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Debre Berhan University
College of Natural and Computational Sciences
Department of Chemistry

MSc Thesis

Investigation of Antibacterial Activity and Phytochemical Screening of Grawa (*VernoniaAmygdalina*) and *MoringaOleifera*Crude Extracts

A Thesis submitted to the School of graduate studies, Debre Berhan University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Organic Chemistry

BY

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APPROVAL SHEET II

We, the undersigned members of the boarded of the examiners of the final open defense by TesfayeSharewwochefo have read and evaluated his thesis entitled "Investigation of Antibacterial Activity and phytochemical screening of Grawa (*Vernoniaamygdalina*) and *Moringaoleifera*Crude Extract" and examined the candidates. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Masters of Science in Organic Chemistry.

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Major Advisor	Signature	Date
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Name of external examiner	Signature	Date
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Name of internal examiner	Signature	Date
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Name of chairman	Signature	Date

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has been submitted in partial fulfillment of the requirements for the degree of masters of Science in organic chemistry at Debre Berhan University. All sources of materials used for this thesis have been duly acknowledged. This paper has never been submitted to and/or presented in any other university, college or institution in candidature of any other degree, diploma, or certificate.

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Date: _____

ACRONMYS AND ABBREVIATIONS

ATCC	American type culture collection
DBU	Debre Berhan University
DMSO	Dimethyl sulfoxide
E.coli	*Escherichia coli*
$FeCl_3$	Iron chloride
H_2SO_4	Sulfuric acid
MDR	Multidrug resistant
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
MO	*Moringaoleifera*
S. aureus	*Staphylococcus aureus*
TD	Traditional medicine
v.amygdalina	*Vernoniaamygdalina*
WHO	World Health Organization

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ABSTRACT

Grawa(*v.amygdalina*) and *moringaoleifera* were the most common traditional medicinal plants used in Ethiopia. The parts of these plants such as stembark, leaves and flowers showed varieties of medicinal properties and cure various human and animal diseases and ailments. The phytochemical screening of the stem bark leaves and flower extracts were qualitatively assessed using standard procedures in this study. Antibacterial activities of ethanol extracts of stem bark, leaves andflower of *v.amygdalina* and *moringaoleifera*were screened against ten bacteria and one yeast these were carried out by the disc diffusion method on Mueller Hinton agar (MHA). The crude ethanol extract of leaf of vernoniaamygdalina/grawa showed13.50+0.75mm zone of inhibition on Candida albican, yeast and12.7+1.2mm zone of inhibition on Listeria monocytogenes, positive bacteria and control drug Chloramphenicol showed 12mm and 17mm zone of inhibition respectively. The ethanol extract showed 13.82+0.67mm zone of inhibition on Pseudomonas aeruginosa,gram,negative bacteria and 16.78+1.23mm zone of inhibition Enterococcus faecalis, gram positive bacteria and control drug Chloramphenicol exhibited 16mm and20mm zone of inhibition respectively. Hence the extracts were highly effective and consistent. The crude ethanol stem bark of venoniaamygdalina extract was subjected to the antimicrobial activity and the results were investigated. The crude extract showed 13.68+1.11 zone of inhibition on the Escherichia coli, gram negative bacteria and 15.75+0.98 zone of inhibition on Staphylococcus epidermidis, gram positive bacteria and the positive control Chloramphenicol showed 10mm and 10 mm respectively. Thus the ethanol stem bark extract showed highly active and consistent on those two bacteriaaginst Candida albican, Listeria monocytogene Pseudomonas aeruginosa and Enterococcus faecalis bacteria.From two part of the plants, the *vernoniaamygdalina*the ethanol extract showed more bacterial activity than the other parts of *moringa oleifera*against nine bacteria(except*Salmonella typhimurium*) and one yeast, indicating that ethanol part of*vernoniaamygdalina*extract have been variety of bioactive compounds.

Key word:vernoniaamygdalina,moringa oleifera

1. INTRODUCTION

1.1.Background

Plants are the richest resource of drug for traditional system of medicines; food supplements modern medicines, pharmaceuticals intermediates, folk medicines, and chemical entities for synthetic drugs. The plants were the main source of drugs for the world population before two hundred and fifty years ago where there was few or no synthetic medicines so that plants derived medicines have made large contribution to human health and to livestock [1].The world health organization (WHO) observes that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concluded that traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness [2].

African traditional medicine abounds in medicinal plants, and the tribal people, wherever they exist, still rely chiefly on herbal medicines. In many parts of Africa, herbal medicine still plays a vital role in health care delivery systems especially in remote places where clinics and hospitals are sparsely located. In these communities, traditional herbalists operate closer to the people, taking advantage of the biodiversity of plant species in such areas to cure various diseases and aliments [3].Herbal medicine also called botanical medicine or phytomedicine refers to the use of any plant part, i.e. seeds, berries, roots, leaves, bark, or flowers for medical purposes. Long practiced outside of conventional medicine, herbalist is becoming more mainstream as scientific research has shown their value in the management of diseases [4, 5].Herbal medicine is the oldest form of healthcare known system to mankind. The use of plants as medicines dates back before the written human history. Almost all countries in the world have an expertise concerned with the therapeutic properties of the local flora [6]. Many drugs commonly used today are of herbal origin. For example, about 25% of the prescriptions of drugs dispensed in the United States contain at least one active ingredient derived from plant material some are made from plant extracts; others are synthesized to mimic a natural plant compound. According to WHO report [7] 119 plant-derived pharmaceutical medicines, where by about 74%is used in modern medicine in many ways is correlated directly with theirtraditional uses as plant medicines by native cultures. Plant products have played an important role in the discovery of new therapeutic agents [8].

A medicinal plant is any plant with one or more of its organ(s) containing substance(s) that could be used for therapeutic purpose (treating and preventing) or from which a precursor for synthesis of useful drugs may be isolated. For centuries, medicinal plants have been widely used by man to treat a variety of illnesses - irrespective of pathogenic origin, hence serving as a fundamental component of indigenous health care systems. Even today, man depends solely on plants for survival and maintenance of health [9].Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs [10].The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed [11]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies[12,13].Medicinal plants are important sources of Traditional Medicine (TM) for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the overgrowing population of the world [14].

Traditional medicine has been practiced in Ethiopia since long time ago, the knowledge largely oral, it has been transferred from one generation to the next through professional healers, traditional medicine still remains the main resource for a large majority (80%) of the people in Ethiopia for treating health problems, traditional medical consultancy has a much lower cost including the consumption of the medicinal plants required than modern medical attention, Ethiopia has about 800 species of plants that are used in such traditional health care system characterized by various shape of the ecological diversities of the country [15].Ethiopia is rich in plant biodiversity. In addition to this, it is found in the tropics where infectious diseases are more prevalent. Report indicated that more than 80% of the people used traditional medicine to treat various systems based on indigenous knowledge [16]. Because of this, various medicinal plants, which have therapeutic potential to treat infected persons, are still given by traditional healers without scientific prescription and optimization of the right doses. *Vernoniaamygdalina*and *Moringa oleifera* are two ofEthiopia medicinal plant. However, most Ethiopian people use *Vernoniaamygdalina* and *Moringa oleifera* having detail knowledge. On the other hand, the chemical composition, phytochemical analysis and antimicrobial activities from stem bark, leaf and flower of *Vernoniaamygdalina* and *Moringa oleifera*and crude extracts have not been exhaustively investigated especially in Ataye, North Shwazone, Amhara Regional State, North Ethiopia. This study focused on the assessment of antibacterial activity

of crude extract (cold maceration) of *Vernoniaamygdalina and Moringa oleifera* and phytochemical screening of medicinal plant.

1.2. Statement of the Problem

The *vernoniaamygdalina* and *moringa oleifera* are known for its use as traditional medicinal plants, and widely used to treat many infectious diseases in some countries especially in Ethiopia. Many studies reported parts of these plant parts (leaf, flower and stem bark) exhibit or show different antimicrobial activities and also there are many secondary metabolites isolated from these plant parts or materials, but based on these result there are no work done on the secondary metabolite and antimicrobial activities of these two plant parts.

1.3. Significance of the Study

The main significance ofthis project is to give adequate information to investigate antibacterial activity and chemical composition of the crude extracts from eachpart of plants.

1.4. Objective

1.4.1. General Objective

The general objective of this project to investigate the antibacterial activity and chemical composition of grawa(*Vernoniaamygdalina*) and *moringa oleifera*crude extract.

1.4.2. SpecificObjective

- ✓ To extractstem bark, leaf and flower of *vernoniaamygdalina*using organic as ethanol using maceration.
- ✓ To extractstem bark,leaf and flower of*moringa oleifera*using organic as ethanol using maceration.
- ✓ To carry out phytochemical screening of ethanol extract of stem bark, leaf and flower of *vernoniaamygdalina*.
- ✓ To carry out chemical test (phytochemical screening) of ethanol extract of stem bark, leaf and flower of *moringaoleifera*.
- ✓ To evaluate the antibacterial activities of the crude extracts of both plants.

2. LITERATURE REVIEW

2.1 Antimicrobial Activity of Natural Products from Plants

Plants are the richest BioSource of natural compounds having antimicrobial [17]. Plant extracts are safe natural substitute to chemical food additives to avoid microbial and oxidative food spoilage. Before large scale production and use of plant extracts, it is important to consider the stability of plant extracts over the period of time under deferent storage conditions [18]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant [19]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenol compounds, and flavonoids, which have been found in vitro to have antimicrobial properties [20].Phytoconstitu ents are the natural bioactive compounds found in plants. They work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions [21]. In many countries different types of plant extracts have been used in traditional medical systems to treat for microbial disease. Phytochemicals present in those plants having antimicrobial and antioxidant properties are the reason for this ability to use them in disease treatments [22]. Antimicrobial activities of some phytochemicals present in such plants have been investigated and the possibility of using them to develop new antimicrobial drugs has also been studied [23]. There is a growing attention for the development of new antimicrobial drugs because pathogenic microorganisms becoming resistance to antibiotics which are in use. Extracts of plants which are obtained from the plant used in traditional medicine are a good source of new antimicrobial drug discoveries. As such investigations on the composition, activity, as well as validation of the use of extracts obtained from medicinal plant is important [24]. Medicinal plants and traditional preparation with antimicrobial activities have been used extensively in the West African regions. These plants of medicinal important have been proven to be very effective even where treatments with antibiotics failed [25].Medicinal plants have been used for many years, for the preparation of traditional medicines from their natural products which possess antimicrobial activities and thus can cure different diseases; the capability of production of definite physiological action on disease causing organisms is due to the presence of secondary metabolici compounds [26].Antibiotics are organic substances produced by microorganisms and are capable of inhibiting the growth of another microorganism at low concentration [27]. The overuse of antibiotics in the treatment of bacterial infections has led to increaseincidencesof resistant pathogens to the available antibiotics [28]. Different types of antimicrobials exist: antibiotics, anti-viral, anti-fungal, anti-

protozoan etc. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources [29].An antimicrobial agent is a secondary metabolite produced by bacteria that hasinhibitory properties against microorganisms which includes antibiotics and synthetic Compounds but with minimal effects on mammalian cells [30].An antimicrobial agent is a chemical compound that in low concentrations can kill or inhibit the growth of a microorganism without causing the host (such as a human or an animal) significant damage. Antimicrobial agents can be naturally produced (like penicillin) by a mould, bacterium, or plants, or synthetically made (like the fluoroquinones). Antimicrobials have had a more positive impact on human health than any other medical discovery. In the early history of antimicrobial agents, it was perceived that infectious diseases had been conquered and were no longer a major threat to human and animal health. However, it was soon observed that bacteria could develop resistance to antimicrobial agents. Due to the spread and persistence of antimicrobial resistance, antimicrobials are losing their effectiveness [31].Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [32]. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria [33].Thus, in the light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [34].

A natural product is a chemical compound or substance produced by a living organism found in nature that usually has a pharmaceutical drug discovery and drug design. Natural products are secondary metabolites or chemical compounds produced by the living organisms and that have the bio-activity. Metabolites are classified into two broad types, primary and secondary. Primary metabolites are essential to growth and life in all living systems and are formed by a limited number of metabolic reactions. Primary metabolites serve as building blocks for the synthesis of macromolecules, proteins, nucleic acids, carbohydrates, and lipids. Secondary metabolites are not essential to the life of the producing organism and are formed from primary metabolites. Many of the secondary metabolites enhance the survival fitness of the organism and may serve for example as chemical weapons used against bacteria, fungi, insects and large animals [35].

2. 2. Genus Vernonia and Its Medicinal Use

The genus name for Vernonia is derived after the English botanist William Vernon who had collected and identified it in the late 1600s [36]. Some species are known as *Ironweed*. Some member species of the genus are edible and are of economic values. They are known for having intense purple flowers though some are grayish. There are numerous distinct subgenera and subsections in this genus. The genus Vernonia comprises about 1000 species of herbs and shrubs in the family Asteraceae (Compositae) [37].Even though it is highly abundant in tropical regions, Vernonia distribution is cosmopolitan being found both in Old and New worlds. Members of the genus Vernonia grow in a wide range of habitats and climatic conditions. They grow well in areas including tropical forest; tropical savannahs; dry and marsh habitats; wet and even frosty regions. Morphologically member species of the genus are composed of liana, herbaceous, shrubs and trees [38].

Vernoniais one of the largest genera of flowering plants in the Asteraceae family, which includes more than 1500 species distributed widely in the tropical and sub-tropical region of Africa, Asia and America. It has two major centers of origin, South America and tropicalAfrica, with approximately five hundred species found in Africa and Asia, three hundred in Mexico, Central and South America and sixteen in the USA. Of the five-hundred species found in Africa, thirty are endemic to Kenya [39, 40]. The majority of these plants are used as ornaments and vegetables, while others are considered as weeds in agriculture. The vegetables have a bitter taste, hence the name “the bitter *genus* Vernonia” [41].

2.3. Genus Moringa and Its Medicinal Uses

The genus Moringa is one of the genera found in the Moringaceae family along with Anoma andHyperanthera. It is well-known as the “drumstick” or “horseradish” family. The Moringa genuscomprises 13 species distributed through southwest Asia, southwest Africa, northeast Africa, andMadagascar. Among the 13 species, current research is limited to *Moringa oleifera*, *Moringa stenopetala*,*Moringaconcanensis*, and *Moringa peregrina.* As the other species are endemic to Madagascar andNortheast Africa, they are being evaluated less as there is less exploration for naturally occurring bioactive substances in these locations. In contrast, M. oleifera, which is native to India, is beingstudied widely. As a result, the species has been cultivated throughout the world, specifically inAsia, Latin America, Florida, the Caribbean, and the Pacific Islands [42].

Moringahas been used in the traditional medicine passed down for centuries in many cultures around the world, for skin infections, anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation and diabetes [43].Moringa species is one of the world's most useful plants; it is a fast-growing, much more drought-tolerant and multi-purpose tree that it has been described as a 'miracle tree' [44]. Among the wide range of uses it provides are human food, fuel wood, livestock forage, medicine, dye, water purification, soil and water conservation, quality of cooking oil, green manure and the tree is used as source of income for Moringa growers [45].

The nutritional characteristics of the Moringa tree are excellent so it can easily be used as a fresh forage material for cattle. The leaves are rich in protein, carotene, iron and ascorbic acid and the pod is rich in the amino acid lysine [46]. Moringa is especially useful as forage for cattle both economically and productively given the problems facing typical cattle breeders. The most interesting point in Moringa is all part of the trees except the wood are edible and these edible portions are extremely nutritious. Therefore, Moringa can be serves for human's nutrition and animals feed. Moringa contains many essential nutrients, for example, vitamins, minerals, amino acids, beta-carotene, antioxidants and omega 3and 6 fatty acids [47].

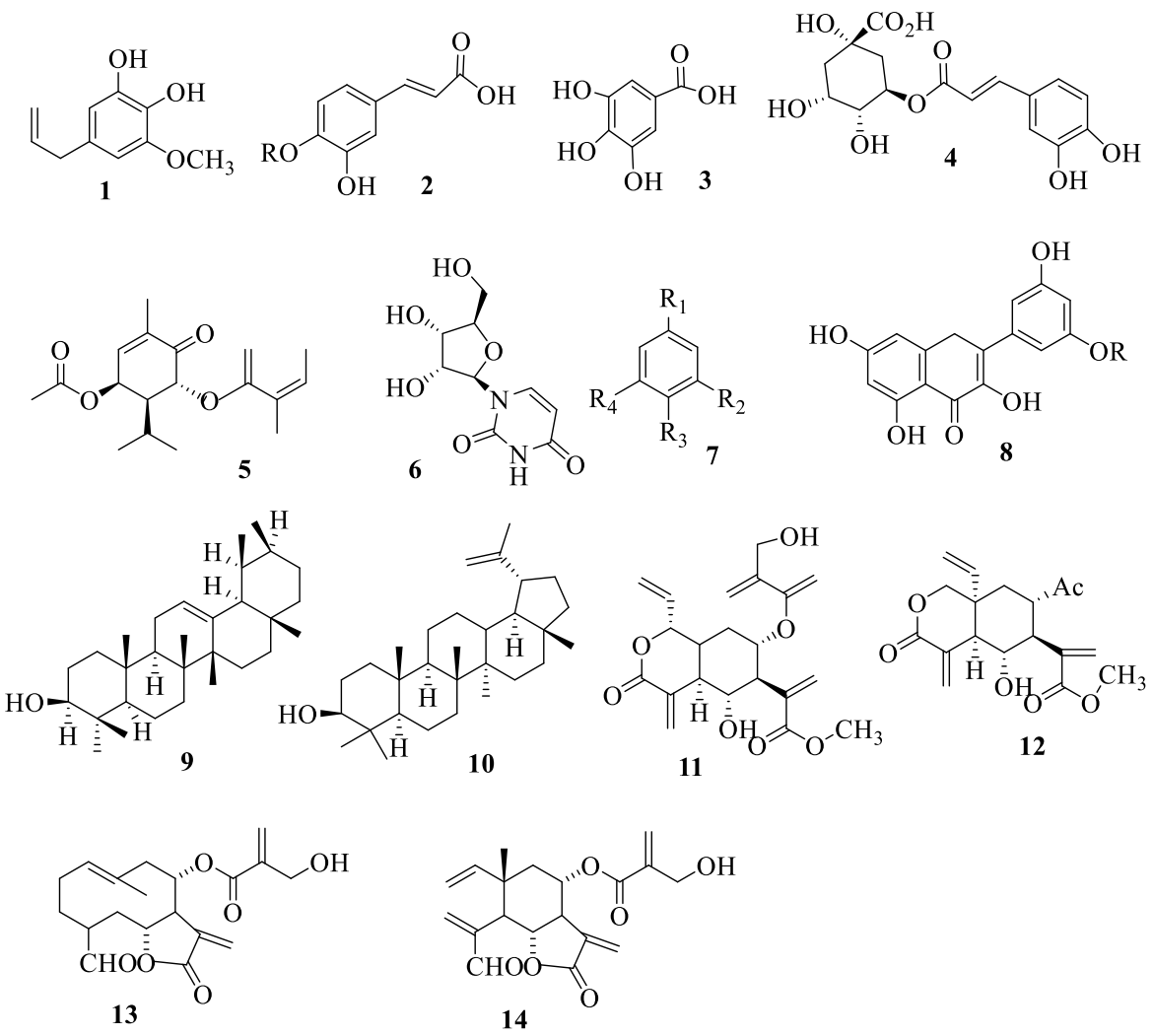
2.4. Chemical Compositions and Biological Activities of Genus Vernonia

Among the diverse biological activities, antibacterial studies are the most reported in the genusvernonia. Antibacterial activity was found to be common to all species in all extracts followed by analgesic, antipyretic, anti-inflammatory and anti-parasitic activity. Antibacterial compounds are mainly lipophilic and will partition from an aqueous phase into bacterial membrane structures, causing expansion of the membranes, increased fluidity, disordering of the membrane structure and inhibition of membrane embedded enzymes [48].

The variety of secondary metabolites extracted from Vernoniaspecies, explains the diversity of their biological activities. some of bioactive compound from vernonia species are: 2-hydroxy-3-methoxy-5-(2-

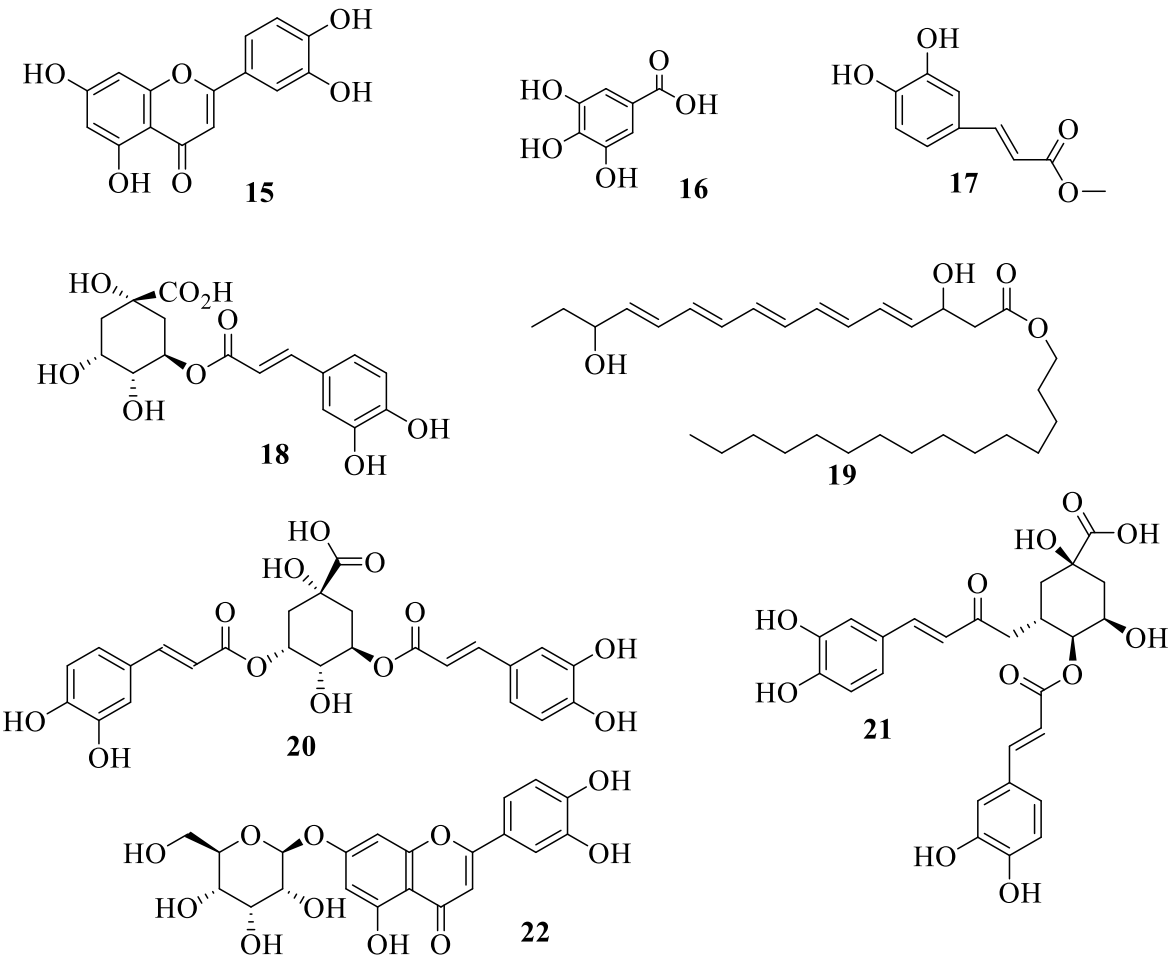
propenyl) phenol (1), isoferulic acid, caffeic acid (R=CH_3, R=H respectively) (2), gallic acid (3), chlorogenic acid (4), vernonione (5) uridine (6),vanillic acid (COOH,OCH_3,OH,H**)** and mega llate (COOH,OH,OH,OH) for (R1,R2,R3, R4)respectively (7), methylquercetin (R=CH_3) and q

uercetin (R=H)(8),α amyrin(9), lupeol(10), vernodalol(11) lasiopulide(12) ,vernopicrin (13) an d vernomelitensin (14) skeletal type (scheme1) as follow.



Scheme 1: Bioactive compounds isolatedfrom Vernonia genus

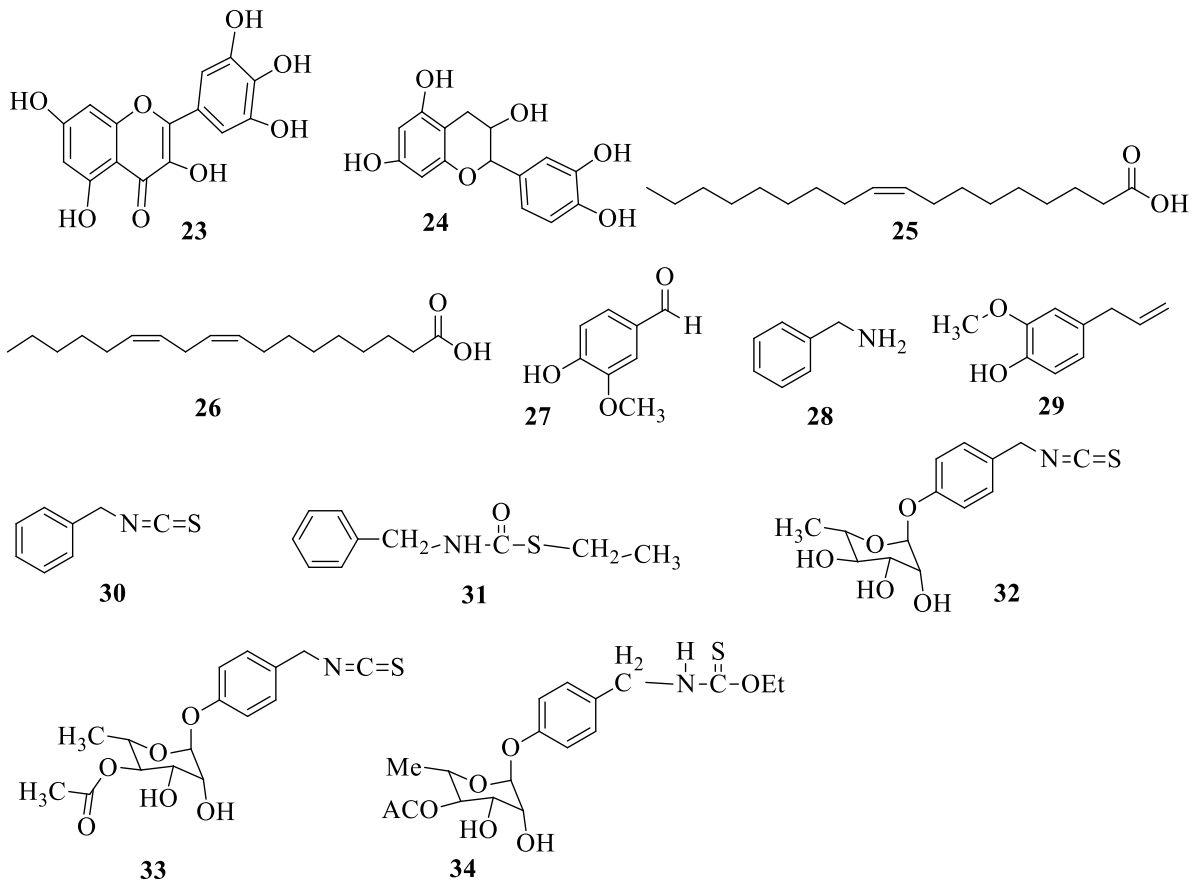
Various compounds have been isolated from genus of *Vernonia*with the flavonoids being among the major classes of compounds [49]. The presents Vernonia species identified to behav ing bioactive compounds from flavonoids are luteolin (15), gallic acid (16), methyl caffeate (17), chlorogenic acid(18),urticifolene(19),3,5 dicaffeoylquinic acid (20),3,4 dicaffeoyl quinic aci d (21) and luteolin7-O-B glucoside (22) in skeletal structure (scheme2) [50].



Scheme 2: Vernoniaspecies identified bioactive compound from flavonoid.

2.5.Chemical Compositions and Biological Activities of Genus Moringa

Moringa species contain various phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The diversity of thesephytochemicals in the genus contributes to its numerous pharmacological uses. The extract obtained from the leaves of moringa in 80% ethanol contains growth enhancing principles (hormones of the cytokines type). The extract can be used in the form of a foliar spray to accelerate the growth of young plants. Use of growth hormone spray will also cause the plants to be firmer and more resistant to pests and disease [51].Different types of compounds were identified from the genus moringa like myricetin (23), epicatechin (24) oleic acid (25) linoleic acid (26) vanillin(27),benzylamin(28), eugenol(29), benzyl isothiocyanate (30), aglycon of deoxy-niazimicine (N-benzyl, S-ethylthioformate (31) ,4-(-L - rhamnopyranosyloxy) benzyl isothiocyanate (32), 4 (4'O acetyl α L rhamnopyranosyloxy) benzylisothiocyanate (33) niaziminin (34), [52].



Scheme3: Structures of selected phytochemicals identifiedfrom moringa species.

2.6. Botanic Description of *VernoniaAmygdalina*

Vernoniaamygdalina(VA) is a shrub that grows up to 3 meters high with petiolate leaf of about 6 mm diameter and elliptic shape. It is scientifically characterizedas belong to the Kingdom Plantae. It is an angiosperm of the order Asterales, familyAsteraceae, and genusVernoniaand species *V. amygdalina*. The full binomial name is*Vernoniaamygdalina*Del. It is extensive in East and West Africa especiallyZimbabwe, Cameroon and Nigeria. Furthermore, it is also found in Asia and isparticularly popular in Malaysia and Singapore. In Nigeria, it is also called as“Chusardoki or fatefate” in Hausa, “Ewuro” in Yoruba language, “Onugbu” in Igbolanguage, “Ityuna” in Tiv, “Oriwo” in Bini language, “Etidot” in Cross River State ofNigeria while in Malaysia and Kenya, it is named as South African leaf and DaunBismillah in malay language. In China, it is more commonly known as Non-tree South. It has 18% protein, 8.5% fiber in a dry matter and anexcellent composition ofmicroelements and macro elements. It is also publically known as bitter leaf due to its bitter taste but the bitterness can be subsided by soaking the leaves in few changes ofwater or by boiling. The anti-nutritional factors like

alkaloids, saponins, glycosidesand tannins are the reasons that cause the bitter taste. It can be utilized as an active anticancer agent, anti-malaria, anti-bacteria and even anti-parasites [53-56].

*V. amygdalina*is a perennial soft wooded shrub that can reach up to 10 m in height with 40 cm stem diameter. The bark is densely pubescent at the young stage, as the plant gets matured, the bark turns to grey then to brown in color, smooth, and disclosed. The leaves are arranged alternately with each other, simple with 0.2 to 4 cm long petioles. The leaf blade is ovate-elliptical to lanceolate and measures 4-15 cm x 1-4 cm, cuneate or rounded at base, shortly acuminated at the apex of the leaves and the margins are clearly toothed to coarsely serrate, finely pubescent but oftenglabrescent. The inflorescence of this plant is in the form of head, arranged in terminal, compound, and umbel-like cymes. The head stalk measures about 1 cm long and pubescent. The flowers are bisexual, regular, strongly exerted from the involucres which are cylindrical to broadly ellipsoid. As the flower developed into fruit, the fruit is a 10-ribbed achene measuring 1.5 – 3.5 mm long, pubescent and glandular and brown to black in color, crowned by a much longer [57].

2.7. BotanicDescription of *Moringa Oleifera*

Moringa oleifera is commonly termed the “drumstick tree”. Other common names include horseradish tree, ben oil tree, or benzoil tree. Some parts of moringa tree (leaves, pods, seeds, flowers, fruits and roots) are eaten as food and some are taken as a remedy. *Moringa oleifera* is fast-growing, deciduous tree. Its maximum height is 10–12 m, while its trunk can reach a diameter of 45cm. The flowers are approximately 1.0–1.5 cm long and 2.0 cm wide. Flowering starts within the first six months after planting. The fruit is a droopy, three-sided brown capsule, 20–45 cm in size and contains dark brown, spherical seeds of about 1 cm diameter. The seeds have three thin, whitish wings, which are responsible for the smooth distribution of the seed by water and wind [58].

*Moringa oleifera*also known as Aleko (Konso), Shiferaw (Amharic), Kalan'gi (Hamer-Bena), Ben-oil tree, cabbage tree, horse-radish tree (English). It is deciduous tree that reaches height of up to 10m, usually smaller, pale feathery foliage. *M. oleifera*originates from India and was introduced to Ethiopia long ago. The tree is now naturalized in many parts of southernEthiopia. Konso people plant *M. oleifera*around their homesteads and also in the terraced fields [59].

2.8. The Uses of *V.Amygdalina*

*Vernoniaamygdalina*is a valuable medicinal is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavor, and can be used as an active anticancer, antibacterial, anti-malarial and anti-parasitic agent. These plantcontains complex active components that are useful pharmacologically. In ethno medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort. Many West African countries such as Cameroon, Ghana and Nigeria use the stem and root as chewing sticks [60]. It is also documented that *V. amygdalina*has been used traditionally in blood clothing and has elicited a substantial reduction in the level of glucose in the blood at post-prandial time point. The plant is commonly known as bitter leaf and is a popular African vegetable. In Nigeria the macerated leaves of the plant are used in making soup while the water extract serves as tonic drink for prevention of certain illnesses. The leaves have found relevance in traditional folk medicine as anti-helminthes and anti-malarial activity. In Ethiopia the leaves of the plant are used to treat skin wound by Zay people. It is one of the traditionally used anti fertility plants in Ethiopia. Hydro alcoholic extract of the leaves was reported to be used in Ethiopia as traditional medicine for fertility regulation. Preliminary study also confirmed that the plant has anti-fertility effect [61].The characteristic bitter taste is believed to have after taste of sweetness. The peeled stem is often used as chewing stick for cleaning the teeth and is very effective to prevent dental carries [62]. The herb not only lowers the body sugar level sufficiently; it also plays a role in the repair of pancreas. If 10 handfuls of fresh leaves are squeezed in 10 liters of water and consumed two glasses thrice a day for a month, diabetes is cured. Nutritionally, *V. amygdalina*is used mainly in soup making in the tropics and also as an appetizer and febrifuge [63] and has successful been used as a supplement in weaning foods [64].

2.9. The Usesof *MoringaOleifera*

The different parts of the MO tree, including roots, bark, leaves, flowers, fruits, and seeds are traditionally used in various therapeutic applications, including, abdominal tumors, hysteria (a psychological disorder), scurvy, paralysis, helminthic bladder, prostate problems, sores and other skin infections. The therapeutic potential and medicinal properties of MO are extensively reviewed [65].*Moringa oleifera*is esteemed as a versatile plant due to its multiple uses. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of

traditional diets in many countries of the tropics and sub-tropics. The leaves of *M. oleifera*are a good source of protein, vitamin A, B and C and minerals such as calcium and iron [66].

In addition, toits substantial uses and nutritional benefits, *M. oleifera*also has a great potential as medicinal plant. The flowers, leaves and roots are used for the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulants in folk remedies. The roots of the young tree and also root bark are rubefacient and vesicant[67].Leaves of*M. oleifera*are traditionally used as purgatives and in the treatment of headaches, hemorrhoids', fevers, inflammation of noise and throat, bronchitis, eye and ear infections, and to combat vitamin Cdeficiency. The leaf juice is believed to control glycaemia and is applied for swollen glands. Leavesof*M. oleifera*are cooked and eaten like spinach or used to prepare soups and salads. Fresh leaves havebeen reported to contain vitamin C and vitamin A, more than those reported in carrots and oranges [68].

Ethiopia has environment conduciveness and labor potential country. Many products can associate in market for economy. For example, Moringa leaf powder in tea bag, *Moringa* f ortified fruit juice/honey, *Moringa* in capsule/tablets, Moringafortified confectionaries and Moringafresh leaf and so on. Moringaseeds are effective against skininfectingbacteria. The leaf juice has a stabilizing effect on blood pressure. The leaf juice controls glucose levels in diabetic patients. Fresh leaves and leaf powder are recommended for tuberculosis patients because of the availability of vitamin A that boosts the immune system. If leaf juice is used as diuretic; it increases urine flow and cures gonorrhea. Leaf juice mixed with honey treats diarrhea, dysentery and colitis (colon inflammation). Fresh leaves are good for pregnant and lactating mothers; they improve milk production and are prescribed for anemia. Paste made from bark treats boils. Paste from ground bark can be applied to relieve pain caused by snake, scorpion and insect bites. Oil is sometimes applied externally for skin diseases. Markets for Moringaleafexist at both the local and international levels.Thus, Moringaproducthas ample scope for economic development in Ethiopia [69].

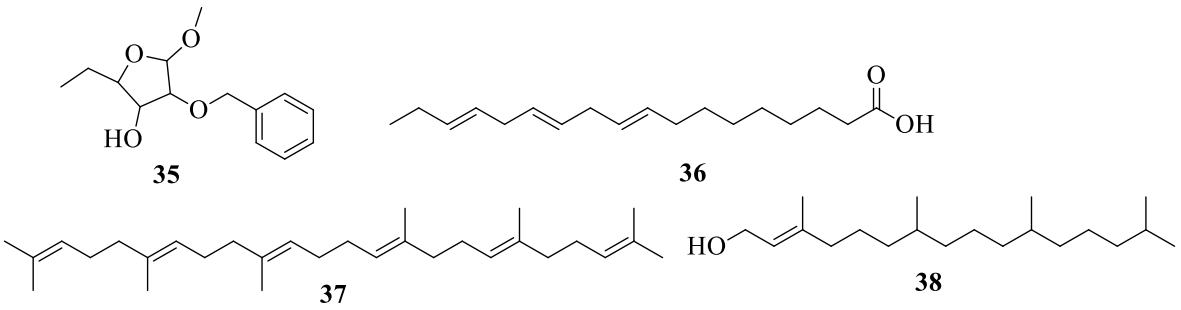
According to Fuglie [70] the many uses for Moringa include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood),fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, bio pesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). Moringa seed oil (yield 30-40% by weight), also

known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products[71].

2.10. ChemicalCompositions and Biological Activities of *VernoniaAmygdalina*

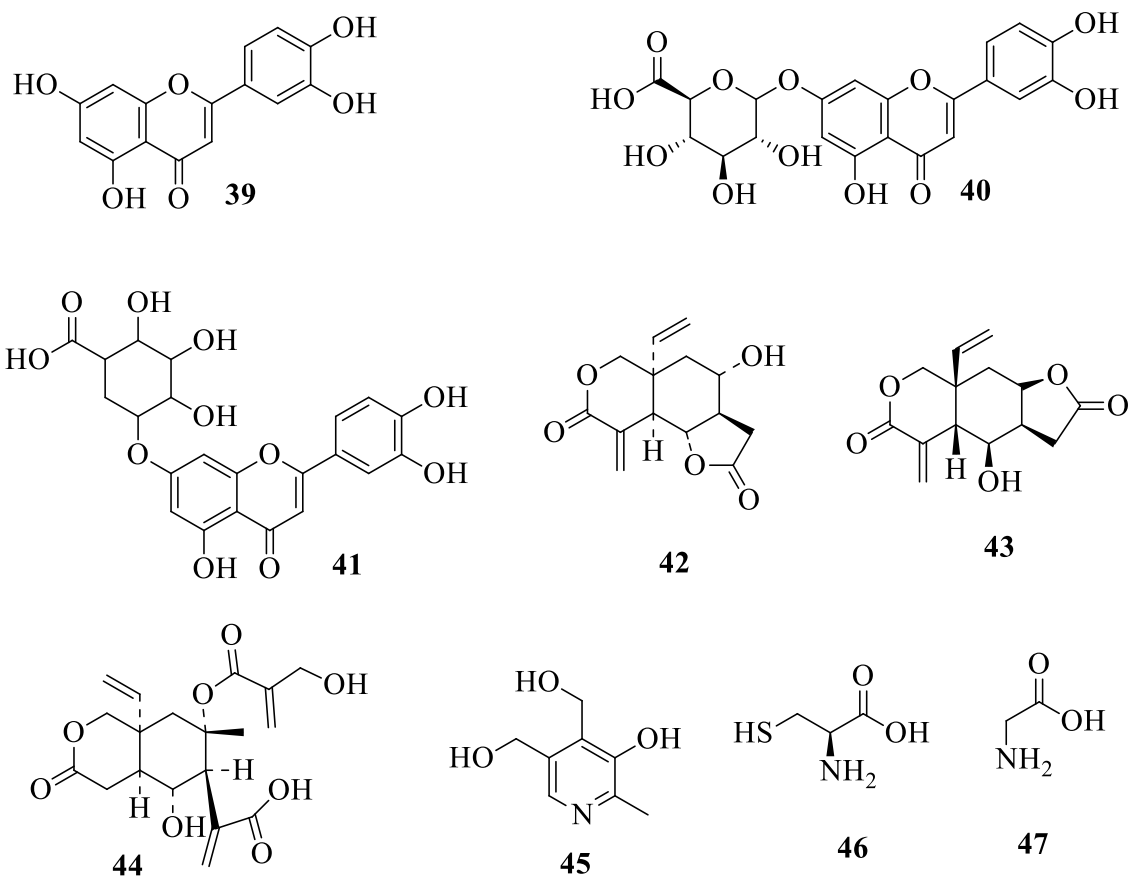
Different studies on *Vernoniaamygdalina*show that it has potential natural product for treatment of various disorders. Its leaf extract was tested for its anticancer activity on mice and was found to be a good candidate for cancer therapy [72] and it was also found to reduce cholesterol induced hyperlipidemia [73]. In addition to its use in treatment of human and animal diseases, extract from *Vernoniaamygdalina*was found to have good potential in preventing plant fungal diseases, which highly damages many of crop plants [74]. In many parts of Ethiopia, leaves of *Vernoniaamygdalina*are used traditionally in washing pots used for preparation of homemade beverage "Tella" [75]. In Nigeria and other tropical African countries, *Vernoniaamygdalina*is used as leafy vegetable. It supplements weaning foods by essential proteins, vitamins and minerals. It also has appetizing and febrifuge effects. *Vernoniaamygdalina*is also a well-known animal feed in Nigeria [76], and though it is not cultivated as animal feed, cattle eat its leaves from naturally growing plants in Ethiopia also. Extracts from leaves have been found to possess antimalarial activity against plasmodium [77, 78] and possess activity against sexually transmitted diseases. Chewing a stick of *V. amygdalina*has been found to have antibacterial activity [79] and water soluble anti-cancer agents have also been discovered from the plant [80].

Traditional health workers in Africa recommend the aqueous extracts of Vernoniaamygdalina as treatment for varieties 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 [81].The peeled stem of *V. amygdalina*can be used as chewing stick for teeth cleaning purposes and it was reported to be very active as it contributed to antiquaries, gum healing, anti-sickling, hemostasis; to stop the blood flow and antimicrobial activity and plaque inhibiting effect[82,83].Structures of compounds abundantly present in the plant V. amygdalina are ethyl-2-O-benzyl-d-arabinofuranoside(35),9,12,15 -octadecatrienoic acid(36), squalene(37),phytol(38) skeletal type(Scheme4).



Scheme 4: Structure of compounds abundantly present in the plant V. amygdalina.

Isolated flavonoids (luteolin (39), luteolin7-O-glucuronide (40), and luteolin 7-O-β glucoside (41)), sesquiterpenes lactones (vernolepin (42), vernomenin (43) and vernodalol (4 4)) and triterpenes (pyridoxine (45), cysteine (46) and glycine (47)) from *V.amygdalina* [84].



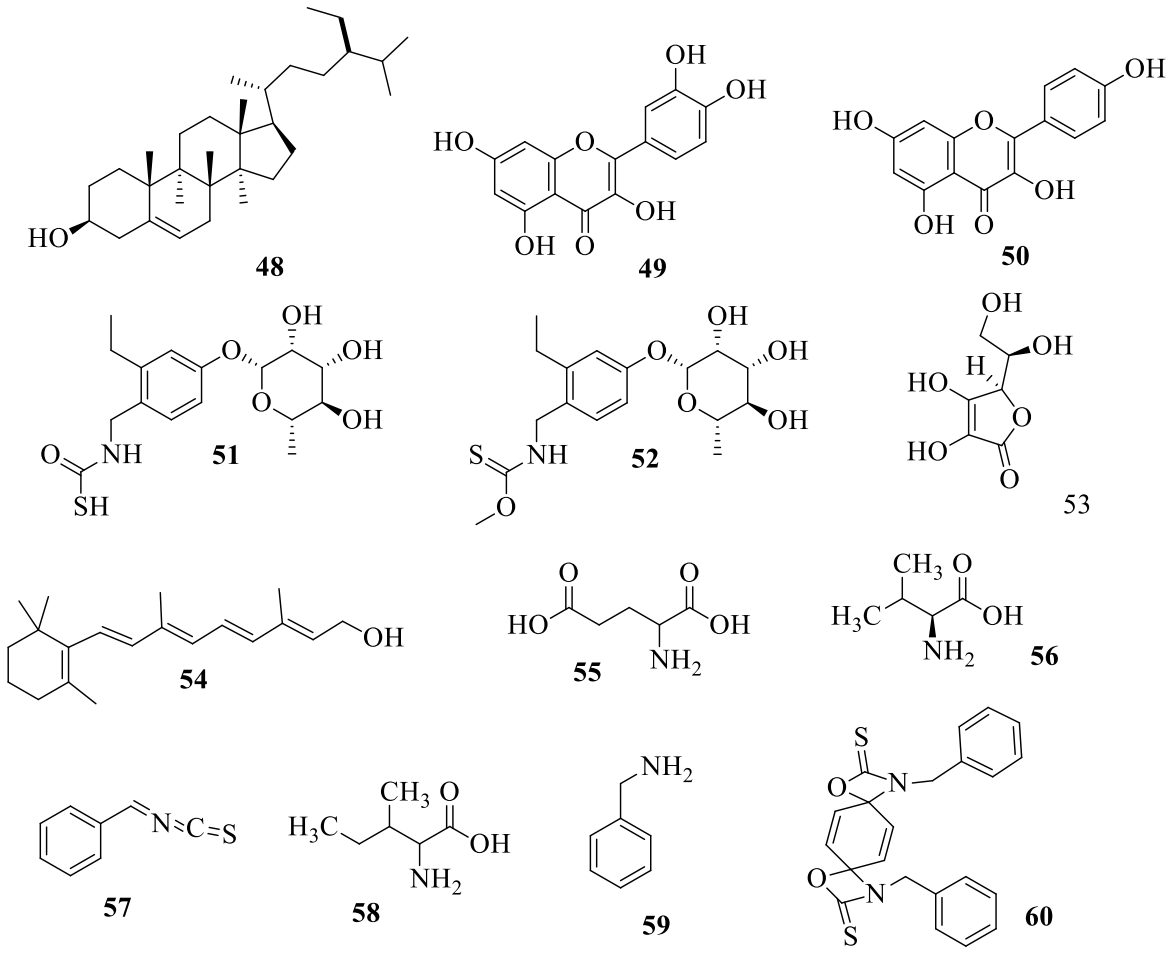
Scheme5: Isolatedflavonoid, sesquiterpenes lactones and triterpenes from *vernoniaamygdalina.*

2.11. Chemical Compositions and Biological Activities of *MoringaOleifera*

Phytochemical refers to only those chemicals which may have an impact on health, or flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients. Phytochemicals of moringa species reveal that the plant family is rich in compounds containing

the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates[85].

Many compounds have been found in *M. oleifera*mainly including phenolic acids, gallic acid, ellagic acid, chlorogenic acid, ferulic acid, glucosinolates, flavonoids, quercetin, vanillin and kaempferol, responsible for its multiple values [86]. The profile of these secondary metabolites varies in different parts of *M. oleifera*Chlorogenic acid, quercetin and kaempferol are major compounds in *M. oleifera*leaves extracts, and the*M. oleifera*leaves also contain abundance of phenolic acids, flavonoids, glucosinolates and isothyocinates [87]. Flowers contains phytosterol, triterpenes, phenolic compounds in high concentration.Procyanidin, sterols, triterpenoids, glycosides, tannins, alkaloids, β-sitosterol and octacosanoic acid are extracted from stem barks[88].Some of the bioactive compound is extracted from *moringa olifera* are β-sitosteol(48), quercetin (49),kaempferol(50),ziazimmicin(51) ,niazinin A(52),ascorbic acid(53), vitamin A(54).Quercetin, a known flavonoid with hepato-protective activity, has been isolated from the alcohol and aqueous extract of Moringa flower. It is used for the treatment of inflammations, muscle diseases, tumours, and enlargement of the spleen. It is reported to cause decrease in serum cholesterol, phospholipids, and triglyceride, hence it is applied in regulation of the ratio of cholesterol to phospholipids. The flowers contain pterygospermin, an antibiotic that is highly effective in the treatment of cholera [89]. The Structure of compound from leaf of *moringa oleifera* extracted as: glutamic acid (55), valine (56), isothiocyan (57) and isoleucine (58), moringine (benzylamine) (59) and Pterygospermin (60) skeletal as follow.



Scheme 6: Structure of compoundextracted from*moringaoleifra* (leaf, stem bark and follower).

3. MATERIALS AND METHODS

3.1. Collection and Identification of the Plant Materials

The leaf, stem bark, and flower of *v. amygdalina* and *moringa oleifera* were collected fromEfratanaGydm inAtaye district in North ShwaZone, Amhara region, Ethiopia, which is located about 270 km away from the capital city, Addis Ababa, in March, 2012 E.C. The fresh leaf, stem bark and flower were wrapped in plastic sheets during transportation. After collection, the leaf, stem bark and flower were washed in clean water and dried in an open air protected from direct exposure to sun light. 1 Kg of fresh leaf, stem bark and flower of the plant were air dried for two weeks at room temperature and then grounded using mortar and pestle. The powdered sample was weight (WO) using electrical weighing balance in the laboratory. The resulting powder was kept in polyethylene bag to avoid it from certain environmental conditions (moisture, air and other surrounding dusts) until used for further analysis.

3. 2. Experimental Sites

The crude extracts were done at Debre Berhan University, Chemistry laboratory, whereas antibacterial activity test was done in Biology Department (Microbiology laboratory) at the same University.

3.3. Apparatus and Instruments

The apparatus and instruments used in this study were: filter paper (Whatmans No.1 filter paper), grinder (mortal, pestle), pipettes (different size), beakers (different size), electronic bala nce, conical flask, flasks (different size),measuring cylinder, Rotatory evaporator, polyethylene bag, plastic containers,autoclave,petri dish,micropipette,micropipette tips,forceps,spreader,a n alytical balance, Separating funnel, Cotton,elements for personal biosecurity(gloves, gowns,go ggles or protective eye wear, chemical/biological safety hood), aluminum foil, inoculating loop , incubator ,ruler, sample vial and laminar air flow hood.

3.4. Chemicals, Reagents and Media

The chemicals and reagents used in the study were: distilled water, organic solvents (ethanol, chloroform, petroleum ether), Chloramphenicol, appropriate media for bacteria (Muller hinter agar), concentrated and dilute Sulfuric acid, concentrated and dilute hydrochloric acid, DMSO, 1% of aqueous Iron chloride ($FeCl_3$), 10% ferric chloride solution ($FeCl_3$), sodium hydroxide, nutrient broth, concentrated sulfuric acid, Mayer's reagent (mercury chloride in Potassium Iodide).

3.5. Extraction of the Plant Materials (leaves, stem bark and flower)

A portion of powdered two plant materials (400g each) of stembarks, leaves and flowers were weighted and soaked in 500ml of 96% of ethanol for labeled in each of 1L capacity round bottom flask covered with aluminum foil and kept for 72 h with intermittent shaking at room temperature. The cold extracts thus obtained were filtered with whatmansNo 1 filter paper**.** The filtrates were concentrated using rotary evaporator at 40^0C. All filtrates were air dried at room temperature. Then the product was labeled and placed at room temperature and weighted for further analysis.

3.6. Phytochemical Screening tests

Detections of common secondary metabolites were performed on crude extracts of leaves, stem bark and flowers of *vernoniaamygdalina* and*moringaoleiferausing* the preceding analytical procedures [90, 91].

Flavonoids*:* 3ml of the extract was pipette out and 10ml of distilled water was added to it and it was shaken and 1ml of 10% Sodium hydroxide was also added to the mixture. A yellow color was observed showing the presence of flavonoid [92].

Test for saponins (Froth Test): 2ml of each extract, 5ml of distilled water was added in a test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of froth indicates the presence of Saponins.

Tannins: Ferric chloride solution 5% ferric chloride solution was added to 3ml of the extract and the colored produced is noted. Condensed tannins usually give a dark green color; hydrolysable tannins give blue-black color.

Test for alkaloids (**Meyer's reagent)**: About 0.5ml of test solution was taken with 1ml of the 2NHCl. Aqueous layer formed was decanted to which one or few drops of Meyer'sreagent were added. A white precipitation shows the presence of alkaloids

Test for Phenols: To 1ml of various crude extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green or blue black color indicated the presence of phenols.

Test for Steroid(Salkowski reaction):1ml of solvent extract was diluted with chloroform and followed by 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of steroids.

Terpenoids (Salkowski test*):* 2ml of crude extract was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was added carefully to form a layer. Reddish-brown coloration of the interface indicates the presence of terpenoids.

3.7. Antibacterial test

3.7.1. Microorganisms used and inoculums preparation

The antimicrobial activity was evaluated against yeast and ten bacterial strains. They were obtained from the American type culture collection (ATCC). The Bacterial strain was selected as representative of both classes of gram negative and gram positive, the microbial strains gram positive *Staphylococcus epidermidis* (ATCC12228),*Staphylococcus aureus* (ATCC25923), *Lis teriamonocytogenes* (ATCC19115), *Streptococcus pyogenes* (ATCC19615)115),*Enterococcus faecalis* (ATCC29212) and gram negative *Salmonella typhimurium* (ATCC13311), *Escherichi a coli* (ATCC25922),*Salmonella enteritidis* (ATCC13076), *Pseudomonas aeruginosa* (ATCC2 7853), *Shigela sonnei* (ATCC25931),and yeast strain *Candida albican* (ATCC10231).Inoculu ms of thesebacteria were prepared in nutrient agar medium to obtain isolated colonies. After incubation at 37°C overnight, well. The colonies were selected with an inoculating needle or loop, and transferred to a tube of sterile nutrient broth and incubated at 37°C for 24h. From these grown bacteria 0.1ml of the bacteria suspension was spread on presterile Muller Hinton agar medium. The surface of the medium was allowed to dry for 35 m inutes but not longer than 15 minutes to allow for absorption of excess moisture. The antibacter ial disks were applied to the plates after 10 minutes of inoculation[93].

3.7.2. Antibacterial Activity Test

Antibacterial activity of ethanol extracts of stem barks,leaves and flowers of *vernoniaamygdalina*and*moringaoleifera* were evaluated by using the paper disc diffusion method against five Gram positive bacteria (*S. aureus, Staphylococcus epidermidis,Enterococcus faecalis, Streptococcus pyogenes* and *listeria monocytogenes)* and five gram negative bacteria (*E.coli, Pseudomonas aeruginosa, Salmonella enteritidis,Salmonella typhimurium* and *Shigelasonni*) and also one yeast(*Candida albican*). The paper disks which is prepared from the Whatmans filter paper Number 1 (6mm in diameter) and sterilized in autoclave at 121°C for 15minutes were placed individually with sterile forceps, and then gently pressed down onto the agar. 20µl of the concentrations (150 and 200mg/ml) of each samples were pipette to the discs in two replications. Antibiotic discs containing Chloramphenicol was used as positive controls. Then the plate was inverted and incubated at 37°C for 24 h. After incubation, clear zones formed around the discs indicated the presence of antibacterial activity. The diameter of the zones of complete inhibition (including the diameter of the disk) was measured and recorded in millimeters. The measurement was made with a ruler on the undersurface of the plate without opening the lid [94, 95].

4. RESULTS AND DISCUSSION

4.1. Phytochemical Screening Test

Phytochemical screening of the extracts of *Vernoniaamygdalina* and *Moringaoleifera* (stem bark, leaf and flower) is presented as follows.

Table 1: Phytochemical constituents of ethanol extract of *Vernoniaamygdalina*.

No	Secondary Metabolites	Crude extracts	Plant parts		
			Stem bark	Leaf	Flower
1	Flavonoids	Ethanol extract	+ve	+ve	+ve
2	Saponnins	Ethanol extract	+ve	+ve	-ve
3	Tannins	Ethanol extract	+ve	+ve	+ve
4	Alkaloids	Ethanol extract	+ve	+ve	+ve
5	Phenols	Ethanol extract	+ve	+ve	+ve
6	Steroids	Ethanol extract	+ve	-ve	-ve
7	Terpenoids	Ethanol extract	+ve	+ve	+ve

+ Ve = Present - Ve = absent

Phytochemical screening of ethanol extracts of these plant species revealed the presence of flavonoids, tannins,alkaloids and phenols in all plant parts (stem bark, leaf and flower).Sapponinswere present in stem bark and leaf but absent in flower**.** It also revealed that the present of steroids in stem bark but absent in the leaf and flower of ethanol extracts of *Vernoniaamygdalina*.

Table*2*: Phytochemical constituents of ethanol extract of *Moringaoleifera*.

No	Secondary Metabolites	Crude extracts	Plant parts		
			Stem bark	Leaf	Flower
1	Flavonoids	Ethanol extract	+ve	+ve	+ve
2	Saponnins	Ethanol extract	-ve	+ve	-ve
3	Tannins	Ethanol extract	-ve	+ve	-ve
4	Alkaloids	Ethanol extract	+ve	-ve	+ve
5	Phenols	Ethanol extract	-ve	+ve	-ve
6	Steroids	Ethanol extract	+ve	-ve	+ve
7	Terpenoids	Ethanol extract	-ve	+ve	+ve

+ Ve = Present - Ve = absent

Phytochemical screening of ethanol extracts of these plant species revealed the presence of flavonoids was in all plant parts (stem bark, leaf, and flower).Sapponins , tannins and phenols were present in leaf but absence in stem bark and flower.Alkaloidsand steroids presence in stem bark and flower but absence in leaf. It also revealed that the presence of terpenoids in leaf and flower but absence of stem bark of ethanol extract of *Moringaolifera*.

4.2. Antimicrobial Activity Test

The results of bacterial test for crude ethanol extract of stem bark, leaf and flower of *vernoniaamygdalina*and *moringaoleifera*were expressed as mean value ± standard Deviation (SD) of growth inhibition zone diameters obtained with two trials. The ethanol extract of stem bark, leaf and flower of *vernoniaamygdalina* and *moringa oleifera*were subjected to the antibacterial activity and the results were investigated. Chloramphenicol and DMSO were used as positive and the negative control respectively. The results were tabulated in table below. The solvent ethanolextract was showed antimicrobial activity on 11 microorganisms with zone of inhibitions ranging from 7-17mm.

Table 3: Results from disc diffusion assay showing the antibacterial activity of crude ethanol extract of the flower of *moringaoleifera*against 11microorganisms**.**

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			Moringa oleifera	Chloramphenicol
1	Candida albican	150	7.33±0.41	12
		200	8.54±0.71	
2	*Listeria monocytogenes*	150	-	17
		200	8.40±.53	
3	*Salmonella typhimurium*	150	-	15
		200	-	
4	*Staphylococcus aureus*	150	-	14
		200	-	
5	*Escherichia coli*	150	8.45±0.64	10
		200	9.46±0.42	
6	*Streptococcus pyogenes*	150	-	22
		200	8.75±0.78	
7	*Pseudomonas aeruginosa*	150	-	16
		200	7.52±0.72	
8	*Staphylococcus epidermidis*	150	7.88±00	10
		200	8.5±0.65	
9	*Salmonella enteritidis*	150	-	23
		200	9.5±0.72	
10	*Enterococcus faecalis*	150	-	20
		200	-	
11		150	-	8
		200	-	

-Indicates no inhibition zone

The ethanol extract showed 8.54±0.71mm zone of inhibition on *Candida albican*, yeast and the control drug Chloramphenicol exhibited 12mm zone of inhibition.The ethanol extract showed 9.46±0.42mm zone of inhibition *Escherichia coli,gram negative bacteria* and the control drug

Chloramphenicol exhibited 10mm zone of inhibition. And also in gram positive bacteria,*Staphylococcus epidermidis*, the extract showed8.5±0.65 mm zone of inhibition and the control drugChloramphenicol 10mm, hence the plant ethanol extract is moderately effective and consistent on *Escherichia coli, Candida albican* andStaphylococcus epidermidis bacteria's. But, there were no zone of inhibition showed on both gram positive bacteria's *Enterococcusfaecalis* and*Staphylococcus aureus* andpositive controls Chloramphenicol showed 20mm and 14mm zone inhibitions respectively. And also there was no zone of inhibition on gram negative bacteria's,*Shigelasonnei* and*Salmonella typhimurium*and control drug Chloramphenicol showed 8mm,15mm zone of inhibition respectively. These indicates that the ethanol flower extract of *Moringaoleifera was not* effective against *Enterococcusfaecalis,Staphylococcus aureus,Salmonella typhimurium* and *Shigelasonne*ibacteria's (Table 3)

Table 4: Results from disc diffusion assay showing the antibacterial activity of crude Ethanol extract of the leaf of *vernoniaamygdalina* against 11 microorganisms.

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			V.amygdalina	Chloramphenicol
1	*Candida albican*	150	12.65±0.92	12
		200	13.50±0.75	
2	*Listeria monocytogenes*	150	8.55±0.92	17
		200	12.7±1.2	
3	*Salmonella typhurium*	150	-	15
		200	-	
4	*Staphylococcus aureus*	150	-	14
		200	10.50±0.61	
5	*Escherichia coli*	150	7.12±0.22	10
		200	7 .32±0.31	
6	*Streptococcus pyogenes*	150	8.73±0.45	22
		200	11.64.6±0.82	
7	*Pseudomonas aeruginosa*	150	10.71±1.11	16
		200	13.82±0.67	
8	*Staphylococcus epidermidis*	150	11.65±0.92	10
		200	12.6±1.21	
9	*Salmonella enteritidis*	150	-	23
		200	8.7±0.9	
10	*Enterococcus faecalis*	150	10.67±0.99	20
		200	16.78±1.23	
11	*Shigelasonnei*	150	-	8
		200	7.2±0	

-Indicates no zone of inhibition

The crude ethanol extract of leaf of *vernonia amygdalina*/grawa showed13.50±0.75mm zone of inhibition on *Candida albican*, yeast and12.7±1.2mm zone of inhibition on *Listeria monocyto genes*, positive bacteria and control drug Chloramphenicol showed 12mm and 17mm zone of

inhibition respectively. The ethanol extract showed 13.82±0.67mm zone of inhibition on Pseudomonas aeruginosa,gram,negative bacteria and 16.78±1.23mm zone of inhibition Enterococcusfaecalis,gram positive bacteria and control drug Chloramphenicol exhibited 16mm and20mm zone of inhibition respectively.Hencethe extractswere highlyeffective and consistent against *Candida albican*, *Listeria monocytogene Pseudomonas aeruginosa* and *Ente rococcusfaecalisbacteria*. The other showed 7 .32±0.31 zone of inhibition on *Escherichia coli, gram negative* bacteria and 8.73±0.45mmzone of inhibition on *Streptococcus pyogenes*, grampositivebacteria,both zone of inhibitions are moderately effective. Also the extract showed no zone of inhibitions on *Salmonella typhurium*, gram negative bacteria and the positive control Chloramphenicol showed 15mm zone of inhibitions (Table 4). This indicates the ethanol leaf extract of *vernoniaamygdalina* is not active against *Salmonella typhurium*, gram negative bacteria.

Table 5: Results from disc diffusion assay showing the antibacterial activity of crude ethanol extract of the leaf *moringaoleifera* against 11 microorganisms.

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			Moringaoleifera	Chloroamphinicol
1	*Candida albican*	150	7.13±0.05	12
		200	9.10±0.14	
2	*Listeria monocytogenes*	150	-	17
		200	10.61±0.85	
3	*Salmonella typhurium*	150		15
		200	10.65± 0.91	
4	*Staphylococcus aureus*	150	-	14
		200	-	
5	Escherichia coli	150	-	10
		200	-	
6	*Streptococcus pyogenes*	150	-	22
		200	8.36±0	
7	P*seudomonas aeruginosa*	150	-	16
		200	-	
8	*Staphylococcus epidermidis*	150	-	10
		200	8.49+0.70	
9	*Salmonella enteritidis*	150	-	23
		200	8.06+0.08	
10	*Enterococcus faecalis*	150	-	20
		200	8.16+0.22	
11	*Shigelasonnei*	150	-	8
		200	7.11±0.12	

-indicates no zone of inhibition

The crude extract of leaf of *moringaoleifra was* subjected to antimicrobial activity and the results were investigated as tabulated in the table5. Chloramphenicol was used as positive control. The crude extract showed antibacterial activity against*Candida albicanSalmonella*

typhimurium, and *Listeria monocytogenes*bacteria with zone of inhibition ranging from 9-11mm, but no inhibition zone on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococc usaureus*bacteria.

Table 6: Results from disc diffusion assay showing the antibacterial activity of crude ethanol extract of the stem bark of*moringaoleifera* against 11 microorganisms.

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			Moringaoleifera	Chloramphenicol
1	*Candida albican*	150	8.45±0.64	12
		200	10.65±0.92	
2	*Listeria monocytogenes*	150	-	17
		200	-	
3	*Salmonella typhimurium*	150	-	15
		200	-	
4	*Staphylococcus aureus*	150	8.45±0.64	14
		200	10.49±0.70	
5	*Escherichia coli*	150		10
		200	7.44±0.63	
6	*Streptococcus pyogenes*	150	-	22
		200	-	
7	*Pseudomonas aeruginosa*	150	8.55±0.45	16
		200	9.65±0.92	
8	*Staphylococcus epidermidis*	150	8.44+0.62	10
		200	10.72±1.01	
9	*Salmonella enteritidis*	150	-	23
		200	10.73±1.02	
10	*Enterococcus faecalis*	150	10.71±0.99	20
		200	14.75±1.01	
11	*Shigelasonnei*	150	7.44+0.64	8
		200	10.49±0.70	

-Indicates no zone of inhibition

The crude ethanol stem bark of *moringaoleifera* extract was subjected to the antimicrobial activity and the results wereinvestigated (table6). The crude extract showed 14.75±1.01 zone of inhibition on the*Enterococcus faecalis*, grampositive bacteria and10.72±1.01 zone inhibitionontheStaphylococcus epidermidis, gram positive bacteria and positive control Chloramphenicol showed 20mm and 10mm respectively. The two negative bacteria (*Pseudomonas aeruginosa* and*shigelasonnei*) and one positive bacteria (*Staphylococcus aureus*) showed 9.65±0.92, 10.49±0.70 and 10.49±0.70 zone of inhibition and positive control Chloramphenicol 16mm,8mm and 14mm zone on inhibition respectively. The only yeast, *Candida albican* showed 10.65±0.92 zone of inhibition and control drug Chloramphenicol 12mm zone of inhibition. Hence the ethanol extract of stem bark of *moringaoleifera*showed moderately effective and consistent on those five bacteria's one yeast. The other two negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) less effect. But *Streptococcus pyogenes* (gram positive bacteria), *Listeria monocytogenes* (gram positive bacteria) and *Salmonella typhimurium* (gram negative bacteria) were not active ethanol stem bark of *moringaoleifera*.

Table 7: Results from disc diffusion assay showing the antibacterial activity of crude ethanolextract of the bark of*v.amygdalina* against 11 microorganisms.

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			v.amygdalina	Chloramphenicol
1	*Candida albican*	150	7.12±0.14	12
		200	7.21±0.14	
2	*Listeria monocytogenes*	150	-	17
		200	9.45±0.64	
3	*Salmonella typhimurium*	150	-	15
		200	7.22±0.28	
4	*Staphylococcus aureus*	150	-	14
		200	8.33±0.42	
5	*Escherichia coli*	150	8.87±0.55	10
		200	13.68±1.11	
6	*Streptococcus pyogenes*	150	7.46±0.32	22
		200	7.61±32	
7	*Pseudomonas aeruginosa*	150	-	15
		200	-	
8	*Staphylococcus epidermidis*	150	7.69±0.41	10
		200	15.75±0.98	
9	*Salmonella enteritidis*	150	7.36±0.95	23
		200	10.49±0.67	
10	*Enterococcus faecalis*	150	-	20
		200	10.67±0.89	
11	*Shigelasonnei*	150	7.78±0.69	8
		200	10.21±0.82	

-Indicates no zone of inhibition

The crude ethanol stem bark of*venoniaamygdalina*extract was subjected to the antimicrobial activity and the results were investigated. The crude extract showed 13.68±1.11 zone of inhibit ion on the *Escherichia coli,* gram negative bacteria and 15.75±0.98 zone of inhibition on *Staph ylococcus epidermidis*, gram positive bacteria and the positive control Chloramphenicol showed 10mm and 10 mm respectively.Thus the ethanol stem bark extract showed highly active and consistent on those two bacteria. The *Salmonella enteritidis* and *Shigela sonnei* (gra m negative bacteria) and *Streptococcus pyogenes* (gram positive) showed10.49±0.67,10.21±0. 82and 7.61±32 and control drug Chloramphenicol 23mm,8mm and 22mm zone of inhibition respectively. Hence crude ethanol stem bark of grawa extract was moderately effective for thes e three bacteria. And also the *Salmonella typhimurium, Enterococcus faecalis, Staphylococcus aureus* and *Listeria monocytogenes* were less effective or not consistent. But Pseudomonas aeruginosa was gram negative bacteria no inhibition zone (table 7).

Table 8: Results from disc diffusion assay showing the antibacterial activity of crude ethanol extract of flower*ofv.amygalina* against 11 microorganisms.

No	Name of Microorganisms	Concentration (mg/ml)	Zone of inhibition	
			v.amygdalina	Chloramphenicol
1	*Candida albican*	150	9.45±0.63	12
		200	11.65±0.87	
2	*Listeria monocytogenes*	150	9.49±0.69	17
		200	11.61±0.86	
3	*Salmonella typhimurium*	150	-	15
		200	-	
4	*Staphylococcus aureus*	150	7.12±0.11	14
		200	7.15+0.12	
5	*Escherichia coli*	150	8.45±0.65	10
		200	9.49±0.72	
6	*Streptococcus pyogenes*	150	8.28±0.56	22
		200	12.57±0.79	
7	*Pseudomonas aeruginosa*	150	10.65±0.97	16
		200	15.46±0.55	
8	*Staphylococcus epidermidis*	150	8.45+0.63	10
		200	12.57±0.87	
9	*Salmonella enteritidis*	150	8.15±0.45	23
		200	12.67±0.79	
10	*Enterococcus faecalis*	150	8.49±0.59	20
		200	12.49±0.67	
11	*Shigelasonnei*	150	9.47±0.72	8
		200	12.56±1	

-Indicates no zone of inhibition

The crude extract of flower of*vernoniaamygdalina*was subjected to antimicrobial activity and the results were investigated as tabulated in the table 8. Chloramphenicol was used as positive control. The crude extract showed antibacterial activity against four-gram-negativeand five grampositive bacteria's and one yeast (*Candida albican*) highly active and consistent withzone

of inhibition ranging from 7-16mm, but no inhibition zone on *Salmonella typhimurium*,gram negative bacteria.

5. CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In this study, the seven phytochemical screeningtestof ethanol crude extracts of each plant parts revealed the presence of flavonoids, terpenoids tannins, alkaloids and phenols.The *vernoniaamygdalina*was rich in alkaloids, flavonoids,terpenoidstannins, and phenols in the five test plant parts. The *moringaoleifera* was rich in flavonoid in the test plant parts. The crude stem bark, leaf and flower part of *vernonia amygdalina* extract ethanol exhibited antibact erial activity against *Salmonella typhimurium* (ATCC13311), *Candida albican* (extracted from patient), *Shigela sonne*i (ATCC25931), *Listeria monocytogenes*(ATCC19115), *Escherichia col i (ATCC25922)*, *Staphylococcus epidermidis* (ATCC12228), *Pseudomo nas aeruginosa* (ATC C27853), *Streptococcus pyogenes* (ATCC19615), *Staphylococcus aureus* (ATCC25923), *Salm onella enteritidis* (ATCC13076) and *Enterococcus faecalis* (ATCC29212), but not against in *S almonella typhimurium*(ATCC13311) on flower and leaf of *vernonia amygdalina* and *Pseudom onas aeruginosa* (ATCC27853) on bark of*vernoniaamygdalina*. Thecrude stem bark, leaf and f lower part of *moringa oleifera* extract ethanol exhibited antibacterial activity against *Candida albican* (extracted from patient), *Shigela sonnei* (ATCC25931), *Staphylococcus epidermidis* (A TCC12228), *Streptococcus pyogenes*(ATCC19615), Staphylococcus aureus(atcc25923), *Salmo nella enteritidis* (ATCC13076) and *Enterococcus faecalis* (ATCC29212). But not against in *Sa lmonella typhimurium* (ATCC13311) on flower and stem bark of moringa and *Staphylococcus aureu*s (ATCC25923) leaf and flower of *moringaoleifera*.

5.2.RECOMMENDATIONS

Based on the finding of this study the work mentioned below is suggested for further investigation on *vernoniaamygdalina*and*moringa oleifera.*

- ✓ Identifying the in vitro antimicrobial activity of plant other plant parts.
- ✓ Carrying out different applications of phytochemical constituents by using different parts of the plant.
- ✓ Identifying the microbial association of the plant to carrying out the beneficial and harmful aspect of the associated microbes.

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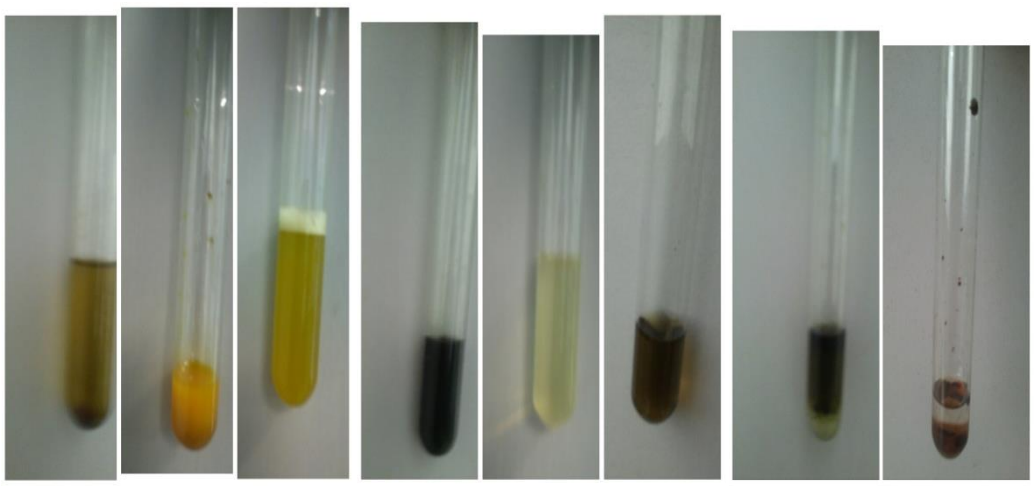
7. APPENDIX'S

Appendixfigure1. Photography of the *vernoniaamygdalina*and*moringaolei*feramaterials.

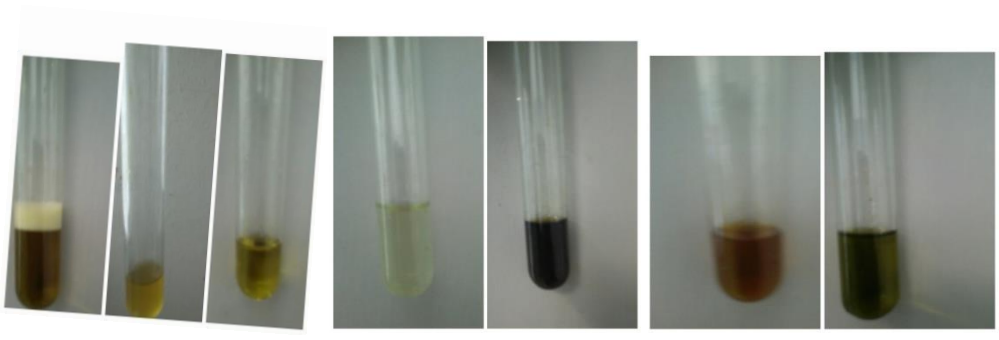
Bark of *V.mygdalina*Leaf of *V.amygdalina* Flower of *V.Aamygdalina*

Stem bark of *moringaoleifera*Leafof*moringa oleifera*Flower of *morigaoleifera*

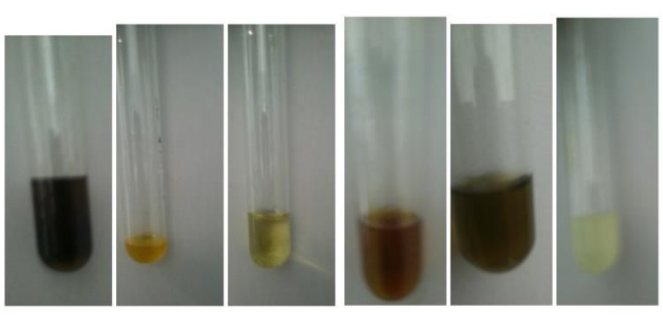
Appendix *Figure 2*: secondary metabolite of Grawa and moringa oleifera.



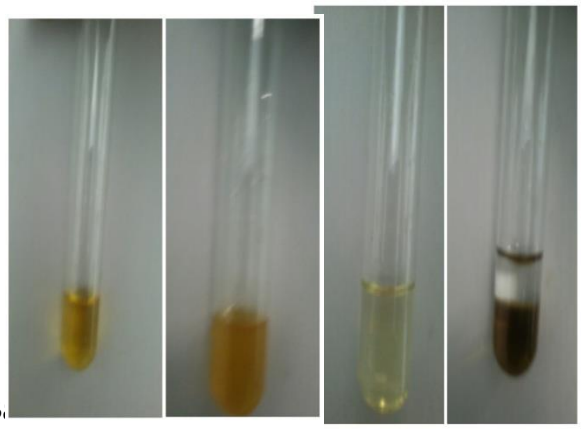
Bark ofgrawa



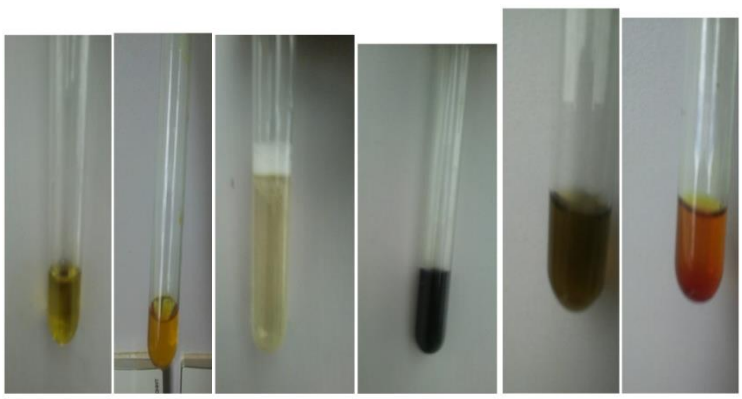
Leaf of grawa



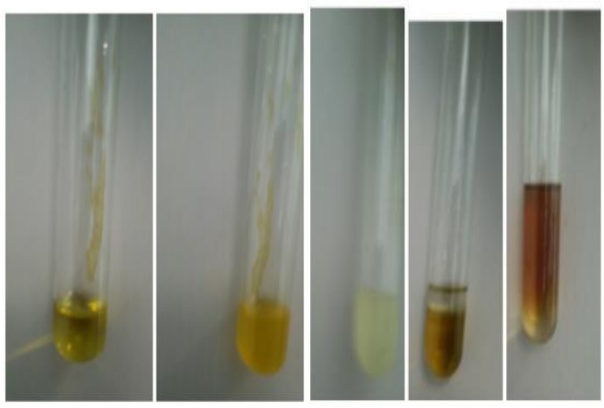
Flower of grawa

B 

Bark of m. oleifera



Leaf of m.oleifera



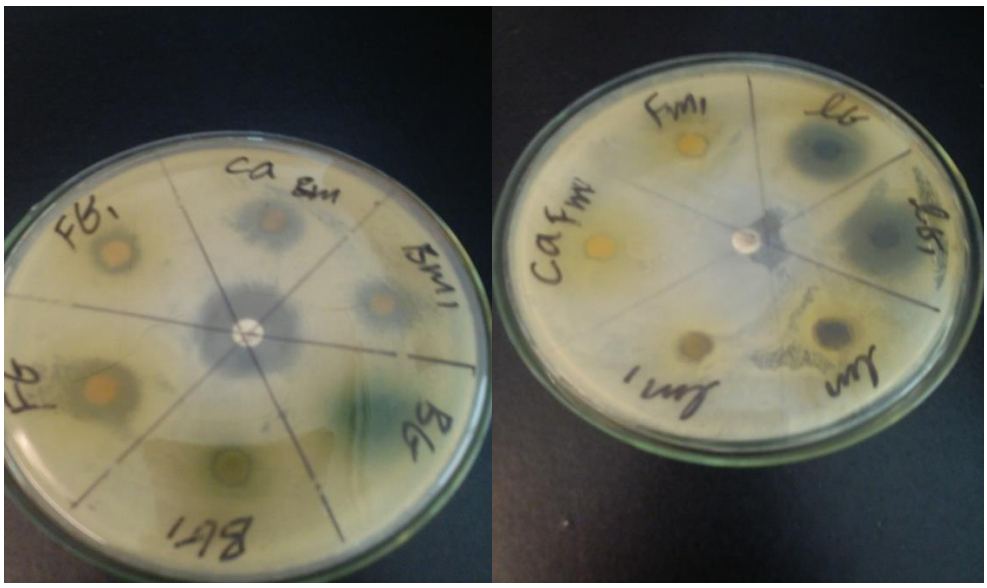
Flower of m.oleifera

Appendix Figure3:Some Petri dishes showing the activity of parts of *vernoniaamygdalina* and *moringa olifera* on one yeast ten bacteria's.

Theabrviation ten bacteria and one yeast and also the plants v*ernoniaamygdalin*a /grawa and *moringaoleifera* as follow.

Ca-*Candida albican*(yeast)
Lm-*Listeria monocytogenes*
Sal- Salmonella typhurium
Ae-*Salmonella enteritidis*
Sa- Staphylococcus aureus
Ef- Enterococcus faecalis
Sh-Shigelasonnei
Sp-*Streptococcus pyogenes*
Pa-*Pseudomonas aeruginosa*
Se-*Staphylococcus*
Ec- *Escherichia coli*

Fm-flower of moringa (200mg/ml)
FG-flower of grawa(200mg/ml)
BG-bark of grawa(200mg/ml)
Bm-bark of moringa(200mg/ml)
LG-leaf of grawa(200mg/ml)
Lm-leaf of moringa (200mg/ml)
Fm1-flower of moringa (150mg/ ml)
FG1-flower of grawa(150mg/ ml)
BG1-bark of grawa(150mg/ ml)
Bm1-bark of moringa(150mg/ ml)
LG1-leaf of grawa(150mg/ ml)
Lm1-leaf of moringa(150mg/ml)



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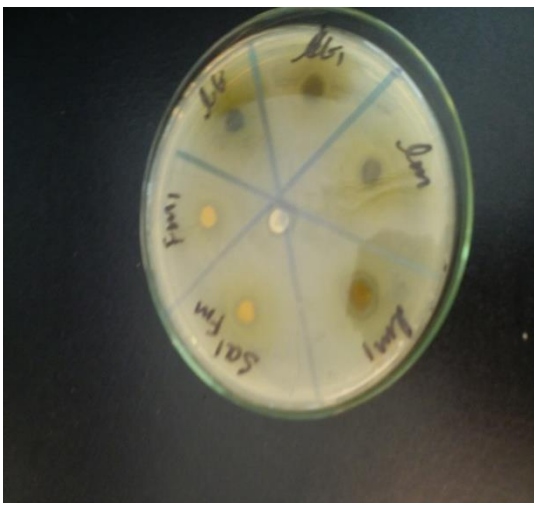
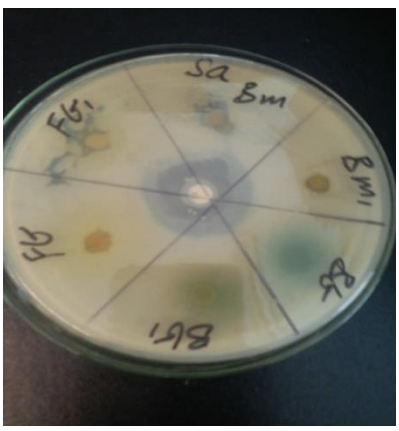
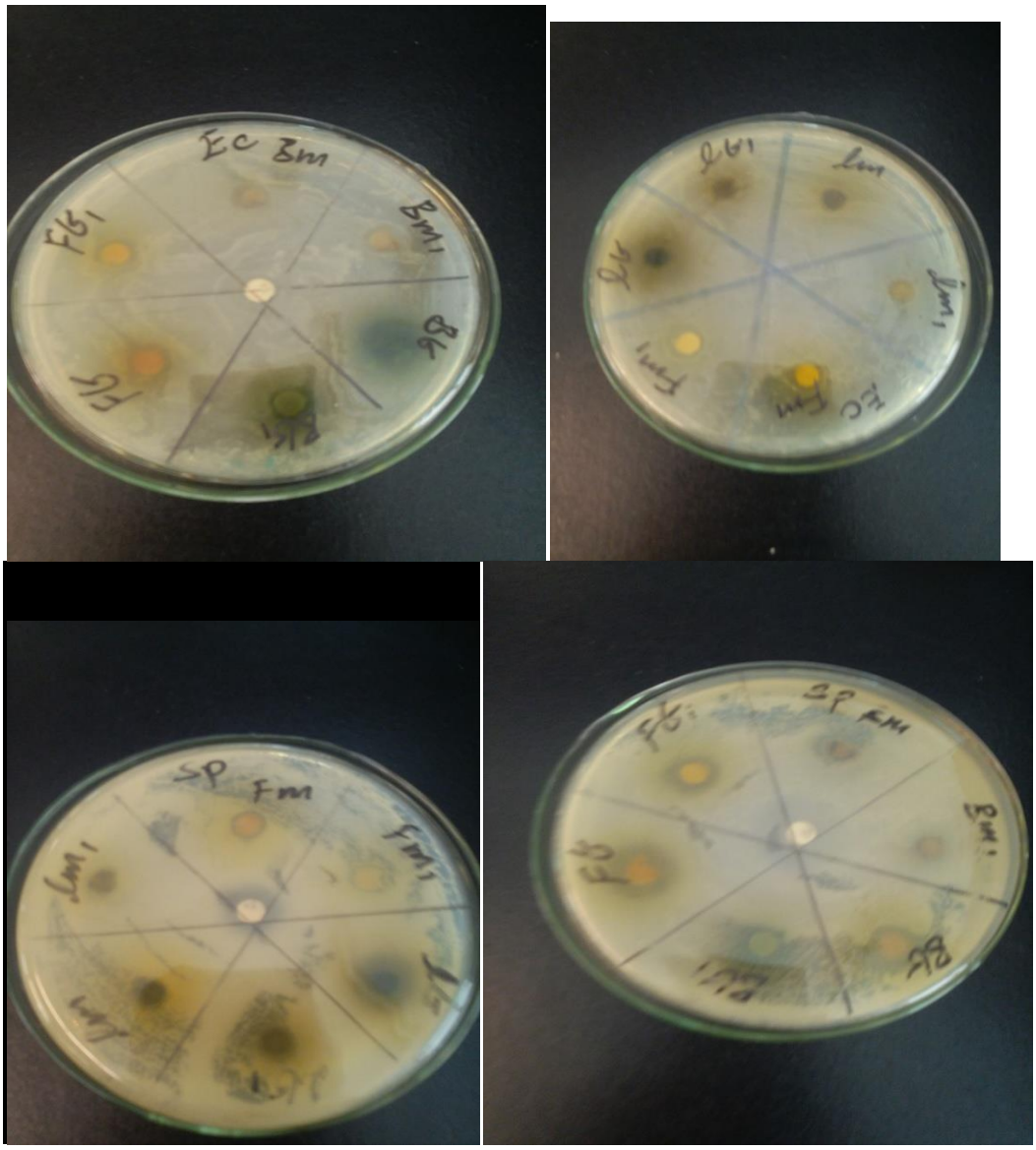
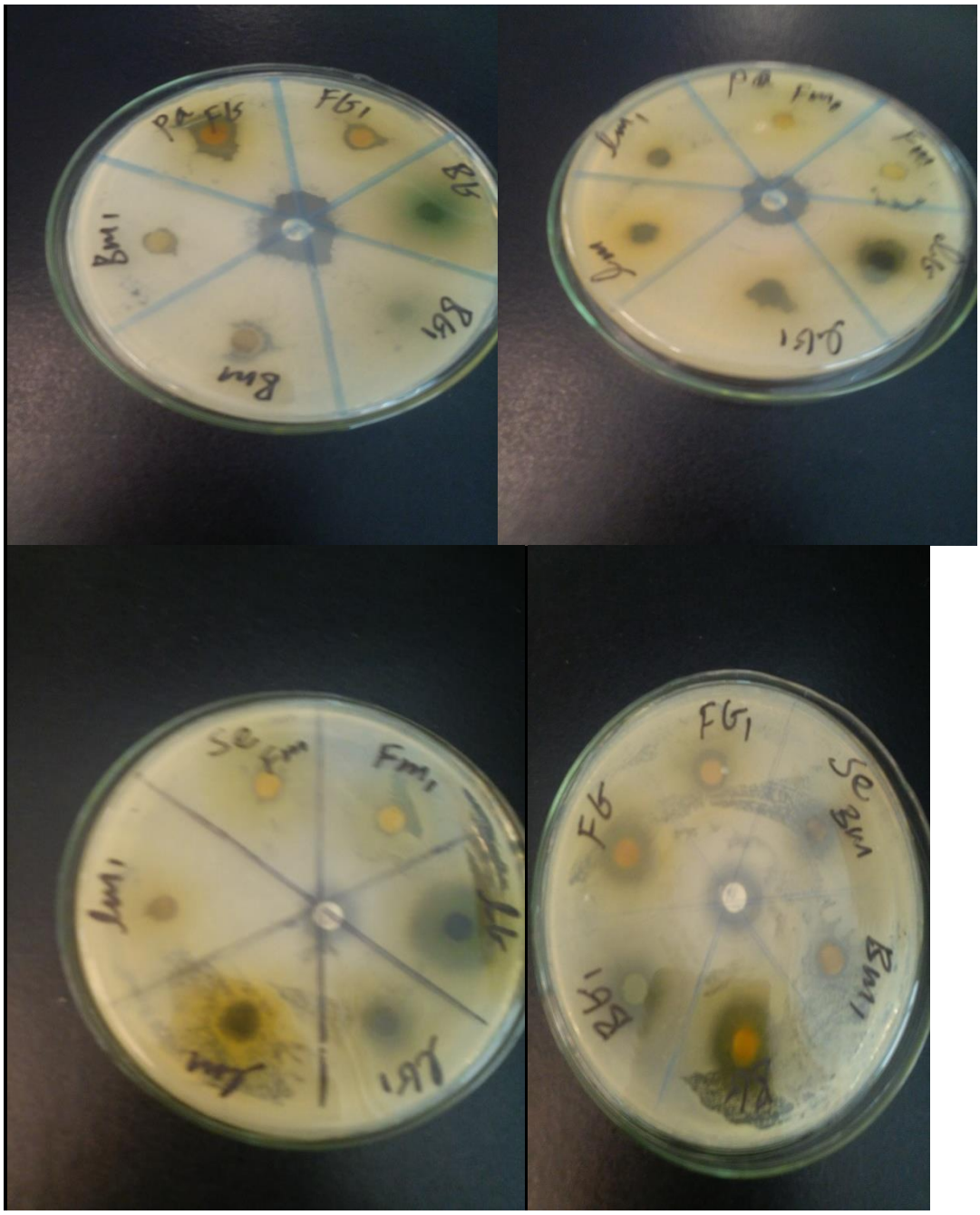





EC Bm
FB1
BM1
BS
FM1
Lm1
SP Fm
FM1
Lm1
SP FM
BM1
BS


Pa Fg
FG1
Bs
Bm1
Bs1
Bm
Fm1
Lm1
Se Fm
Lm
Se Bm
Bm1