southern Asia (i.e. Afghanistan, Pakistan, Nepal, India, Myanmar, Thailand and Vietnam).

C. procera is drought-resistant, salt-tolerant to a relatively high degree, and it disperses seeds through wind and animals. It quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially sandy soils in areas of low rainfall; assumed to be an indicator of over-cultivation (Randall, 2012)

2.3.2. Botanical description of *Vernonia amygdalina*

This plant is belongs to family Compositae or Asteraceae and is used widely as vegetable and medicinal plant. It has the common name bitter leaf (Ibrahim *et al*., 2000). 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 odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting. *Vernonia amygdalina* commonly called bitter leaf is a perennial shrub of 2-5 m in height that grows throughout tropical Africa. It is a highly appreciated vegetable in west and central Africa where it is commonly used in traditional medicine. Local names Afrikaans (rivierbloutee); Amharic (grawa); English (vernonia tree, bitter leaf); Luganda (mululuza, muburizi); Tigrigna (grawa) (Ajibesin *et al*., 2008; Bukenya-Ziraba and Kamoga, 2007; Giday *et al*., 2009; Stave *et al*., 2007; Teklehaymanot, 2009).

Table 2: The local names of *V. amygdalina* in various countries

Country	**Local Name**
England	Better leaf
Cameron	Atednkol, Suwaaka
China	Ikaruga chrysanthemum tonsils, non-tree
Democratic Republic of Congo	Mpasinyioso
Democratic Republic of São Tomé	South LiboMucambu, LiboQue
Ethiopia	Buzut, Giraw, Grawa, Ibicha
Ghana	Awonoo, Awonwene, Jankpantire
Kenya	Olulusia,
Nigeria	Ewuro, Ejije, Onugbu, Olugbu, Shiwaka
Rwanda	Umubilizi
Swaziland	Liny South Africa
Tanzania	Mtugutu
Uganda	Labwori, Lubilili, Lubirizi, Omubirizi
Zimbabwe	Musikavakadzi
Gabon	Ndoki
Malaysia	South Africa leaf

The plant has acquired relevance recently, having been proven in human medicine to possess potent antimalarial and antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Tula *et al.,* 2012).

Various workers had reported the phytochemical and antibacterial activity of the plant parts against food borne pathogen (Ibrahim *et al.*, 2009), urinary tract pathogens (Uzoigwe and Agwa, 2011) and other clinical isolates (Oboh and Masodje, 2009; Ibrahim *et al.*, 2011: Tula *et al.*, 2012).

2.3.2.1. Classification of *Vernonia amygdalina*

Vernonia amygdalina is a species under the genus *Vernonia* that contains about 1000 species. More than 500 of these *Vernonia* plants are distributed in Africa and Asia, approximately 300 in Mexico, Central and South America and around 16 can be found in the United States.

However, the taxonomic ranking of this genus at all levels; ranging from its species up to the generic term can be a challenge. Austin (2000) has found that even taxonomy of *V. amygdalina* from different geographical area (Ethiopia and Cameroon) could be different. *V. amygdalina* produces a variety of flavonoids and bitter sesquiterpene lactones, which contribute to the bioactivities of this plant (Nangendo *et al*., 2002).

Table 3: Classification of *Vernonia amygdalina* (*Grawa*)

Taxonomic Rank	Taxon
Kingdom	Plantae
Class	Dicotyledonae
Sub class	Asteridae
Order	Asterales
Family	Asteraceae
Genus	*Vernonia*
Species	*Vernonia amygdalina*

2.3.2.2. The Geographical Distribution *Vernonia amygdalina*

Vernonia amygdalina occurs naturally along rivers and lakes, in forests margins, woodland and grassland up to 2800 m altitude, in regions where mean annual rainfall is 750-2000 mm. It requires full sunlight and prefers humid environment. It grows on all soil types but prefers humus-rich soils. *V. amygdalina* can be commonly found along drainage lines and in natural forests or at home and commercial plantations (Alem and Woldemariam, 2009).

V. amygdalina is a common homestead farming vegetable and fodder tree in Nigeria (Ndaeyo, 2007) and has been used as an ingredient to prepare Nigerian (Ogbono soup) or Cameroon (Ndole) dish after removal of its bitter taste through soaking in several changes of water or by boiling (Abosi and Raseroka, 2003; Onabanjo and Oguntona, 2003).

In Ethiopian highland, *V.amygdalina* has been classified by the farmer as a multi-purpose fodder tree with high biomass yield, easy propagation, high adaptability and high compatibility with other crops that do not compete with them for soil nutrients or moisture but instead help to improve the soil fertility and growth of perennial crops (Mekoya *et al*., 2008). However, studies found that some white, fragrant and bee-infested flowers growing on copious corymbs panicles would be formed under drastic growth environment and the seeds from these flowers could then be thrived well in slightly acidic soil with low organic matter and high water holding capacity. Water is the key factor for the growth of its leaves. Thus, high yield can be obtained during rainy season (Kayode, 2004).

2.4. Plant Extracts as Antibiotics

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes (Nascimento *et al*., 2000). A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross–resistance with agents already in use may be minimal (Morrison, 2009). Plants produce many secondary metabolites such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins.

Plant compounds, as bioactive agents have proven to be the best alternative for chemical compounds (Cock *et al*., 2009). Plant based antimicrobials strategies are urgently needed to evaluate the therapeutic or antimicrobial properties of plants extract (Chowdhury *et al*., 2013).

2.5. Benefits of *Calotropis procera* and *Vernonia amygdalina*

Calotropis procera and *Vernonia amygdalina* are commonly used in indigenous practices to treat fungal and bacterial diseases (Aliero *et al.*, 2001)

2.5.1. Antimicrobial Activities

Skin, hair, nail and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses (Amer *et al.*, 2006). Plants produce a great deal of secondary metabolites, many of them with antifungal and antibacterial activity. The sap of the leaves of *Calotropis procera* and *Vernonia amygdalina* was found to show inhibitory capacities against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeroginosa* (Ijeh *et al.*, 2010).

2.5.2. Source of Alkaloids

Alkaloids rank among the most efficient and therapeutically significant plant substances. They are chemically very diverse group of organic nitrogen compounds. Generally, they are extremely toxic though they do have a marked therapeutic effect in minute quantities. Pure isolated plant alkaloids and their synthetic derivates are used as basic medicinal agents all over the world for their antispasmodic and bactericidal effects (Ciocan and Bara, 2007).

2.5.3. Source of Food, Bio-fuel, and Fiber

Calotropis is cultivated for use as a medicine in some parts of China (Parrotta 2001). It has also been used to produce fibre and has been suggested as suitable for bio-fuel, having a potential crop yield of about 90 tons per hectare twice per year (Parsons and Cuthbertson 2001).

*V. amygdalina*is bitter leaf eaten as raw vegetables and cooked in soups. Roots and twigs chewed as appetizer (Aswalam *et al*., 2008)

2.5.4. Medicinal Values

All plants containing active compounds are important. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant.

These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species (Ibrahim *et al.,* 2009). The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct. The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. The plant has acquired relevance recently, having been proven in human medicine to possess potent antimalarial and antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Izevbigie *et al.,* 2004). Various workers had reported the phytochemical and antibacterial activity of the plant parts against food borne pathogen, urinary tract and other clinical isolates (Ibrahim *et al.,* 2009)

2.6. General Characteristics of the Selected Bacterial Species

A pathogen is usually defined as a microorganism that causes, or can cause, disease. We have defined a pathogen as a microbe that can cause damage in a host. So these bacteria's listed below can cause a diseases for human.

2.6.1. *Staphylococcus aureus*

Staphylococcus aureus is a gram–positive, non–motile, non–spore forming and catalase positive coccus with an average diameter of 0.8–1 micron.

The cells can be found alone, in pairs, as chains of 2–3 cells or as tetrads and clusters. *Staphylococcus aureus* is a bacterium that belongs to the family of Staphylococcoceae. *S. aureus* is a ubiquitous bacterium colonizing 20–30% of the human population (Van Belkum *et al*., 2009).

The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina. *S. aureus* causes infections in almost every organ and tissue of the human body. The most commonly affected part of the body due to *S. aureus* infection is the skin (Daum, 2007). Skin infections are the most common form of *S. aureus* infection. This can manifest in various ways, including small benign boils, folliculate, impetigo, cellulitis, and more severe, invasive soft-tissue infections(Tong *et al.*, 2015). *S. aureus* is extremely prevalent in persons with atopic dermatitis. It is mostly found in fertile, active places, including the armpits, hair, and scalp. Large pimples that appear in those areas may exacerbate the infection if lacerated. This can lead to staphylococcal scalded skin syndrome

2.6.2. *Escherichia coli*

Escherichia coli (commonly abbreviated E. coli) is a Gram–negative, facultative anaerobic, rod–shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of endodermis. A typical *Escherichia coli* cell is about 1 μm wide and 2 to 3 μm long (Burton and Engelkirk, 2004). Since its identification in 1885, *Escherichia coli* has become one of the most widely studied bacterial species. *E. coli* strains are comparatively easy to grow and manipulate in the laboratory, are easy to genetic manipulation, and naturally acquire mobile genetic elements. *E. coli* isolates form part of the beneficial normal flora of the intestine; some species have evolved pathogenic mechanisms to cause disease in humans and animals. *E. coli* species can cause enteric or extra intestinal infections in humans.

Infections are primarily urinary tract caused by uropathogenic *E. coli species* and sepsis/meningitis caused by neonatal meningitis *E. coli species,* (Clements *et al*., 2012). Enteric *E. coli* are part of the natural flora of many animals.

Human infections occur through consumption of contaminated food products (undercooked meat, or contaminated fresh produce such as salad leaves), drinking water contaminated with animal or human waste, or through direct person – to – person spread from poor hygiene (Berger *et al*., 2010).

2.6.3. *Pseudomonas aeruginosa*

Pseudomonas is a genus of Gram-negative, aerobic Gamma proteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches (Madigan *et al.,* 2005*).*

Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*. Because of their widespread occurrence in water and plant seeds such as dicots, the *pseudomonad*s were observed early in the history of microbiology *(*Palleroni, 2010) *Pseudomonas* infection is caused by strains of bacteria found widely in the environment; the most common type causing infections in humans is called *Pseudomonas aeruginosa.*

Serious *Pseudomonas* infections usually occur in people in the hospital and/or with weakened immune systems. Infections of the blood, pneumonia, and infections following surgery can lead to severe illness and death in these people. However, healthy people can also develop mild illnesses with *Pseudomonas aeruginosa*, especially after exposure to water. Ear infections, especially in children, and more generalized skin rashes may occur after exposure to inadequately chlorinated hot tubs or swimming pools. Eye Patients in hospitals, especially those on breathing machines, those with devices such as catheters, and patients with wounds from surgery or from burns are potentially at risk for serious, life-threatening infections. Infections have occasionally been reported in persons using extended-wear contact lenses.

In hospitals, where the most serious infections occur, *Pseudomonas* can be spread on the hands of healthcare workers or by equipment that gets contaminated and is not properly cleaned. *Pseudomonas aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. One of its most worrying characteristics is its low antibiotic susceptibility (Van Eldere, 2003*).*

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in the Research Laboratory of Plant Pathology and Central Laboratory at Haramaya University, which are found in East Hararghie zone 515 km East of Addis Ababa, Ethiopia. Haramaya University is located at latitude of $9^{o}20'$ north and $42^{0}3'$ longitude east. The university has a total area of about 46 km^2. It has a moderate average temperature of $16^{o}C$ and the mean maximum and minimum annual temperature is 24.02 and $9.73^{o}C$ respectively. The mean annual rainfall is 780 mm. (Seifemichael, 2014). The 1980 m elevation of the area (*Weina dega*) ensures that it enjoys a relatively moderate and pleasant climate throughout the year. *Eucalyptus globlus*, *Eucalyptus camaldulensis* (exotic), *Vernonia amygdalina* (indigenous), *Spathodea nilotica* (exotic), *Jacaranda mimosifolia* (exotic), *Pinus radiate* (exotic), *Cordia africana* and *Grevillea robusta* are dominant plants and vegetations in Haramaya university main campus (Haile *et al.*, 2012)

3.2. 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*), which were the target of this study, were randomly collected from different areas. 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.

3.3. Preparation of Crude Extracts from *C. procera* and *V. amygdalina*

The collected plant parts of *C. procera* and *V. amygdalina* were separately washed thoroughly with tap water followed by 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 then ground into fine powder with the help of suitable sterile grinder. Then it stored in sterile airtight containers according to Singh *et al*. (2007).

3.3.1. Preparation of Crude Extracts using Organic Solvents (Methanol, Ethanol and Hexane)

Twenty grams 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 (2002), the suspended plant materials were kept on a rotary shaker rotating at 190-220 rpm for 72 hrs at room temperature (Newton *et al*., 2002). Muslin cloth was then 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^{o}C and the gummy residue was further dried in a water bath at 40^{o}C – 50^{o}C for 24 hrs until the solvents were removed (Amit *et al*., 2015). After the evaporation of solvents, the remaining crude extracts were weighed using a balance and the resulting weights recorded. These crude extracts were further diluted with 10 ml of sterile distilled water and kept in sample vials with stoppers at 4°C until they were used against the test pathogens (Dewanjee *et al*., 2007).

3.3.2. 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, 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 (De Flora and Izzotti, 2007)

3.4. Percentage Yield Determination of Crude Extracts Obtained from *C. procera* and *V. amygdalina* Leaves, Roots and Stem Barks using Different Solvents (Hexane, Methane, Ethanol, and Water)

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 followed;

$$\%\text{yield}\left(\frac{w}{w}\right) = \frac{\text{extract obtained (in g)}}{\text{Grounded plant sample taken (in g)}} X\ 100$$

3.5. Sterility Test of the Plant Extracts

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 37^0C 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 (Ronald, 1995). Then the extracts were assayed for antimicrobial activities.

3.6. 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 ^{o}C and 15 psi for 15 minutes before cultured bacteria.

3.7. Sterilization of Materials

All materials used must be sterilized. Glasses were washed with detergents, rinsed properly with tap water and dried. They were then sterilized in the oven at 160^{o}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^{o}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 (Arekemase *et al*., 2013).

3.8. Standardization of the Bacterial Cell Suspension used as Inoculum

All bacterial cultures were first grown on 5% sheep red blood agar plates at 37^{o}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.

3.9. The Test Bacterial Species

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.

3.10. Inoculation of Mueller Hinton Agar Plates with the Test Organisms and Applying Extracts on the Plates using Paper Disks

Inocula of the respective bacterial species were streaked on to nutrient agar 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 of the extracts. 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 (Estimone *et al*., 1998).

Finally, the plates were incubated at 37^{o}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 (Obeidat *et al*., 2012)

3.11. Test for Antibacterial Activities of Crude Extracts

The antimicrobial activities of aqueous crude extract (ACE); methanol crude extract (MCE), ethanol crude extract (ECE), and hexane crude extract (HCE) were determined by the filter paper disc method as described by Omenka and Osuoha (2000). Sterile filter paper discs (6.0 mm diameter) were cut and aseptically impregnated with the respective 100 µl of the solutions of crude extracts. Then the impregnated paper discs were dried at 40^{o}C for 30 minutes were then placed on Mueller – Hinton agar (MHA) plates inoculated with dense inoculum suspension of each test pathogen. The plates were incubated at 37^{o}C for 48 hrs and examined for zone of inhibitions.

3.12. *In-Vitro* Evaluation of the Aqueous Extracts

The aqueous extracts obtained from different parts (stem bark, leaf and root) of both plants (*C. procera* and *V. amygdalina*) were assayed for antimicrobial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The three test pathogens were exposed to aqueous extracts of different plant parts by an adaptation of the paper disc method (Smania *et al*., 1995). 100µl of the solution of each plant extract at a concentration of 20, 30, 40, 50 and 60mg/ml were placed on 6.0 mm paper discs and placed on Mueller – Hinton agar (MHA) plates. The negative control also received the same amount (100 µl) of sterile distilled water.

All tests were done in three replications. The inhibition zones were observed after 24 hrs of growth at 37^{o}C.

3.13. *In-Vitro* Evaluation of the Ethanol, Methanol and Hexane Extracts

Eighteen extracts obtained using ethanol, methanol and hexane from *V. amygdalina* and *C. procera* plant parts (leaf, stem bark and root) were evaluated for their antimicrobial activities. The growth media were prepared following standard procedures.

All procedures employed in this test were the same as those described in section 3.11 except that the plates without plant extracts were used as controls. Size of colony diameters was measured after full growth of the control plates (Roman, 2010).

3.14. Positive and Negative Controls

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), while sterile distilled water (100 µl) was used as a negative control (Hailu *et al*., 2005).

3.15. Reconstitution of the Antibiotic

The antibiotic was reconstituted by dissolving 50mg of powder/ granules in a 500 ml of sterile distilled water to get a concentration of 0.1 mg/ml. The prepared antibiotic dilution was tested for its antimicrobial activities and compared with those of the crude extracts at concentrations 20, 30, 40, 50 and 60 mg/ml (Arekemase *et al.,* 2013**)**

3.16. 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 (Bauer *et al.,* 1996).

The Mueller-Hinton agar media were first prepared as described in section 3.5 and sterilized by autoclaving. The sterilized media were allowed to cool at 50^{o}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 ranged from 22 – 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 x 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^{o}C for 24 hrs. The lowest concentration, which inhibited the growth of the respective organisms, was taken as MIC.

3.17. 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.

4. RESULTS AND DISCUSSION

4.1. Percentage Yield of Crude Extracts

MCE, HCE, ECE and ACE 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 4 and Table 5. As indicated in the tables, the yield (amount) of the crude extracts ranged from 1.45% to 13.75% for *C. procera* and from 5.05% to 19.95% for *V. amygdalina*. MCE of the leaves of *V. amygdalina* gave the maximum yield (~~19.91~~ 19.95%) and MCE of the stem of *C. procera* gave maximum yield (13.75%), followed by ECE of the stem bark of *V. amygdalina* (13.75%) and MCE of leaves of *C. procera* (12.10%). The lowest yield was obtained from aqueous extract of the roots of both plants *V. amygdalina* (5.05%) and *C. procera (*1.45%).

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 (2013) who reported that the yields of MCE of *V. amygdalina* leaves were 14.1% (w/w). As Cowan (1999) 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. Both tables (Table 4 and Table 5) show that the percentage yields of most crude extracts obtained in both plants using the same extraction solvent with different plant parts and the same plant part with different solvents varied in extraction potential yield.

Extraction from roots using ethanol and hexane, from leaf and ~~root~~ stem bark using hexane and from leaf and stem bark of using water show no significant difference in yield of crude extracts of *C. procera*. Similarly in the case of *V. amygdalina,* extraction from leaves and stem barks using ethanol, from leaves and roots using hexane and from stem barks and roots using water showed no significant difference in yield of crude extracts. 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 (Wynn and Fougere, 2006).

Table 4: 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^{Ba}	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 5: 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.

4.2. 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 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.

4.2.1. Antimicrobial Activities of Crude Extracts of the Stem of *C. procera* Against the Test Organisms

The antibacterial activities of the crude extracts of the stem were tested on *E. coli*, *S. aureus* and *P. aeruginosa.* As indicated in Table 6 below, the zone of inhibition of the ethanol, methanol, hexane and water stem extracts were in the range of 12.70 - 24.50 mm, 9.53 - 21.00 mm, 9.50 - 21.00 mm and 8.66 - 14.00 mm, respectively. Table 6 clearly shows that all stem extracts at concentrations of 30, 40, 50 and 60 mg/ml show significant antibacterial activities against all the three tested organisms. The zone of inhibition for *S. aureus* (24.50 mm) was significantly higher ($p<0.05$) than those observed for *E. coli and P. aeruginosa*. At all solvents, stem extracts at a concentration of 20 mg/ml showed no significant antibacterial activity against all the tested bacteria.

Table 6: 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 Crude extracts	Zone of Inhibition(mm) EsE	MsE	HsE	WsE	SDW	Chloramphenicol (0.1 mg/ml)
E.coli	20	0	0	0	0	0	
	30	16.66 ± 0.57^{Cd}	14.46 ± 0.50^{Ec}	9.50 ± 0.50^{Ab}	8.96 ± 0.90^{Aa}	0	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}	0	
	50	21.33 ± 0.12^{Fd}	16.23 ± 0.32^{Gc}	12.30 ± 0.31^{Cb}	10.32 ± 0.42^{Da}	0	
	60	22.66 ± 0.57^{Gd}	18.80 ± 0.72^{Hc}	16.00 ± 0.28^{Db}	13.16 ± 0.28^{Fa}	0	
P. aeruginosa	20	0	0	0	0	0	
	30	12.70 ± 0.26^{Ac}	9.53 ± 0.50^{Ab}	16.66 ± 0.57^{Ed}	8.66 ± 0.57^{Aa}	0	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}	0	
	50	19.12 ± 0.23^{Ec}	12.00 ± 0.00^{Cb}	20.55 ± 0.00^{Gd}	10.01 ± 0.66^{Da}	0	
	60	21.83 ± 0.76^{Fc}	13.50 ± 0.05^{Db}	23.50 ± 0.50^{Hd}	11.33 ± 0.58^{Ea}	0	
S. aureus	20	0	0	0	0	0	
	30	12.83 ± 0.76^{Ab}	14.56 ± 0.28^{Ec}	16.83 ± 0.76^{Ed}	9.00 ± 0.90^{Ba}	0	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}	0	
	50	17.45 ± 0.22^{Db}	19.77 ± 0.93^{Id}	18.60 ± 0.65^{Fc}	13.10 ± 0.55^{Fa}	0	
	60	24.50 ± 0.50^{Hc}	21.00 ± 1.00^{Jb}	21.00 ± 1.00^{Gb}	14.00 ± 1.00^{Ga}	0	

Key: EsE = ethanol stem extract, MsE = methanol stem extract, HsE= Hexane stem extract, chloramephenicol = positive control, sterile distilled water (SDW) = 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.

The ethanol stem extract at 40 mg/ml on *E. coli* and at 50 mg/ml on *S. aurous* showed no significant antibacterial activities against test pathogens. At 50 mg/ml on *E. coli* and at 60 mg/ml on *P. auruginosa*, at 30 mg/ml on *P. auruginosa* and *S. aureus*, at 40 mg/ml on *P. auruginosa* and *S. aureus* showed no significant antibacterial activities against test pathogens. The methanol stem extract at 30 mg/ml on *E. coli* and *S. aureus,* at 50 mg/ml on *E. coli* and at 40 mg/ml on *S. aureus* showed no significant antibacterial activities against test pathogens. The hexane stem extract at 30 mg/ml on *P. auruginosa* and *S. aureus* and at 40 mg/ml on *S. aureus*, at 40 mg/ml on *P. auruginosa* and at 50 mg/ml on *S. aureus*, at 50 mg/ml on *P. auruginosa* and at 60 mg/ml on *S. aureus* showed no significant antibacterial activities against test pathogens. The water stem extract at 40 and 30 mg/ml on *E. coli* and *P. auruginosa* showed no significant antibacterial activities against test pathogens. At 50 mg/ml on *E. coli* and *P. auruginosa*, at 50 mg/ml on *S. aureus* and at 60 mg/ml on *E.coli* at 60 mg/ml on *P. auruginosa* and at 40 mg/ml on *S. aureus* showed no significant antibacterial activities against test pathogens. In the other way methanol and hexane, stem extracts at 60 mg/ml against *S. aureus* showed no significant antibacterial activities against test pathogens. This result agreed with the finding of (Abdulmoniem *et al.,* 2012) that recorded stem extracts of *C. procera* 15 mm against *E. coli*, and *S. aureus*, 14 mm on *P. aeruginosa*. Similarly, aqueous stem extracts were recorded 14 mm on *S. aurous*, 11 mm on *E. coli* and 13 mm on *P. aeruginosa (*Abdulmoniem *et al.,* 2012). The distilled water, a negative control, showed no inhibition against all test pathogens. In general, the diameter of zone of inhibition observed due to the antibiotic on the tested pathogens ranged from 26.50 - 29.10 mm.

4.2.2. Antimicrobial Activities of Crude Leaf Extract of C. procera Against the Test Pathogens

The leaf extracts were also tested for their antibacterial properties against the test pathogens. As shown in table 7 below, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous leaf extracts were in the range of 13.80 – 21.50 mm, 10.83 – 16.83 mm, 8.43 – 17.83 mm and 5.16 – 12.66 mm respectively.

Table 7 also shows that all solvent leaf extracts at concentrations of 30, 40, 50 and 60 mg/ml show significant antibacterial activities against all the three tested pathogens except at concentration of 20 mg/ml for all solvent extracts showed no significant antibacterial activities against all test organisms.

The ethanol leaf extract at 40 mg/ml on *E. coli* and at 30 mg/ml on *S. aureus*, at 60 mg/ml on *E. coli* and at 40 mg/ml on *P. auruginosa*, at 50 mg/ml on *P. auruginosa* and at 60 mg/ml on *S. aureus* showed no significant antibacterial activities against test organisms. The methanol leaf extract at 30 mg/ml on *E. coli* and *P. auruginosa*, at 40 mg/ml on *E. coli* and at 60 mg/ml on *S. aureus*, at 30 and 40 mg/ml on *S. aureus* showed no significant antibacterial activities against test organisms. The hexane leaf extract at 30 mg/ml on *E. coli* and *S. aureus*, at 40 mg/ml on *E. coli and* at 50 mg/ml on *S. aureus* showed no significant antibacterial activities against test pathogens. At 60 mg/ml on *E. coli* and at 50 mg/ml on *P. auruginosa*, at 40 mg/ml on *P. auruginosa* and at 60 mg/ml on *S. aureus* showed no significant antibacterial activities against test organisms. The water leaf extract at 30 mg/ml on(*E. coli* and *S. aureus*) and at 60 mg/ml on *P. auruginosa*, at 40 mg/ml on *E. coli* and *S. aureus*, at 50 mg/ml on *E. coli* and *S. aureus*, at 60 mg/ml on *E. coli* and *S. aureus* showed no significant antibacterial activities against test organisms. In the other way methanol and hexane leaf extracts at 50 mg/ml against *P. auruginosa*, hexane and water leaf extracts at 30 mg/ml against *E. coli* showed no significant antibacterial activities against test pathogens. Ethanol and methanol leaf extracts at 50 mg/ml against *E. coli*, hexane and water leaf extracts at 30, 40, 50 and 60 mg/ml against *S. aureus* showed no significant antibacterial activities against test organisms.

This finding was in agreement with the finding of Amit *et al*., (2015) which recorded inhibition zone of methanol leaf extract of *C. procera* as 10.5 mm, 13 mm and 12.5 mm against *E. coli*, *S. aureus*, and *P. aeruginosa* respectively. Abdulmoniem *et al.,* (2012) also recorded methanol leaf extract 17 mm, 16 mm and 15 mm against *E. coli, S. aureus* and *P. aeruginosa* respectively. In addition, the author also reported the inhibition zone of aqueous leaf extract as 14 mm against *S. aureus* and 12 mm against *E. coli* and *P. auroginosa* (Abdulmoniem *et al.,* 2012).

Table 7: Antibacterial activities of crude extracts of the leaves of C. *procera* against the test organisms (mean ± SD, n=3)

Test organisms	Con. mg/ml Crude Extracts	Zone of Inhibition(mm) ElE	MlE	HlE	WlE	SDW	Chloramphenicol (0.1 mg/ml)
E. coli	20	0	0	0	0	0	
	30	13.80 ± 0.34^{Ad}	12.50 ± 0.50^{Bb}	8.43 ± 0.40^{Aa}	8.80 ± 0.72^{Da}	0	
	40	14.51 ± 0.06^{Bd}	13.60 ± 0.54^{Dc}	11.42 ± 0.01^{Db}	9.77 ± 0.22^{Ea}	0	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}	0	
	60	17.43 ± 0.45^{Fd}	16.83 ± 0.76^{Hc}	15.83 ± 0.76^{Gb}	12.66 ± 0.57^{Ga}	0	
P. aeruginosa	20	0	0	0	0	0	
	30	16.66 ± 0.57^{Ed}	12.93 ± 0.90^{Bc}	10.20 ± 0.34^{Cb}	5.16 ± 0.57^{Aa}	0	
	40	17.41 ± 0.41^{Fd}	14.63 ± 0.14^{Ec}	13.00 ± 0.33^{Eb}	6.55 ± 0.77^{Ba}	0	
	50	18.98 ± 0.53^{Gc}	16.01 ± 0.22^{Gb}	16.00 ± 0.02^{Gb}	7.11 ± 0.26^{Ca}	0	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}	0	
S. aureus	20	0	0	0	0	0	
	30	14.53 ± 0.50^{Bc}	10.83 ± 0.76^{Ab}	8.50 ± 0.36^{Aa}	8.33 ± 0.57^{Da}	0	
	40	16.15 ± 0.44^{Dc}	11.01 ± 0.05^{Ab}	9.01 ± 0.05^{Ba}	9.40 ± 0.93^{Ea}	0	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}	0	
	60	19.00 ± 1.00^{Gc}	14.00 ± 0.00^{Db}	12.80 ± 0.72^{Ea}	12.66 ± 0.57^{Ga}	0	

Key: ElE = ethanol leaf extract, MlE =methanol leaf extract, HlE= Hexane leaf extract, chloramephenicol = positive control, sterile distil water(SDW) = 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.

As shown in table 7 above, the best result was obtained from ethanol leaf extract against the three test pathogens at all concentrations of the extracts.

4.2.3. Antimicrobial Activities of Crude Root Extract of C. Procera against the Test Pathogens

The solvent root extracts of *C. procera* were tested for their antimicrobial properties against the test pathogens and zone of inhibition (mm) were measured (Table 8). As can be seen in table 8, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous root extracts were in the range of 8.13 – 16.33 mm, 9.53 – 18.50 mm, 7.46 – 12.83 mm and 6.50 – 8.66 mm respectively. Table 8 also shows that all solvent roots extracts at concentrations of 30, 40, 50 and 60 mg/ml show significant antibacterial activities against all the three tested pathogens except at concentration of 20 mg/ml for all solvent extracts showed no significant antibacterial activities against all test organisms. This result is comparable with the finding of Abdulmoniem *et al.*, (2012) in which the author reported the inhibition zone of root methanol extract 17 mm, 15mm and 17mm against *S. aureus, E. coli* and *P. aeruginosa* respectively. The author also reported the inhibition zone of root aqueous extract as 12 mm, 13 mm and 10 mm against *S. aureus, E. coli* and *P. aeruginosa* respectively.

Table 8, also confirmed that, the ethanol root extract at 30 mg/ml on *E. coli* and *P. auruginosa*, at 40 mg/ml on *E. coli* and *P. auruginosa*, at 60 mg/ml on *E.coli* and at 40 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. The methanol root extract at 60 mg/ml on *E.coli* and *P. aeruginosa*, at 40 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. The hexane root extract at 30 mg/ml on *E. coli* and at 40 mg/ml on *P. aeruginosa*, at 40 mg/ml on *E. coli and* at 50 mg/ml on *P. aeruginosa* showed no significant different antibacterial activities against test pathogens. At 50 mg/ml on *E. coli* and at 60 mg/ml on *P. aeruginosa*, at 30 mg/ml on *P. aeruginosa* and *S.aureus* showed no significant different antibacterial activities against test organisms. The water root extract at 30, 40, and 50 mg/ml on *E. coli* and at 30 and 40 mg/ml on *P. aeruginosa* and at 30 mg/ml on *S. aureus* showed no significant different antibacterial activities against test pathogens.

At 60 mg/ml on *E. coli* and at 50 mg/ml on *P.aeruginosa* and at 40 mg/ml on S. *aureus*, at 60 mg/ml on *P.aeruginosa* and at 50 mg/ml on S. *aureus* showed no significant different antibacterial activities against test organisms.

In the other way methanol and ethanol root extracts at 60 mg/ml against *P. auruginosa*, hexane and ethanol root extracts at 30, 40, 50 and 60 mg/ml against *E. coli*, ethanol and methanol root extracts at 30 mg/ml against *S. aureus* showed no significant different antibacterial activities against test pathogens. The least inhibition zones were recorded in root water extract against *E. coli* and *P. auruginosa*. These results are in close agreement with the finding of Abdulmoniem *et al.,* (2012) reported the least inhibition zones in root water extracts 10 mm and 11 mm against *P. aeruginosa* and *E. coli* respectively.

Table 8: Antibacterial activities of crude root extract of C. *procera* against the test organisms (mean ± SD, n=3)

Test organisms	Con. mg/ml Crude extracts	Zone of Inhibition(mm) ErE	MrE	HrE	WrE	SDW	Chloramphenicol (0.1 mg/ml)
E.coli	20	0	0	0	0	0	
	30	8.13 ± 0.31^{Ab}	10.50 ± 0.50^{Bc}	8.33 ± 0.41^{Bb}	6.50 ± 0.43^{Aa}	0	
	40	10.11 ± 0.00^{Bb}	13.60 ± 0.54^{Fc}	9.89 ± 0.58^{Db}	6.87 ± 0.44^{Aa}	0	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}	0	
	60	13.06 ± 0.11^{Eb}	14.00 ± 0.00^{Gc}	12.83 ± 0.28^{Hb}	7.43 ± 0.40^{Ba}	0	
P,aeruginosa	20	0	0	0	0	0	
	30	8.26 ± 0.27^{Ac}	9.53 ± 0.28^{Ad}	7.46 ± 0.45^{Ab}	6.73 ± 0.30^{Aa}	0	
	40	10.61 ± 0.74^{Bc}	11.33 ± 0.44^{Cd}	8.10 ± 0.11^{Bb}	6.85 ± 0.97^{Aa}	0	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}	0	
	60	14.23 ± 0.40^{Gc}	14.00 ± 0.00^{Gc}	11.50 ± 0.50^{Fb}	8.20 ± 0.20^{Ca}	0	
S. aureus	20	0	0	0	0	0	
	30	12.76 ± 0.68^{Dc}	12.40 ± 0.76^{Dc}	7.50 ± 0.45^{Ab}	6.93 ± 0.35^{Aa}	0	
	40	13.16 ± 0.47^{Ec}	14.01 ± 0.11^{Gd}	9.01 ± 0.00^{Cb}	7.40 ± 0.13^{Ba}	0	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}	0	
	60	16.33 ± 0.57^{Ic}	18.50 ± 0.45^{Jd}	12.23 ± 0.32^{Gb}	8.66 ± 0.41^{Da}	0	

Key: ErE = ethanol root extract, MrE =methanol root extract, HrE= Hexane root extract, chloramephenicol = positive control, sterile distil water(SDW) = 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.

4.2.4. Antimicrobial Activities of Crude Stem Bark Extracts of *V. Amygdalina* against the Test organisms

The antibacterial activities of the stem bark ethanol, methanol, hexane and aqueous crude stem bark extracts were tested against three pathogens *(E. coli, S. aureus and P. aeruginosa)*. Table 9 shows that all solvent stem bark extracts of V. amygdalina at concentrations of 30, 40, 50 and 60 mg/ml show significant antibacterial activities against all the three tested pathogens except at concentration of 20 mg/ml for all solvent extracts showed no significant antibacterial activities against all test organisms. The inhibition zone of the ethanol, methanol, hexane and aqueous stem crude extracts were in the range of 8.00 – 24.40 mm, 8.56 - 21.50 mm, 7.43 - 21.86 mm and 6.26 - 10.00 mm respectively (Table 9). As shown in table 9, the organic solvents (ethanol, methanol and hexane) crude extracts showed higher inhibition zone than aqueous crude extract.

Investigators in the past had clearly shown that ethanolic stem bark extract were more effective than water stem bark of *V. amygdalina* extract (Dutta, 1993; Ibekwe *et al.*, 2001). They attributed this to the high volatility of ethanol, which tends to extract more active compounds from the sample than water. Table 9, also confirmed that, the ethanol stem extract of *V. amygdalina* at 40 mg/ml on *E. coli* and *S. aureus*, at 50 mg/ml on *E. coli* and *S. aureus* showed no significant different antibacterial activities against test organisms. The methanol stem extract of *V. amygdalina* at 40 mg/ml on *E. coli* and at 30 mg/ml on *S. aureus* showed no significant difference antibacterial activities against test pathogens. At 50 mg/ml on *E. coli* and at 40 mg/ml on S. aureus, at 60 mg/ml on *E. coli* and at 50 mg/ml on *S. aureus*, at 30, 40, and 50 mg/ml on *P. aeruginosa* showed no significant different antibacterial activities against test organisms.

The hexane stem extract at 30 and 40 mg/ml on *E. coli* and at 50 mg/ml on *P. aeruginosa*, at 50 mg/ml on *E. coli and* at 60 mg/ml on *P. aeruginosa*, at 30 and 40 mg/ml on *P. aeruginosa* showed no significant different antibacterial activities against test organisms. The water stem extract of *V. amygadalina* at 40 mg/ml on *E. coli* and at 30 and 40 mg/ml on *P. auruginosa* as well as at 30 mg/ml on S. aureus showed no significant different antibacterial activities against test pathogens. At 50 mg/ml on *E. coli* and *P. aeruginosa*, at 60 mg/ml on *E. coli* and *P. aeruginosa* and at 50 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms.

The hexane and the water stem extracts of *V. amygdalina* at 30 mg/ml against *P. auruginosa*, methanol and ethanol stem extracts at 40 mg/ml against *P. aeruginosa*, ethanol and hexane stem extracts at 50 mg/ml against *S. aureus*, methanol and hexane stem extracts at 60 mg/ml showed no significant antibacterial activities against test pathogens.

Generally, all extracts of *V. amygdalina* parts have antimicrobial properties against the three test pathogens. Ethanol, methanol, hexane and aqueous extracts exhibited inhibition zones on *S. aureus* (gram +ve), *E. coli* (gram - ve) and against *P.aeruginosa* (gram –ve) bacteria (Tables 9), which is in agreement with other scientific report (Al-Magboul *et al.*, 1985). According to Arekemase et al., (2013) report, ethanol stem bark extract of *V. amygdalina* showed an inhibition zone of 17.5 mm, 19.5 mm and 17.5 mm at the same concentration (30 mg/ml) against S. aurous, *E. coli, P. aeruginosa* respectively. Aqueous Stem bark extract also showed inhibition zone of 12.1mm, 16.2mm and 12.1mm at a concentration of 30 mg/ml against *E. coli, P. aeruginosa* and *S. aureus* respectively (Arekemase *et al*., 2013).

Table 9: Antibacterial activities of crude extracts of the stem bark of *V. amygdalina* against the test organisms (Mean ± SD, N=3)

Test organisms	Con. mg/ml Crude extracts	Zone of Inhibition(mm) EsE	MsE	HsE	WsE	SDW	Chloramphenicol (0.1 mg/ml)
E.coli	20	0	0	0	0	0	
	30	14.56 ± 0.05^{Ed}	13.10 ± 0.45^{Cc}	10.86 ± 0.05^{Bb}	6.26 ± 0.46^{Aa}	0	
	40	17.34 ± 0.22^{Gd}	15.50 ± 0.34^{Dc}	11.40 ± 0.22^{Bb}	6.90 ± 0.24^{Ba}	0	26.90 ± 0.1^{A}
	50	21.23 ± 0.45^{Hd}	18.13 ± 0.32^{Ec}	13.10 ± 0.73^{Cb}	8.09 ± 0.22^{Ca}	0	
	60	24.40 ± 0.36^{Jd}	20.40 ± 0.10^{Fc}	14.70 ± 0.26^{Db}	9.16 ± 0.28^{Da}	0	
P.aeruginosa	20	0	0	0	0	0	
	30	8.00 ± 0.46^{Ab}	8.56 ± 0.45^{Ac}	7.43 ± 0.40^{Aa}	7.13 ± 0.47^{Ba}	0	
	40	8.60 ± 0.64^{Bc}	8.80 ± 0.11^{Ac}	8.10 ± 0.23^{Ab}	7.23 ± 0.00^{Ba}	0	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}	0	
	60	10.00 ± 0.30^{Db}	10.60 ± 0.34^{Bc}	13.30 ± 0.51^{Cd}	9.00 ± 0.00^{Da}	0	
S. aureus	20	0	0	0	0	0	
	30	15.56 ± 0.57^{Fb}	16.23 ± 0.25^{Dc}	18.80 ± 0.26^{Ed}	7.33 ± 0.41^{Ba}	0	
	40	17.17 ± 0.27^{Gb}	18.03 ± 0.33^{Ec}	19.88 ± 0.11^{Fd}	8.50 ± 0.14^{Ca}	0	
	50	20.77 ± 0.19^{Hc}	20.00 ± 0.23^{Fb}	20.56 ± 0.60^{Gc}	9.43 ± 0.55^{Da}	0	29.10 ± 0.17^{B}
	60	23.76 ± 0.25^{Ic}	21.50 ± 0.45^{Gb}	21.86 ± 0.32^{Hb}	10.00 ± 0.00^{Ea}	0	

EsE = ethanol stem extract, MsE =methanol stem extract, HsE= Hexane stem extract, chloramephenicol = positive control, sterile distil water (SDW) = 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. ,

4.2.5. Antimicrobial activities of crude extracts of the leaves of *V. amygdalina* against the test organisms

The ethanol, methanol, hexane and aqueous crude extracts of the leaves of *V. amygdalina* at concentrations of 20, 30, 40, 50 and 60 mg/ml were evaluated for *in-vitro* antimicrobial activities against the three test organisms. The inhibition zones that resulted from these extracts are shown in Table 10. All crude leaf extracts of *V. amygdalina* showed a significant growth inhibition against all three tested organisms except at concentration of 20 mg/ml for all solvent extracts showed no significant antibacterial activities against all test organisms (Table 10). As shown from Table 10, the inhibition zones of the ethanol, methanol, hexane and aqueous crude extracts of leaves were in the range of 9.20 - 18.40 mm, 9.23 - 18.36 mm, 10.20 - 18.33 mm and 1.30 - 16.36 mm respectively.

Crude extracts obtained using organic solvents (ethanol, methanol and hexane) showed higher inhibition zone than aqueous crude extracts (Table 10). Table 10, also confirmed that, the ethanol leaf extract at 40 mg/ml on (*E. coli* and *P. auruginosa*) and at 30 mg/ml on *S. aureus*, at 50 mg/ml on *(E.coli* and *P. aeruginosa*) and at 40 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. The methanol leaf extract at 50 mg/ml on *E.coli* and at 30 mg/ml on *P. aeruginosa*, at 60 mg/ml on *E. coli* and at 40 mg/ml on *P. aeruginosa* showed no significant different antibacterial activities against test organisms. The hexane leaf extract at 30 mg/ml on *E. coli*, *P. aeruginosa* and *S. aureus*, at 40 and 50 mg/ml on *E. coli and* at 40 mg/ml on *P. aeruginosa*, at 60 mg/ml on *E. coli* and *P. aeruginosa* showed no significant antibacterial activities against test organisms. The water leaf extract at 40 and 50 mg/ml on *E. coli*, at 60 mg/ml on *E. coli* and at 30 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms.

The ethanol and the methanol leaf extracts of *V. amygdalina* at 30 mg/ml against *E. coli*, methanol and hexane leaf extracts at 40 mg/ml against *E. coli* showed no significant different antibacterial activities against test pathogens. Ethanol and hexane leaf extracts at 30 mg/ml against *P. aeruginosa*, methanol and ethanol leaf extracts at 60 mg/ml against *P. aeruginosa* and at 30, 40, 50 and 60 mg/ml against *S. aureus*, all extract solvents except water against *S. aureus* showed no significant different antibacterial activities against test pathogens.

Arekemase *et al*., (2013) also reported that ethanol leaf extracts at 30 mg/ml showed inhibition zone of 20.5 mm against *E. coli*, 10.5 mm against *P. auroginosa,* 22.2 mm against *S. aurous* and aqueous leaf extract at 30 mg/ml showed 17.1 mm against *E .coli*, 17.5 mm against *P. aeruginosa* and 19 mm against *S. aureus.* Kesatebrhan (2013) also reported**,** Methanol leaf extracts of *V. amygdalina* showed 11.8 and 14.7 mm inhibition zone against *E. coli* and *S. aureus* at 40 mg/ml respectively. .

Table 10: Antibacterial activities of crude extract of the leaves of *V. amygdalina* against the test organisms (Mean ± SD, n=3)

Test organisms	Con. mg/ml Crude extracts	Zone of Inhibition(mm) ElE	MlE	HlE	WlE	SDW	Chloramphenicol (0.1 mg/ml)
E.coli	20	0	0	0	0	0	
	30	9.20 ± 0.34^{Ab}	9.23 ± 0.40^{Ab}	10.43 ± 0.37^{Ac}	6.00 ± 0.00^{Ea}	0	
	40	11.67 ± 0.54^{Cc}	11.14 ± 0.00^{Bb}	11.00 ± 0.55^{Bb}	6.83 ± 0.84^{Fa}	0	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}	0	
	60	16.10 ± 0.37^{Ed}	14.53 ± 0.47^{Fc}	12.00 ± 0.00^{Db}	8.43 ± 0.40^{Ga}	0	
P.aeruginosa	20	0	0	0	0	0	
	30	10.43 ± 0.40^{Bb}	13.50 ± 0.30^{Dc}	10.63 ± 0.23^{Ab}	1.30 ± 0.10^{Aa}	0	
	40	12.45 ± 0.11^{Cc}	14.90 ± 0.14^{Fd}	11.00 ± 0.53^{Bb}	2.00 ± 0.21^{Ba}	0	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}	0	
	60	16.93 ± 0.25^{Fc}	16.43 ± 0.40^{Hc}	12.43 ± 0.40^{Db}	3.63 ± 0.37^{Da}	0	
S. aureus	20	0	0	0	0	0	
	30	12.66 ± 0.41^{Cc}	12.36 ± 0.35^{Cc}	10.20 ± 0.26^{Ab}	8.43 ± 0.20^{Ga}	0	
	40	14.18 ± 0.11^{Db}	14.00 ± 0.34^{Eb}	14.76 ± 0.33^{Ec}	11.60 ± 0.54^{Ha}	0	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}	0	
	60	18.40 ± 0.40^{Hb}	18.36 ± 0.32^{Ib}	18.33 ± 0.30^{Gb}	16.36 ± 0.40^{Ja}	0	

Key: ElE = ethanol leaf extract, MlE =methanol leaf extract, HlE= Hexane leaf extract, chloramephenicol = positive control, sterile distil water (SDW) = 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($p<0.05$)

4.2.6. Antimicrobial activities of crude extracts of the roots of *V. amygdalina* against the test organisms

The *in-vitro* assays of antibacterial activities obtained from roots of *V. amygdalina* using four different solvents are presented in Table 11. As can be seen from the table, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous root extracts were in the range of 6.63 – 14.80 mm, 8.93- 20.00 mm, 8.66 – 19.76 mm and 7.16- 10.43 mm, respectively. All crude extracts of *V. amygdalina* roots showed a significant growth inhibition against all three tested organisms except at a concentration of 20 mg/ml for all solvent extracts showed no significant antibacterial activities against all test organisms (Table 11).

Table 11; show that, the ethanol root extract at 30 mg/ml on *E. coli*, at 40 mg/ml on *P. auruginosa* and at 50 mg/ml on *S. aureus*, at 40 mg/ml on *E. coli*, showed no significant different antibacterial activities against test organisms. At 50 mg/ml on *P. auruginosa*, at 60 mg/ml on *S. aureus*, at 60 mg/ml on *E.coli* and *P. aeruginosa*, at 30 mg/ ml on *P. aeruginosa* and at 40 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. The methanol root extract at 30 mg/ml on *E .coli* and at 40 mg/ml on *S. aureus*, at 40 mg/ml on *E. coli* and at 30 mg/ml on *P. aeruginosa*, at 40 mg/ml on *P. aeruginosa* and at 50 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. The hexane root extract at 30 and 40 mg/ml on *E. coli* and at 30, 40, 50 mg/ml on *S. aureus*, at 60 mg/ml on *E. coli* and *S. aureus* showed no significant antibacterial activities against test organisms. The water root extract at 30, 40 and 50 mg/ml on *E. coli* and at 30 and 40 mg/ml on *P. auruginosa* and at 30 mg/ml on *S. aureus* showed no significant different antibacterial activities against test organisms. At 50 mg/ml on *P. aeruginosa* and at 40 mg/ml on *S.aureus*, at 60 mg/ml on S. *aureus* and *P. aeruginosa* showed no significant different antibacterial activities against test organisms.

The ethanol and the methanol root extracts of *V. amygdalina* at 50 and 60 mg/ml against *E. coli* showed no significant antibacterial activities against test pathogens. Methanol and hexane root extracts at 30 and 60 mg/ml against *P. aeruginosa* showed no significant antibacterial activities against test pathogens. Methanol and hexane root extracts at 30 mg/ml against *S. aureus,* hexane and water root extracts at 40, 50 and 60 mg/ml against *S. aureus* showed no significant antibacterial activities against test pathogens.

Antimicrobial activities of extracts the roots of *V. amygdalina* against different pathogens were also reported by Arekemase *et al*., (2013) that showed inhibition zones of 17 mm against *E. coli*, 14.5 mm against *P. aeruginosa* and 18.5mm against *S. aureus*.

Table 11: Antibacterial activities of crude extracts of the roots of *V. amygdal*ina against the test organisms (Mean ± SD, n=3)

Test organisms	Con. mg/ml Crude extracts	Zone of Inhibition(mm) ErE	MrE	HrE	WrE	SDW	Chloramphenicol 0.1mg/ml
E.coli	20	0	0	0	0	0	
	30	10.46 ± 0.45^{Cd}	11.43 ± 0.80^{Bc}	8.70 ± 0.26^{Ab}	7.50 ± 0.30^{Aa}	0	
	40	12.00 ± 0.22^{Dc}	12.62 ± 0.99^{Cd}	8.90 ± 0.65^{Ab}	7.95 ± 0.74^{Aa}	0	26.90 ± 0.1^{A}
	50	13.12 ± 0.22^{Ec}	13.30 ± 0.11^{Dc}	9.69 ± 0.33^{Bb}	7.98 ± 0.60^{Aa}	0	
	60	14.80 ± 0.26^{Fc}	14.30 ± 0.51^{Ec}	10.86 ± 0.41^{Cb}	8.00 ± 0.00^{Ba}	0	
P.aeruginosa	20	0	0	0	0	0	
	30	8.40 ± 0.36^{Bb}	12.90 ± 0.36^{Cc}	12.43 ± 0.40^{Dc}	7.16 ± 0.28^{Aa}	0	
	40	10.23 ± 0.11^{Cb}	15.53 ± 0.16^{Fd}	14.00 ± 0.88^{Ec}	7.90 ± 0.31^{Aa}	0	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}	0	
	60	14.66 ± 0.35^{Fb}	20.00 ± 0.43^{Ic}	19.76 ± 0.25^{Gc}	10.43 ± 0.37^{Ea}	0	
S. aureus	20	0	0	0	0	0	
	30	6.63 ± 0.47^{Aa}	8.93 ± 0.30^{Ac}	8.66 ± 0.20^{Ac}	7.86 ± 0.23^{Ab}	0	
	40	8.00 ± 0.51^{Ba}	11.00 ± 0.09^{Bc}	8.98 ± 0.93^{Ab}	8.70 ± 0.04^{Cb}	0	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}	0	
	60	12.43 ± 0.40^{Db}	16.06 ± 0.11^{Gc}	10.43 ± 0.40^{Ca}	10.40 ± 0.40^{Ea}	0	

Key: ErE = ethanol root extract, MrE =methanol root extract, HsE= Hexane root extract, chloramephenicol = positive control, sterile distilled water (SDW) = 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($p<0.05$)

When the growth inhibition effect of the 24 crude extracts against bacterial pathogens is compared with the antibiotics used as the positive control, the effect observed by the positive controls was very high. *S. aureus* was found to be the most susceptible bacteria than the other two against the positive control, chloramphenicol (Table 6 – 11).

The present study was carried out to examine the antibacterial activities of different parts (leaf, stem bark and root) of *C. procera* and *V. amygdalina* extracted by four different solvents i.e., methanol, ethanol, hexane and aqueous, against three important test organisms i.e., *E. coli*, *S. aurues* and *P. aeruginosa.* As indicated in all tables (6-11) 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. Cown (1999) 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 (Preethi, 2010). The antibacterial activities observed could be due to the presence of secondary metabolites. Some other reporters had also reported that various parts of these pants (root, leaf, flower and stem bark) showed antimicrobial activities (Kawo *et al.*, 2009; Kareem, 2008; Bhaskar, 2000). 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, water), incorrect preparation and dosage (concentration) (Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007).

Several investigators had also reported that plants contain antibacterial or antimicrobial substances (Ijeh *et al.*, 2006). 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 (Arekemase, 2011). Similarly, a number of studies have also reported the antimicrobial efficacy of ethanol, methanol and hexane extracts of other plants (Cvetnic and Vladmir, 2004; Gotep, 2010; Lu *et al*., 2011; Malar *et al*., 2011; Meher, 2013; Wojtyczka, 2013).

4.3. 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 data revealed that the MIC values of the different extracts of *C. procera* showed that the highest activity was recorded against *S. aureus* (22 mg/ml) in ethanol extract of the leaf (Table 12). The lowest activity was obtained against all test organisms (28mg/ml) in aqueous extracts of all parts (root, leaf and stem bark) of *C. procera* and ethanol root extracts against *P. auroginosa*. These finding revealed with the finding as reported by (Kawo *et al*., 2009) lower MICs values were recorded for ethanol extracts of *C. procera* leaf against *E. coli* and *S. aureus*. No inhibition was recorded for all root extracts of *C. procera* against all tested pathogens at all concentrations except root ethanol extract (28mg/ml) *against S. aurous* and *P. aeruginosa* and root methanol extracts against *S. aurous*.

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 Kawo *et al.*, (2009). The same reasons also reported by (Igbinosa *et al.*, (2009) for stem bark extracts of *J. curcas*. 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 12: 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
	Leaf	24^{aB}	26^{bB}	26^{bB}	28^{cA}
E. coli	Stem bark	26^{aC}	26^{aB}	26^{aB}	28^{bA}
	Root	0	0	0	0
	Leaf	22^{aA}	24^{bA}	24^{bA}	28^{cA}
S. aureus	Stem bark	22^{aA}	24^{bA}	24^{bA}	28^{cA}
	Root	28^{aD}	28^{aD}	0	0
	Leaf	26^{aC}	26^{aB}	26^{aB}	28^{bA}
P. aeruginosa	Stem bark	24^{aB}	26^{aB}	26^{aB}	28^{bA}
	Root	28^{aD}	0	0	0

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 $p<0.05$.

The MIC obtained from *V. amygdalina* using Agar dilution method for different part extracts are shown in Table 13 as follows. The MIC value of the different extracts of *V. amygdalina* indicated that the highest activity was recorded against *E. coli* (22 mg/ml) in ethanol and methanol extracts of the leaf (Table 13). The lowest activities were obtained against stem water extract, all solvent root extracts against E. coli (28 ml/gm), Stem water, and all solvents root extracts against *S. aurous* (28 mg/ml) and stem water and all solvent roots extracts against *P. auruginosa* (28 mg/ml).

Generally, the MIC values were recorded for the leaf ethanol and methanol extracts 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.

Table 13: 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 parts	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 $p<0.05$.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

In the present study, different parts of *C. procera* and *V. amygdalina* were collected from Dire Dawa district and Haramaya university main campus, respectively. Their antimicrobial properties were studied on test organisms (*E. coli*, *S. aureus* and *P. auruginosa*). For the bioassay study, the bioactive components of the plant parts (leaf, root and stem bark) were extracted with ethanol 99.5%, methanol 99.8%, hexane 99.8% and sterile distilled water. The crude extracts of the four solvents were all tested for their antimicrobial activities against the selected pathogens described above at five different concentrations (20, 30, 40, 50 and 60 mg/ml).

The bioassay study was undertaken using paper disc diffusion method and revealed that both plant crude extracts with organic solvents showed better results as compared to distilled water. The maximum zone of inhibition was recorded in case of stem of ethanol extract at 60mg/ml of *C. procera* against *S. aureus* ranging 24.50 mm of zone of inhibition (table 6). As well as in case of *V. amygdalina* stem of ethanol extract at 60 mg/ml against *E. coli* ranging 24.40 mm of zone of inhibition (table 9). In case of leaf of ethanol extract at 60 mg/ml of *C. procera* against *P. aeruginosa* ranging 21.50 mm of zone of inhibition (table 7), in case of root of methanol extract at 60 mg/ml against *P. auruginosa* ranging 20.00 mm of zone of inhibition (table 11). In case of root of methanol extract at 60 mg/ml of C. *procera* against *S. aurous* ranging 18.50 mm of zone of inhibition (table 8), in case of leaf of ethanol extract at 60 mg/ml against *S. aureus* ranging 18.40mm of zone of inhibition (table 10).

In conclusion, it was found out that leaves and stems of *C. procera* and *V. amygdalina* have significantly best antimicrobial activity as compared with roots of *V. amygdalina* and *C. procera*. Among the different types of test extracts, the stem and leaf of ethanol extract of *C. procera* (table 9) and the leaf of ethanol and methanol extracts of *V. amygdalina*(table 10) showed the least MIC value at concentration of 30mg/ml against *S. aurous*. The growth inhibition effects of the crude extracts were found to be less than positive control (chloramphenicol). However, the distilled water used as a negative control was incapable to show any growth inhibition effect against all the tested pathogens.

5.2. Conclusion

The present study has shown that the leaves, stem bark and roots of *Vernonia amygdalina* and *Calotropis procera* extracts have antimicrobial effects against some pathogenic organisms *(E.coli, S. aurous P. auruginosa*). 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. The result also indicated that all the plant extracts have antibacterial effect on all test pathogens, showing that they contain active ingredients against the organisms.

Based on the pharmacological results of the present study, it could be said 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. Further research is therefore recommended to isolate, purify and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries. The results of the study also support the folklore claim along with the development of new antimicrobial drugs from both the plant parts.

5.3 Recommendation

Based on the result of the experiments performed in the present study, the following points are suggested.

- This study has shown that the stem and leaf extracts of both plants (*C. procera* and *V. amygdalina*) are more effective against the tested bacteria than the root part extracts. Therefore, it is recommended that these plant parts should be used for better antimicrobial activities of these pathogens.

- There is a need for conducting more studies to identify and characterize the medicinal principles in the tested plant, which may serve as novel compounds for development of new and more effective antimicrobial drugs. This would prove very useful especially in this time when drug resistance is a major issue.

- It is worth noting that *in vitro* finding is not always dependable because plants, which are effective *in vitro*, might not work when used *in vivo*. Therefore, it is recommended that further identification of the active constituents is needed to evaluate the efficacy and safety *in vivo* against the test pathogens

- Finally, there is need for further research on the active principles, to purify them and to carry out toxicological studies before they can be formulated into dosage forms for use against potential disease causing microbes.

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APPENDIX

7. APPENDICES

7.1. Appendix Figures

Figure 1: collection of *calotropis procera* Figure 2: collection of *Vernonia amygdalina*

Figure 6: Laboratory activities

Figure 7: showed the antibacterial activities of leaves of *C. procera* and *V. amygdalina* against various pathogens

E. coli *P. aeruginosa* *S. aureus*

Figure 8: showed the antibacterial activity of stems of *C. procera* and *V. amygdalina* against various pathogens

E. coli *P. aeruginosa* *S. aureus*

Figure 9: showed the antibacterial activity of roots of *C. procera* and *V. amygdalina* against various pathogens

E. coli *P. aeruginosa* *S. aureus*

7.2. Appendix Tables

Table 1: ANOVA values for the comparison of the antibacterial effect of the crude extract of the leaf of *C.procera* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	560.74	186.40	972.40	<0.0001	43.53
E. c	Methanol	11	385.269	127.58	405.02	<0.0001	38.03
	Hexane	11	585.00	194.31	752.18	<0.0001	51.56
	Water	11	86.60	27.94	80.81	<0.0001	25.18
Chloramephenicol		11	860.07	286.69	1124.27	<0.0001	39.96
	Ethanol	11	221.85	72.72	158.09	<0.0001	26.65
S. a	Methanol	11	220.41	72.91	350.00	<0.0001	34.21
	Hexane	11	255.65	84.39	273.71	<0.0001	40.91
	Water	11	226.56	75.52	224.87	<0.0001	43.30
Chloramephenicol		11	1003.44	333.65	1070.53	<0.0001	48.56
	Ethanol	11	538.33	178.47	488.96	<0.0001	37.98
P. a	Methanol	11	457.08	151.02	302.05	<0.0001	40.03
	Hexane	11	333.40	109.77	215.60	<0.0001	38.38
	Water	11	138.40	45.62	272.76	<0.0001	46.51
Chloramphenicol		11	755.83	251.40	1246.63	<0.0001	34.90

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*

Table 2: ANOVA values for the comparison of the antibacterial effect of the crude extract of the stem of *C.procera* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	486.91	161.41	484.25	<0.0001	36.79
E. c	Methanol	11	361.52	119.60	352.64	<0.0001	34.00
	Hexane	11	296.90	96.53	603.27	<0.0001	39.23
	Water	11	195.54	64.17	170.76	<0.0001	38.94
Chloramephenicol		11	857.48	285.82	836.56	<0.0001	39.86
	Ethanol	11	894.12	297.32	1101.18	<0.0001	36.79
S. a	Methanol	11	557.88	184.93	480.35	<0.0001	34.00
	Hexane	11	400.20	132.13	277.69	<0.0001	39.23
	Water	11	194.25	62.75	83.66	<0.0001	38.94
Chloramephenicol		11	998.95	332.25	1208.18	<0.0001	39.86
	Ethanol	11	538.41	178.47	488.96	<0.0001	45.19
P.a	Methanol	11	457.08	151.52	302.05	<0.0001	27.98
	Hexane	11	333.409	109.77	215.60	<0.0001	39.41
	Water	11	138.40	45.85	272.76	<0.0001	35.02
Chloramephenicol		11	755.83	251.40	1246.63	<0.0001	34.98

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*

Table 3: ANOVA values for the comparison of the antibacterial effect of the crude extract of the root of *C.procera* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	178.08	59.11	644.90	<0.0001	40.54
E. c	Methanol	11	203.72	67.37	335.47	<0.0001	36.96
	Hexane	11	152.52	50.36	282.43	<0.0001	36.18
	Water	11	36.55	11.72	68.30	<0.0001	27.41
Chloramphenicol		11	906.89	302.09	4027.98	<0.0001	41.61
	Ethanol	11	207.70	68.13	165.17	<0.0001	31.39
S. a	Methanol	11	333.96	110.79	563.37	<0.0001	36.89
	Hexane	11	250.83	82.99	358.24	<0.0001	46.58
	Water	11	211.94	70.20	425.50	<0.0001	47.88
Chloramphenicol		11	1005.12	334.15	1002.46	<0.0001	48.60
	Ethanol	11	272.70	90.64	921.77	<0.0001	42.52
P. a	Methanol	11	233.10	75.82	107.81	<0.0001	37.63
	Hexane	11	360.86	28.83	508.9	<0.0001	54.50
	Water	11	86.97	28.83	508.92	<0.0001	34.92
Chloramphenicol		11	773.84	257.50	1537.33	<0.0001	35.55

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*

Table 4: ANOVA values for the comparison of the antibacterial effect of the crude extract of the leaf of *V.amygdalina* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	510.91	169.63	678.55	<0.0001	48.39
E. c	Methanol	11	482.25	159.86	479.58	<0.0001	48.15
	Hexane	11	74.00	23.55	56.53	<0.0001	23.57
	Water	11	182.66	60.44	362.66	<0.0001	35.95
Chloramphenicol		11	893.66	297.00	891.00	<0.0001	40.66
	Ethanol	11	428.56	138.90	93.85	<0.0001	37.88
S. a	Methanol	11	408.76	132.66	98.45	<0.0001	37.11
	Hexane	11	449.30	141.25	44.21	<0.0001	40.55
	Water	11	440.48	145.35	262.28	<0.0001	51.90
Chloramphenicol		11	1003.44	333.65	1070.53	<0.0001	48.56
	Ethanol	11	381.68	126.12	305.14	<0.0001	39.98
P.a	Methanol	11	302.23	99.27	180.23	<0.0001	33.85
	Hexane	11	92.96	29.02	39.40	<0.0001	25.18
	Water	11	51.94	15.89	29.85	<0.0001	51.33
Chloramphenicol		11	748.92	247.21	271.16	<0.0001	34.04

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*

Table 5: ANOVA values for the comparison of the antibacterial effect of the crude extract of the **stem** of *V.amygdalina* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	709.87	234.56	303.02	<0.0001	42.14
E. c	Methanol	11	855.60	266.70	38.45	<0.0001	49.99
	Hexane	11	102.48	33.21	93.67	<0.0001	24.67
	Water	11	50.01	23.84	40.99	<0.001	31.28
Chloramphenicol		11	871.21	288.89	509.81	<0.0001	40.11
	Ethanol	11	447.32	147.48	241.77	<0.0001	22.85
S. a	Methanol	11	417.56	135.91	110.57	<0.0001	15.38
	Hexane	11	464.59	152.96	214.43	<0.0001	8.36
	Water	11	122.58	38.32	40.19	<0.0001	17.12
Chloramphenicol		11	1003.44	333.66	1070.53	<0.0001	31.74
	Ethanol	11	100.12	32.41	89.61	<0.003	28.82
P.a	Methanol	11	34.36	10.75	40.83	<0.003	16.53
	Hexane	11	100.10	30.76	31.52	<0.0001	26.79
	Water	11	23.96	11.50	72.42	<0.001	17.86
Chloramphenicol		11	748.92	247.21	271.16	<0.0001	34.04

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*

Table 6: ANOVA values for the comparison of the antibacterial effect of the crude extract of the root of *V.amygdalina* using four extracting solvents of different concentration on the test pathogen

	Solvent	DF	SS	MS	F-value	P>F	CV
	Ethanol	11	378.41	124.23	173.95	<0.0001	41.94
E. c	Methanol	11	479.71	158.73	361.44	<0.0001	44.46
	Hexane	11	58.74	17.86	27.80	<0.0001	24.51
	Water	11	60.25	18.13	14.54	<0.001	28.36
Chloramphenicol		11	871.21	288.89	509.81	<0.0001	40.11
	Ethanol	11	574.17	190.39	854.31	<0.0001	61.66
S. a	Methanol	11	603.12	198.79	172.99	<0.0001	52.99
	Hexane	11	298.50	97.44	126.13	<0.0001	44.27
	Water	11	82.25	26.75	107.00	<0.0001	29.56
Chloramphenicol		11	1003.44	333.65	1070.53	<0.0001	48.56
	Ethanol	11	520.78	171.89	269.99	<0.0001	56.28
P. a	Methanol	11	697.24	230.30	291.22	<0.0001	47.36
	Hexane	11	405.08	134.59	832.56	<0.0001	38.57
	Water	11	160.91	52.52	126.06	<0.0001	38.56
Chloramphenicol		11	748.92	247.21	271.16	<0.0001	34.04

Key: *E.c = Escherichia coli, P. a = pseudomonas aeruginosa, S.a = Staphylococcus aureus*