Debre Berhan University

College of Natural and Computational Sciences

Department of Chemistry

Graduate Project (Chem 692)

Investigation of the Antimicrobial activity, Antioxidant properties and Phytochemical Analysis of Osyris quadripartite

A Graduate Project Submitted to the College of Graduate Studies, Debre Berhan University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Organic Chemistry

By: Ermiyas Solomon Kebede

Advisor: Minbale Gashu (PhD)

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Letter of approval sheet

Debre Berhan University

College of Graduate studies

Department of Chemistry

This is to certify that the thesis prepared by Ermias Solomon, entitled: Investigation of the Antimicrobial activity, Antioxidant properties and Phytochemical Analysis of Osyris quadripartite, submitted in partial fulfillment of the requirement of the Degree of Masters of Science in Organic Chemistry compliance with the regulation of the University and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this thesis work entitled **"**Investigation of the antimicrobial activity, antioxidant properties and Phytochemical Analysis of Osyris quadripartite" is based on my work done under the esteemed supervisor of Dr. Minbale Gashu and the data presented in this work are original and authentic .They have not been published before in any journal .Beside that, I have properly acknowledged the reference materials I used in this thesis work.

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I would like to thank God for all giving me strength, peace and wellbeing throughout this work .Next to this; I would like to extend my gratitude and sincere thanks to my advisor Dr. Minbale Gashu for his valuable guidance in all of the research time , starting from the writhing of the proposal up to the end of the thesis. I thank Mr. Hirpo Hinsene for his support and cooperation in my work in chemistry laboratory. I thank also Mr. Belayhun, technician at Biology Department, for helping me with the anti-microbial activity study.

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List of Abbreviations

WHO	World Health Organization
HIV	Human Immune Virus
MGB- sub unit	Muramic acid, glucosamine and pentapeptaid linkage
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
PUFA	Polyunsaturated Fatty Acid
O.wightiana	*Osyris wightiana*
UV-Vis spectroscopy	Ultraviolet spectroscopy
DMSO	Dimethyl sulfoxide
DPPH	1, 1-diphenyl -2-picrylhydrazil
MHA	Muller-Hinton Agar
S.aureus	Stylaphylococcus aureus
S. thyphimurium	Salmonella. thyphimurium
L. monocytogen	Listeria monocytogen
E. coli	Escherichia coli
Ab_{sample}	Absorbance of sample solution
$Abs_{control}$	Absorbance of control
IC_{50}	Inhibition concentration of the sample to scavenge 50%

Abstract

Osris quadripartite (African sandal wood in English, *Qeret* in Amharic) belongs to santalacea family which locally used to treat diarrhea, sexually transmitted diseases, anemia, cancer and wounds. The antimicrobial activity of ethyl acetate and petroleum ether extracts of the root, stem bark and leaf of *O. quadripartite,* evaluated using disc diffusion method, and showed an effect on the growth of *S.aures* and *S.typhimurium* at a concentration of 100μg/ml and 150μg/ml but not on *L. monocytogenes* and *C. albican.* Petroleum ether extract of all plant parts has also a slight effect on *E.coli* at a concentration of 100μg/ml and 150μg/ml. Methanol extract of root, stem bark and leaf has no effect on all tested microbial. The DPPH radical scavenging activity of methanol extract of the root has highest antioxidant capacity (IC_{50}=102.9μg/ml) than stem bark (IC_{50}=168.57μg/ml) and leaf (IC_{50}=214.81μg/ml) respectively. Ethyl acetate extract of root has the highest antioxidant activity (IC_{50}=340.5μg/ml) than leaf (IC_{50}=452.6μg/ml) and stem bark (IC_{50}=859.29μg/ml) respectively. The strong antioxidant activity of methanol extract is due to the presence of more phenolic compounds which has better hydrogen donating ability.

Methanol extract of root, stem bark and leaf of *O.quadripartita* contained terpenoid, sterol, flavonoid, tannin and phenol. Saponin was present in methanol extract of stem bark and root but not on the leaf. Tannin and phenol were also found in ethyl acetate extract of all plant parts. In petroleum ether extract only flavonoid was found in root stem bark and leaf. The root and stem bark of *O.qudripartita* was very important for dyeing in textile industry and coloring material in food industries.

Key words: O.quadripartita, Antimicrobial, anti-oxidant and dye.

1. INTRODUCTION

1.1 Background of the study

According to world health organization traditional medicine can be defined as knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies to treat diagnose or prevent illness. Herbal drugs are unprocessed parts of plant while herbs include crude plant material such as flowers , leaves , fruits , seed , stem , wood , roots or other parts which may entire , fragmented or powdered[1] . According to the WHO more than 65 % of the world population have incorporated medicinal plants for treating diseases .The active compounds from this medicinal plants are antimicrobial agents that have the ability to fight bacteria and fungi. Bacterial infections are widespread and cause sickness .These bacterial pathogens continue to be the threat to human health and welfare as a result of new or resistant pathogens [2].

In Ethiopia, traditional medicine has played a significant role in treating different public health ailments. Reports revealed that about 80% of the Ethiopia population still depends on traditional medicinal plants due to its cultural acceptability, economic affordability and efficacy against certain type of disease as compared to modern medicine [3]. Plants have a great importance in lives because they fulfill human needs such as food, shelter, clothing, fuel, ornaments, flavoring and medicine. They provide natural products that are used in treating infectious diseases. Plant derived products with therapeutic properties are known as herbal medicines [4].

Osyris quadripartite (Africa sandal wood), named *Qeret* in Amharic, belongs to *Santalacea* family. It is a shrub or small hemi-parasitic tree growing to a height of six meter. The plant is indigenous to East Africa and South Africa regions [5]. Different parts of the plant such as root, stem barks and leaf are useful [6]. The root and bark are used to make a tea and added as tonic in soups .The leaf have emetic properties .A paste of the leaf buds is applied to cuts and wounds. The wood and roots contain essential oil that is used in perfumery .The bark is a source of tannins .The roots are used for making red dye. A decoction of the bark and heartwood are used to treat sexually transmitted diseases and anemia. The paste of the fruit is applied to the forehead to relieve headache [7]. In Debre Sina, North Shoa Zone, fresh leaf and ground stem bark of *O. quadripartite* is applied in treating infected part of the body including cut or wound.

1.2 Statement of the problem

The *O. qudripartita* is known for its traditional medicinal use to treat many infections disease in some countries including in Ethiopia. Researchers reported that the leaf of the plant species showed antibacterial, antioxidant and cytotoxic activities and also there are many secondary metabolite isolated from this plants. There is no research work reported on the stem bark and root of the plant. This study was intended to deal with assay of antimicrobial and antioxidant activities of the plant extracts of its root, stem bark and leaf and their chemical components.

1.3 Significance of the study

This study is important to give baseline information about the antimicrobial, antioxidant activities and dying effect of the plant part for further uses.

1.4 Objectives of the study

1.4.1 General objective

To study the antibacterial, antioxidant and phytochemical composition of *O. quadripartite.*

1.4.2 Specific objectives

- To extract the root, stem bark, and leaves of *O. quadripartite* using different organic solvents
- To evaluate and compare the antimicrobial activities of the crude extracts of the root, stem bark , and leaf against five organisms, namely, *Salmonella typhimurium* (ATCC13311), *Listeria monocytogenes*(ATCC19115), *Escherichia coli (ATTC 25922)*, *Staphylococcus aures (ATTC 25923),* and *Candida albican*
- To evaluate and compare the antioxidant activity of the crude extracts of the plant parts (root, stem bark and leaf)
- To undergo qualitative phytochemical analysis of extracts of the plant
- To investigate the dyeing properties of the plant

2. LITERATURE REVIEW

2.1. Medicinal plants in Ethiopia

The various climate and topographic condition of the countries like Ethiopia are to a rich biological diversity. Ethiopia believed to be home for about 6,000 species of higher plants with approximately 10% endemism. In Ethiopia 95% of traditional medicinal preparation are of plants origin. A bout 80% of Ethiopia depends on medicinal plants for primary health care [8].

2.2 Natural products found in Medicinal plants

Natural products are secondary metabolites which are produced by living organisms but are not involved in the primary metabolites .They have a particular activities on the human body for the treatment of a particular disease. They can be slightly modified to improve the activities or decrease the side effect. In 400 B.C people use the bark and the leaves of Willow tree to relive pain and fever. In 1897 Fllix Hoffmann synthesized aspirin. In 1962, A. Barclay isolated Taxol from the bark of the Pacific Yewthre (Taxus brevifolia). Quinine was isolated in 1820 by Caventou and Pelletier, from the bark of the Cinchona tree. It is used for the treatment of fever, pain, and Malaria. In 1934, I.G. Rarben synthesized chloroquine [9].

Natural products certainly have the potential to be used as source of antimicrobial drug discovery. There are around over 1340 plants with definite antimicrobial activities and also over 300,000 antimicrobial compounds have been isolated from plants [10]. The plants are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, and polyphenols, which are generally superior in their antimicrobial activities [11]. The common metabolites that have a potential to have antimicrobial activities are listed below [12].

2.2.1 Alkaloids

Alkaloids are among the largest group of secondary metabolite being extremely divers in terms of structure and biosynthesis pathway, including more than 20,000 different molecules distributed throughout approximately 20% of known vascular plants. Alkaloids are low molecular weight nitrogen containing compounds and, due to the presence of heterocyclic rings containing nitrogen atom, are typically alkaline. Alkaloids are known by their numerous pharmacological effects on vertebrate. The metabolite can be divided into different classes according to their precursor encompassing more than 20 different classes (e.g pyrrolidine alkaloid, tropane alkaloid, piperidine alkaloids, pyridine alkaloids, quinolizide alkaloid, and indol alkaloids) [13].

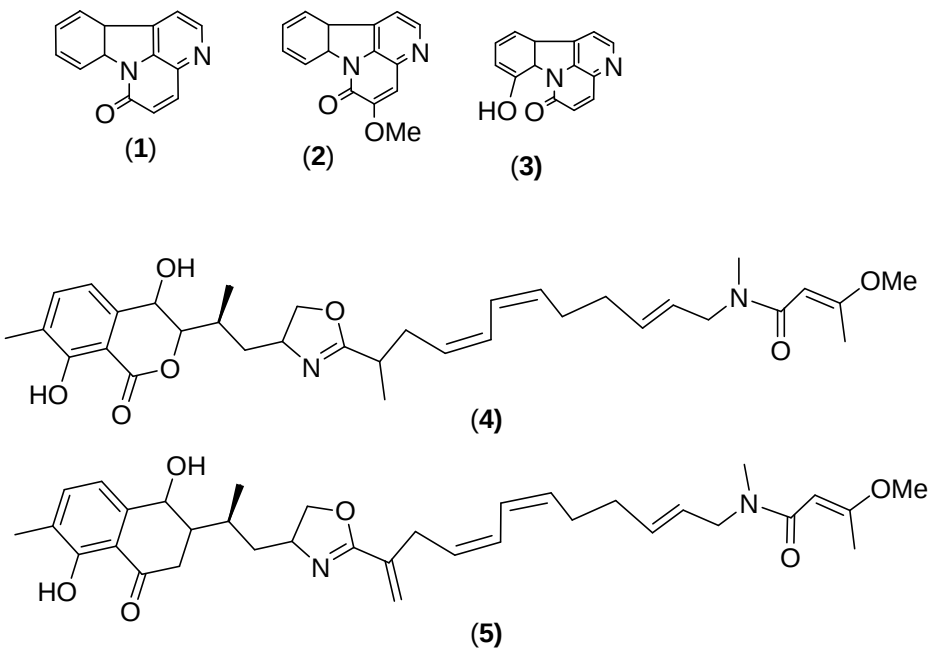


Figure 1: Structures of some alkaloids

2.2.2 Fatty acids

More than 1000 natural acetylic metabolites have been isolated from a wide variety of plant, fungal species and marine algae and invertebrates many of them display important biological activities, namely antitumor, antibacterial, antimicrobial, antifungal, and other medicinal properties [14].

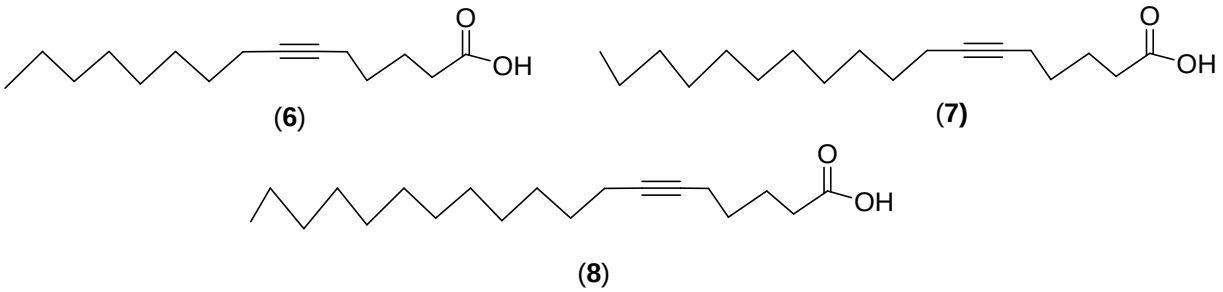


Figure 2: Structure of some fatty acids

2.2.3 Phenolic compounds

Phenolic compounds are the most widely distributed secondary metabolites. It is uncommon in bacteria fungi and algae. Higher plants synthesized several thousand known different phenolic compounds leaves of vascular plants contain esters, amides and glycosides of hydroxycinnamic acid ,glycolated flavonoids; especially flavonols , proanthocyanidins and their relatives [15].

The basic structure of phenolic compounds comprises of an aromatic ring with one or more -OH groups. Phenolics which found in food materials can be divided in to three major groups: simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids. Based on the number of carbons, the phenolics commonly found in the plant can be classified in to several groups (Table-1) [16]. Flavonoids are derivatives of 2-phenylbenzyl-γ-pyrone which classified as flavones , isoflavones , flavonols , anthocyanidins , flavanones , falvanols , Chalcones , and aurones [17]. Flavonoids occur as aglycones, glycosides and methylated derivatives .In plants, flavonoids (i.e flavonoids without attached sugar) occur in a variety of structural forms. All contains fifteen carbons atom in their basic nucleus: two six-member rings linked with a three carbon unit which may or may not be a part of third ring .The ring labeled A, B, C. They can act as antioxidants and metal chelators. They also possess anti-inflammatory, antiallargic, hepatoprotective, antithrombotic, antiviral, and anti-carcinogenic activities [18].

Table 1 Class and basic structure of phenolic compounds.

Class	Number of carbon	Basic structure
Simple phenolics ,Benzoquinones	6	C_6
Phenolics acids	7	C_6-C_1
Acethophenones ,Phenylacetic acid	8	C_6-C_2
Hydroxycinnamic acid ,Phenylpropanoids Coumarins ,Isocoumarins ,Chromones	9	C_6-C_3
Napthoquinones	10	C_6-C_4
Xanthones	13	C_6-C_1-C_6
Stilbenes ,Anthraquinones	14	C_6-C_2-C_6
Flavonoids ,Isoflavonoids	15	C_6-C_3-C_6
Lignans ,Neolignans	18	$(C_6$-$C_3)_2$
Biflavonoids	30	$(C_6$-C_3-$C_6)_2$
Lignins	many	$(C_6$-$C_3)_n$
Condensed tannins (Proanthocyanidins of flavolans)	Many	$(C_6$-C_3-$C_6)_n$

Tannins are the third important group of polyphenolics. In medicine , especially in Asia , the tannin containing plant extract are used against diarrhea, as diuretics, against stomach and duodenal tumors, as anti-inflammatory, antiseptic, haemostatic pharmaceutical, antiviral, antibacterial, antitumor and tannins can selectively inhibit HIV replication. It can precipitate heavy metals and alkaloids (except morphine), they can be used inpoisoning with these substance. Tannins are used in the dye stuff industries as caustics for cationic dyes and also in the production of inks. In the food industries tannins are used to clarify wine, beer antioxidant in the fruit juices and as coagulant in rubber production [19].

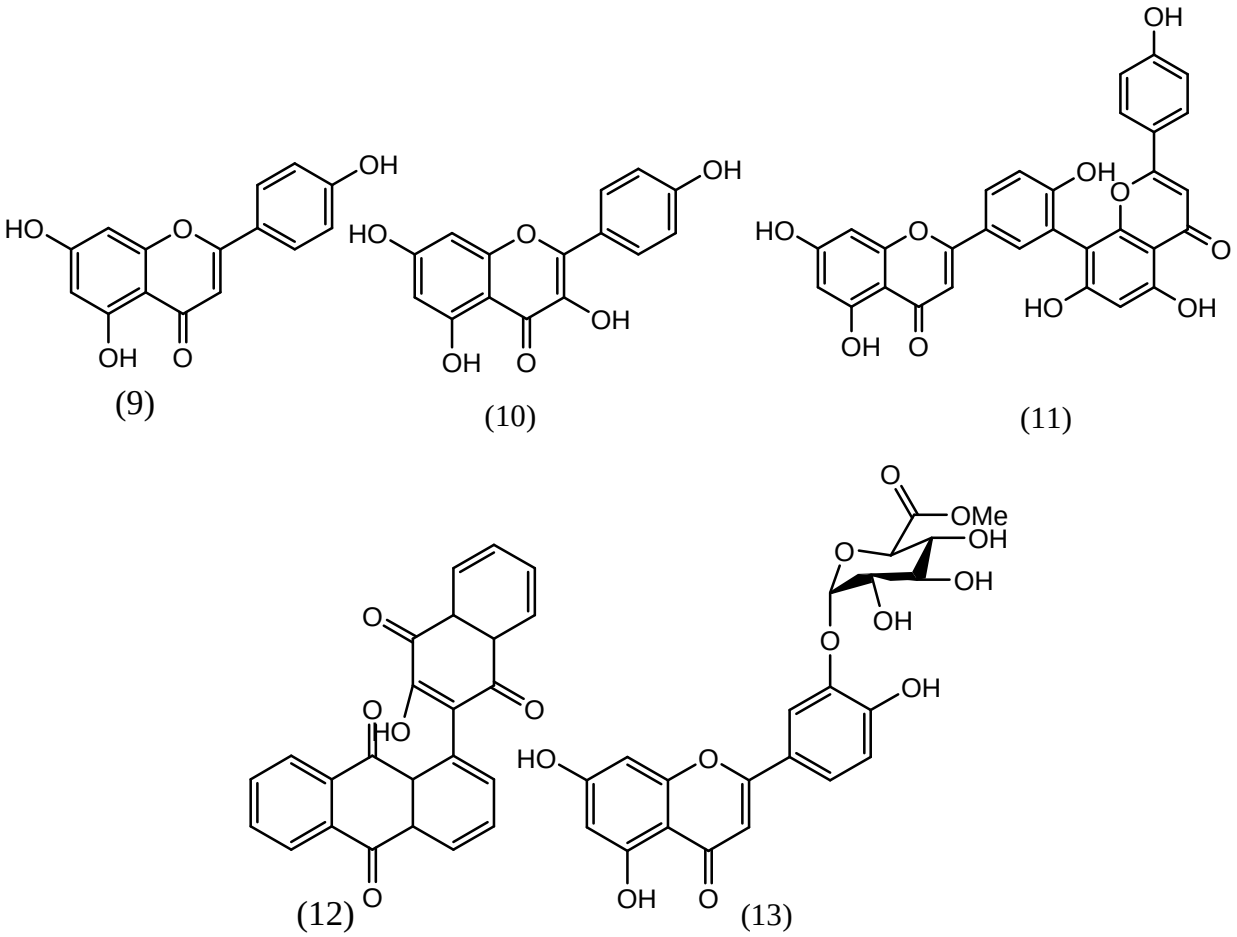


Figure 3: Structure of flavonoids.

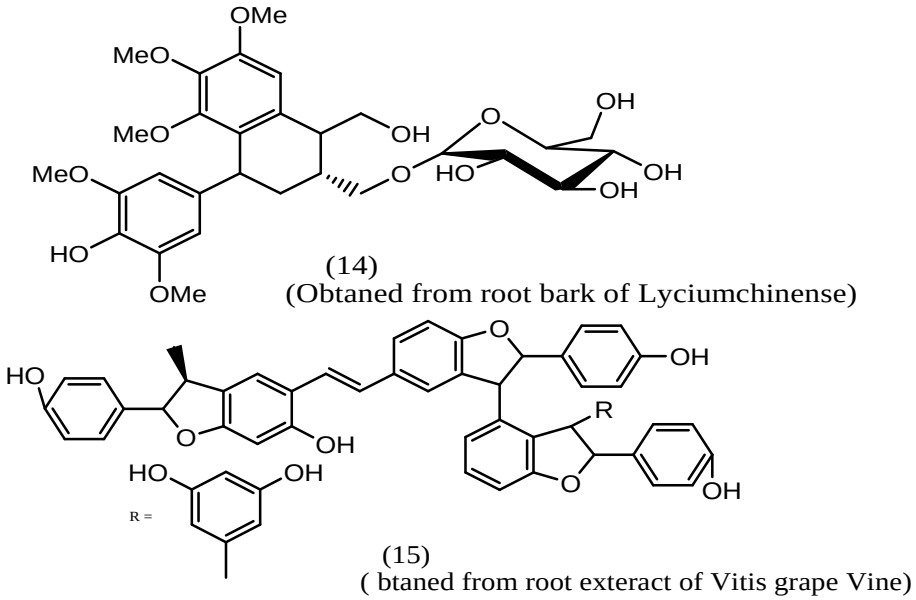
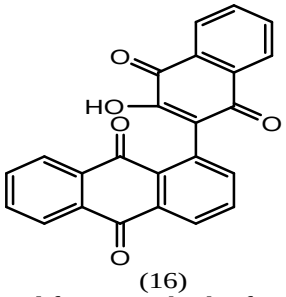
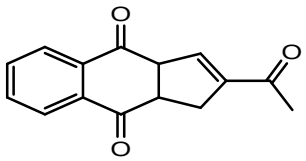


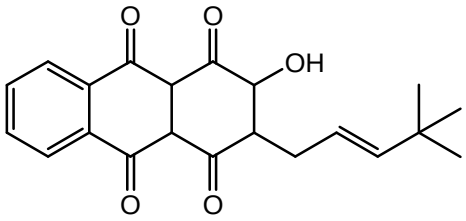
Figure 4: Structures of Lignin



(16)
(Obtaned from root bark of newbouldia leevis)



(17)



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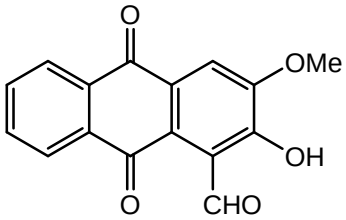
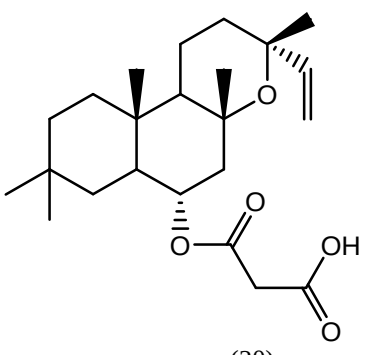


(19)

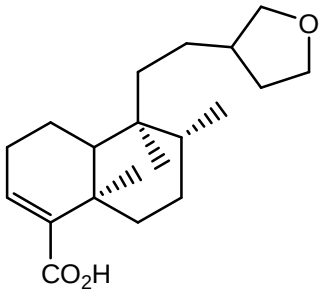
Figure 5: Structure of Quinones

2.2.4 Terpenoids

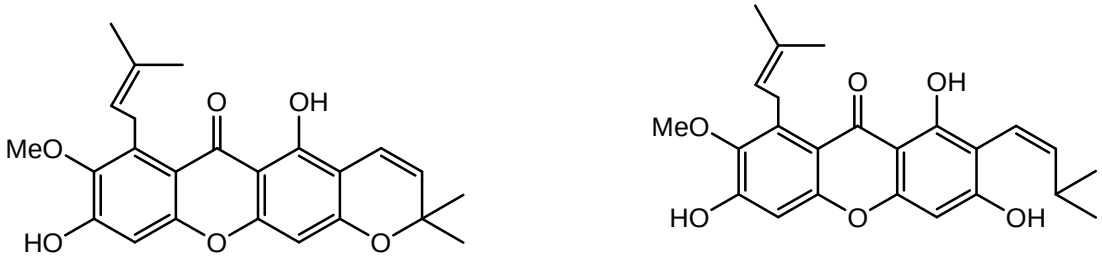
Terpenoids are substances which give plants and flowers their fragrance. They occur widely in the leaves and fruity of higher plants, conifers, citrus and eucalyptus. The term terpenes is used to denote compounds containing an integral number of C_5 units and chemically all terpenoids are derived from basic branched C_5 unit (isoprene or 2-methyl-1,3-butadine). According to the number of such C_5 unit present in the molecule ,terpenoids are classified in to hemi-, mono-, sesqui-, di-, sester-, tri -,and tetraterpenoids (carotenoids) having 1,2,3,4,5,6 and 8 isoprenoids C_5 residue respectively. A vast number of terpenoids have high potential on anti-inflammatory effect [20].



(20)
(Obtaned from aerialpart of Stemodial foliosa)



(21)
(Obtaned fromsteam bark of Lrvingia gabonensis)



(22) (23)

Figure 6: Structure of terpenoids

2.3 Bioactivity of Phytochemicals

Phytochemicals may either be used as chemotherapeutic or chemo preventive agent. Chemoprevention referring to the use of agents to inhibit reverse or retard tumor genesis. Chemo preventive phytochemicals are applicable to cancer therapy [21].The mechanism of action is considered to be the disturbance of the cytoplasm membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents [22].

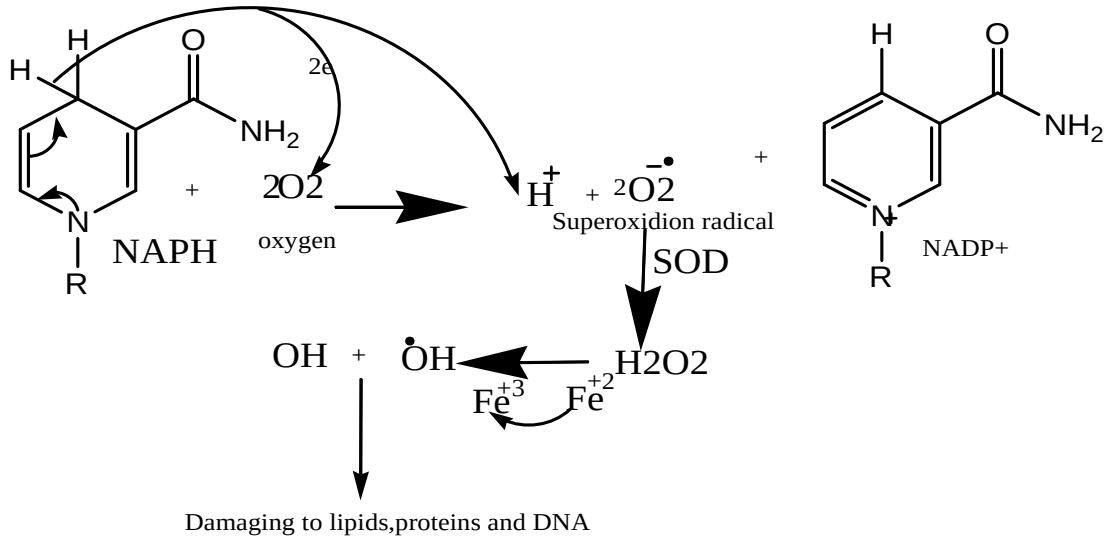
2.3.1 Antimicrobial activities and mechanism of action.

Phytochemicals employed by plants to protect them against pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine [23]. Plant can also exert either bacteriostatic or bactericidal activity. The variation of antimicrobial activity results is because of concentration of plant constituents present in plant organs due to one geographic location to another depending on age of the plant, nutrient concentration of the soil, extraction method, harvesting times, and antimicrobial analysis technique [24]. The antimicrobial effect also depends on the physicochemical properties of antimicrobial agents (acid dissociation constant, solubility and hydrophobicity/lipophilicity ratio), the environmental factors (PH, water activity, temperature) and microbiological factors (species genus, strain of the target microorganism) [25,26,27,28,29]. The well-known mechanism of action of antimicrobial agents are interference with cell wall, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of intermediary metabolic pathways and disruption of the cytoplasm membrane [30]. The cell wall synthesis include the cytoplasmic synthesis of building block composed of N-acetyl muramic acid (M) linked to N-acetyl glucosamine (G) with an attached pentapeptide (P) side chain (MGP sub units). Linkage of an MGP subunit to a lipid II molecule allows subsequent translocation across the cytoplasm membrane to the cell exterior space. The trans glycosylase enzymes assemble the MGP subunit in to linear backbone by catalyzing glycosidic linkage between the M and G component of the MG subunits. Trans peptidase enzyme then act to cross-link the peptide side chain with pentaglycine bridge, cleaving

the terminal 2 D–alanines of the peptide side chain in the process, there by producing the mature lattice like peptideoglycan that provides the bacterium with its shape and osmotic stability [31]. The β-lactam antibiotics interact and efficiently inhibit the bacterial Trans peptidase directly. The β-lactam block the conversion of immature to mature peptidoglcan .This indicated that the streochemical similarity of the β-lactam moiety with the D-alanine substrate which makes the growing bacteria highly susceptible to the lysis and death [32]. Vanomycin inhibit peptidoglycan synthesis. It binds to the carboxyl terminal of acyl-D-alanyl-D-alanin residues of the pentapeptide moiety of lipid II .This specific binding sterically hindered the transglycosylase enzyme [33]. Aminoglycoside acts by binding to specific ribosomal subunits (inhibit protein synthesis) .This drugs can combine with other binding sites on 30s ribosome and they kill bacteria by inducing the formation of aberrant as well as by causing misreading. Chloramphenicol inhibits both gram negative and gram positive bacteria. It inhibits peptide bond formation by binding to peptidyl transferase enzymes on the 50s ribosoms. Trimethoprim and sulfonamides interact with folic acid metabolism in the microbial cell by competitively blocking the biosynthesis of tetrahydrofolate necessary for the ultimate synthesis of DNA, RNA and bacterial cell wall proteins [34]. Quninolones are specific inhibitor of DNA gyres such as supercoling and relaxation that require DNA breakage and reunion [35]. Cationic antimicrobial agent, such as polymyxin B increases the permability of the outer membrane to lysozyme and hydrophobic compounds [36].

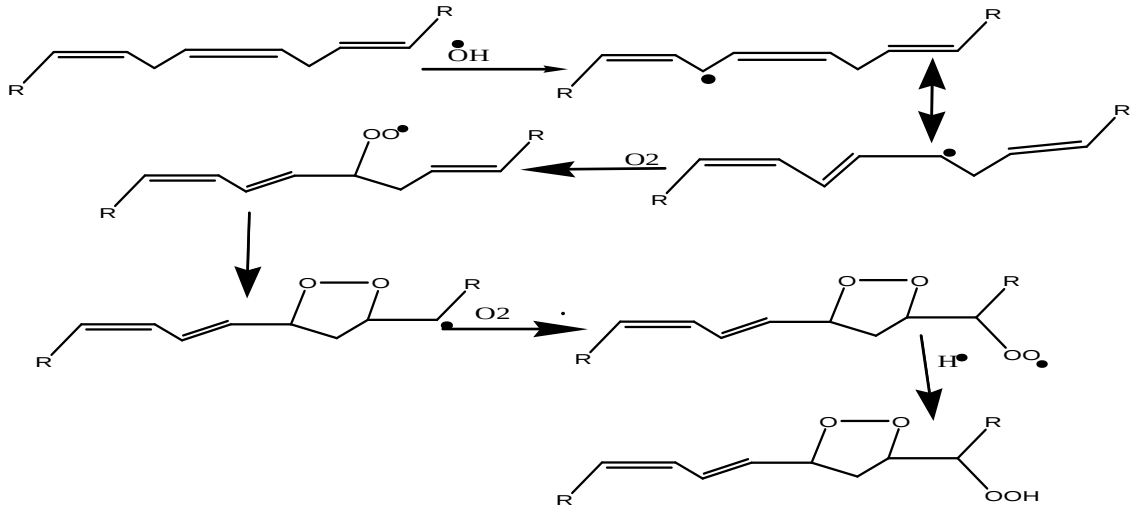
2.3.2 Antioxidant activity and mechanism of action

Antioxidant protect cell against the damaging effect of reactive oxygen species called free radical such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and which results in oxidative stress leading to cellular damage [37]. Natural antioxidants play a key role in health maintenance and prevention of disease, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neuron degenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damaging and ageing [38]. Antioxidants exert their activity by scavenging the free oxygen radical there by giving rise to a fairly' stable radical' where the free radicals tends to trap electrons from the molecule in the immediate surroundings [39]. Reactive oxygen species began with rapid uptake of oxygen, activation of NADPH oxidase [40] and the production of the super oxide anion radical (Sheme-1) [41].



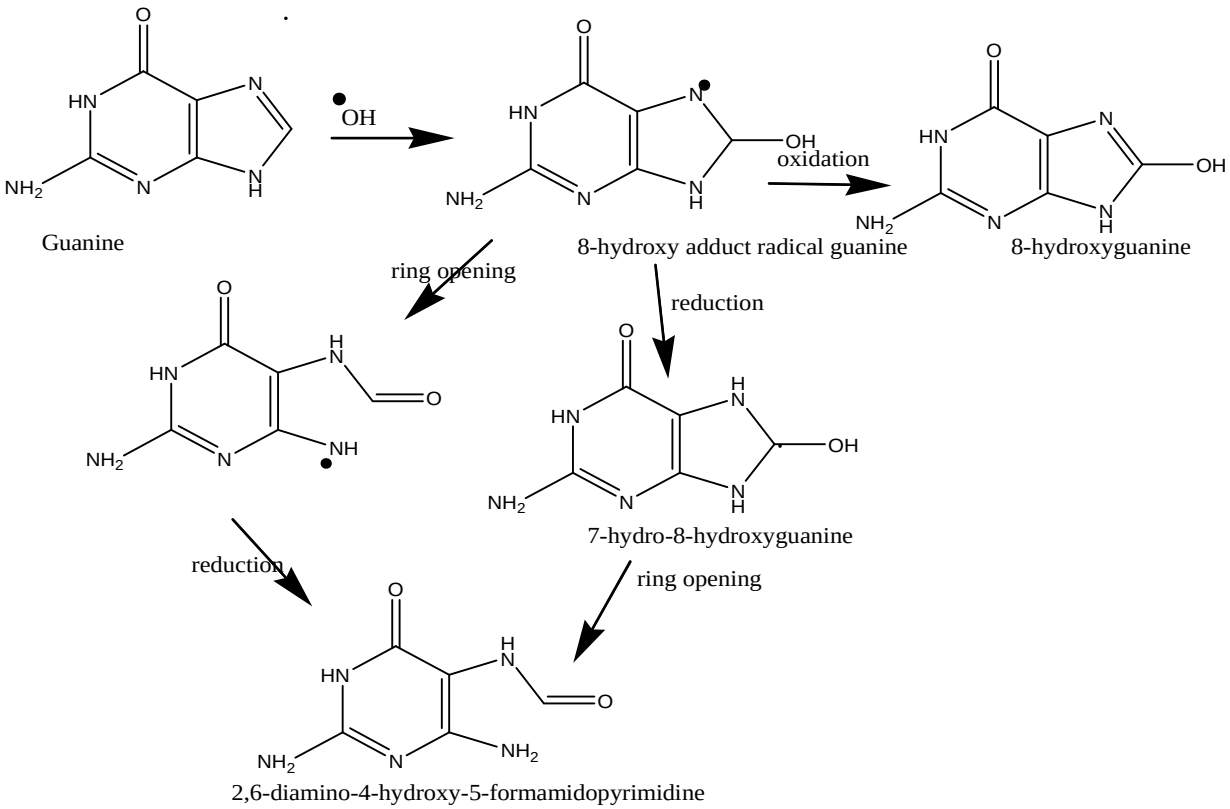
Scheme-1: Production of superoxide anion radical and Hydroxyl radicals

Free radicals attack all major class of biomolecules, mainly the polyunsaturated fatty acid (PUFA) of cell membranes [42, 43] and, the DNA resulting in abnormality leading to diseases including: tumor inflammation, asthma, rheumatoid arthritis, cardiovascular disorders, cystictibrosis, and neurodegenerative diseases [39]. The oxidation of PUFA generates fatty acid radicals which rapidly add oxygen to form fatty acid peroxy radicals. Peroxy radicals are the carrier of the chain reaction. The peroxy radicals further oxidized PUFA molecules and initiate new chain reaction, producing lipid hydroperoxidase [44, 45] widely associated with loss of fluidity and cell lyses [46] with the tissue injuries and diseases (Scheme-2) [47].

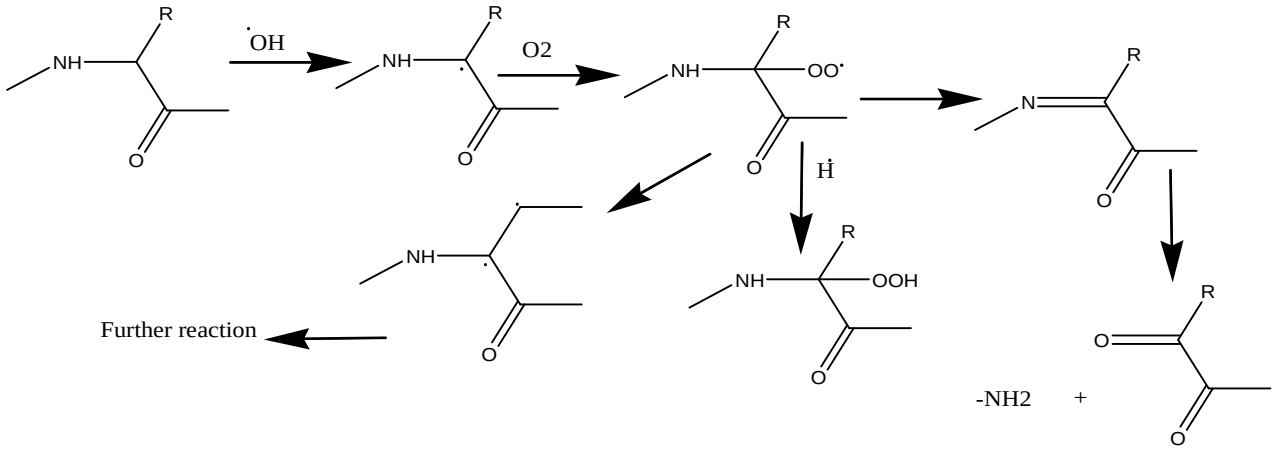


Scheme-2: Reaction of hydroxyl radicals with PUFA

The hydroxyl radical is highly reactive and reacts with biological molecules such as DNAs, proteins and lipids, which result in [48,49,50] modification on bases of the nucleic acid (scheme- 2), producing mutagenesis and carcinogenesis [51] and producing inactivation and denaturation of proteins (scheme - 3)[52].

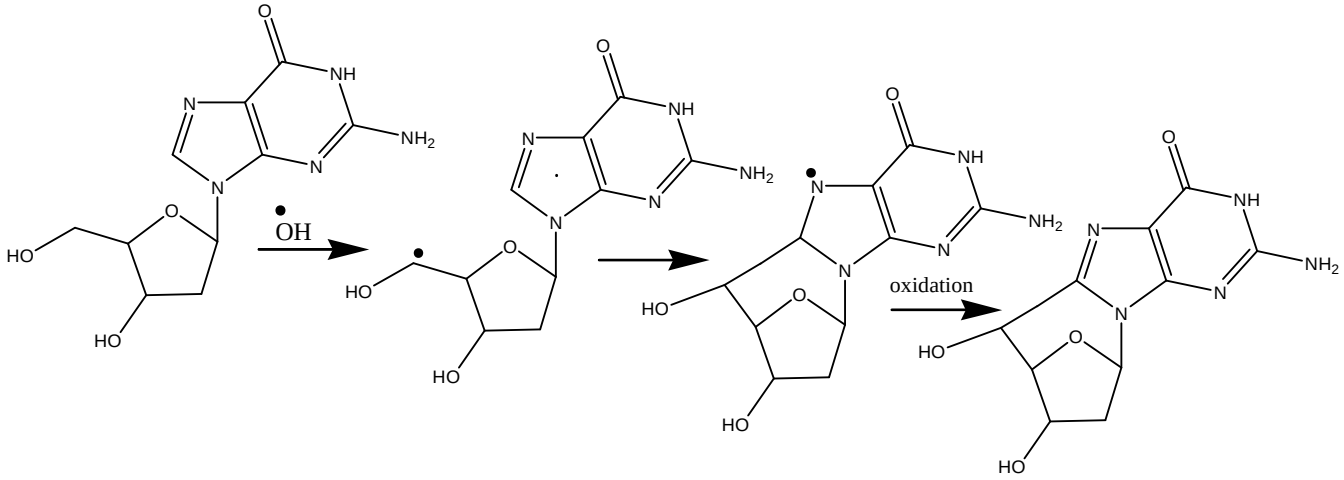


Scheme-3: Reaction of hydroxyl radical with guanine of the DNA



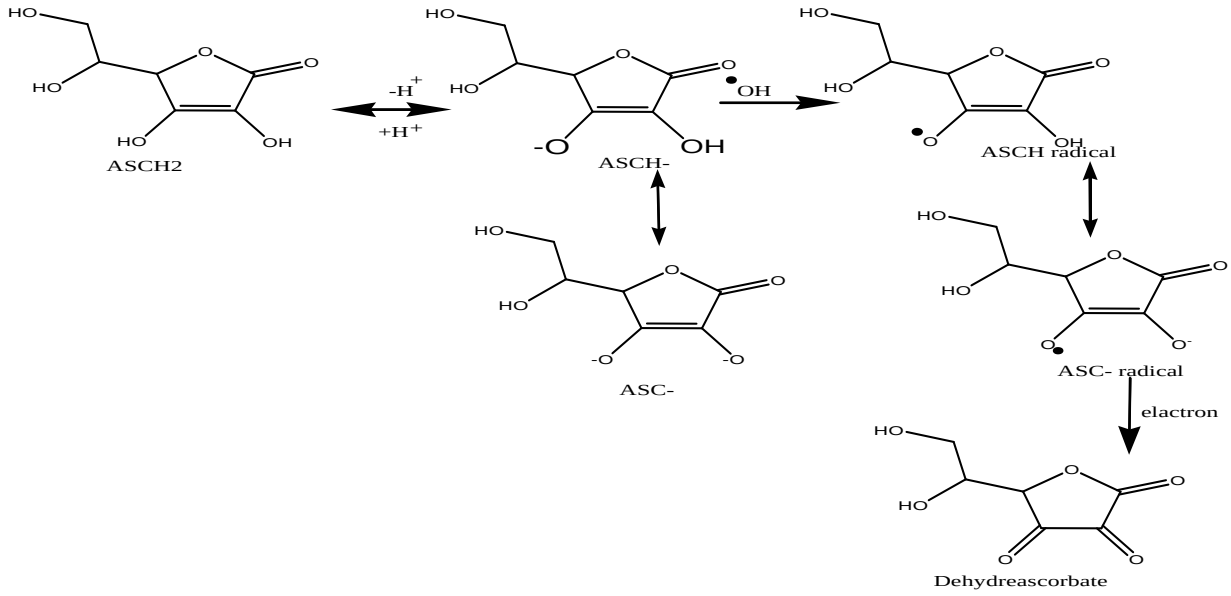
Scheme-4: Reaction of hydroxyl radicals with α-amino acids

In the glycosides, altering cellular functions such as those associated with the activity of interleukins and the formation of prostaglandins, hormones and neurotransmitters (Sheme-5) [51].

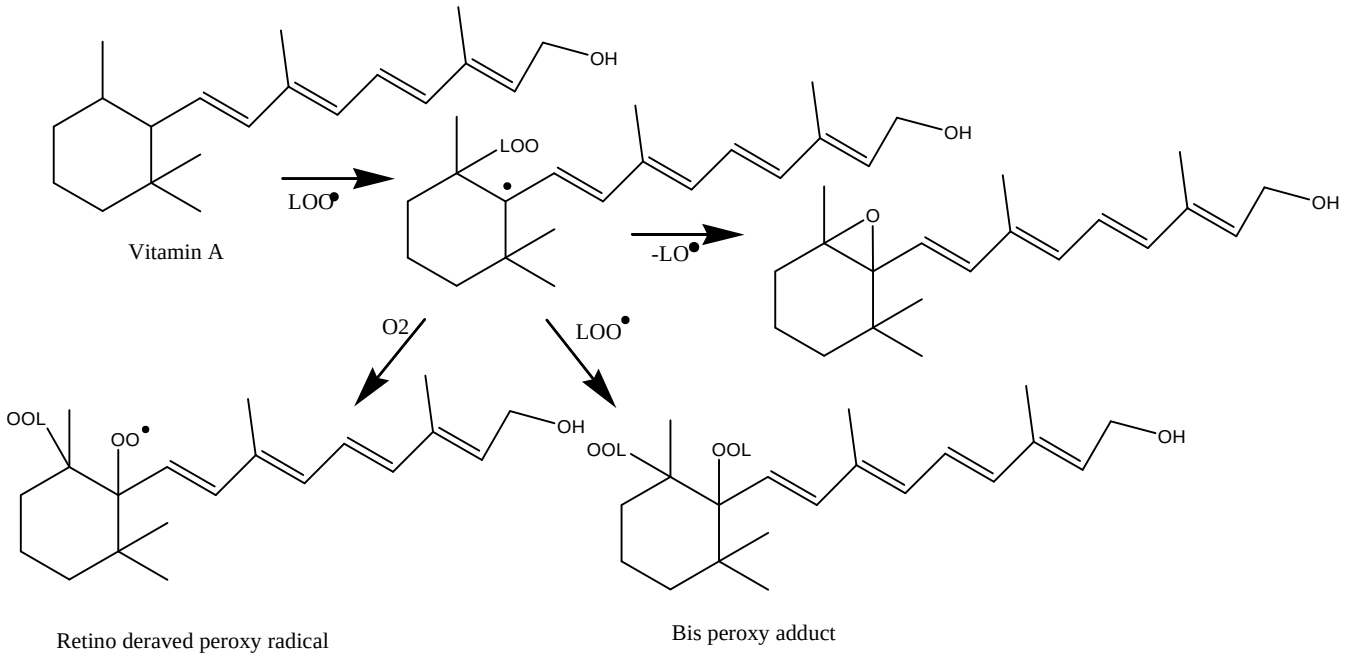


Scheme-5: Reaction of hydroxyl radicals with sugar moiety of DNA

Plants based free radical scavenging molecules including phenols, flavonoids, terpenoid are strong in antioxidant activity [53, 54]. Plants such as citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which possess the ability to scavenge the free radical in human body. Antioxidants are often added to food to prevent the radical chain reaction oxidation, and these act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process [55].



Scheme-6: Mechanism of radical scavenging activity of ascorbic acids



Scheme-7: Mechanism of radical scavenging activity of vitamin A

2.4 Description of the plant *Osyris quadripartita*

The genus *Osyris* includes more than 34 species which belongs to the family of *Santalaceae.* The species *Osyris quadripartite* (locally called Qeret in Amharic) is an ever green tree or shrub reaching a height 1m up to 7 meter with many branches. The plant is native to Africa, South West Europe and Asia .This plant is widely distributed in Ethiopia [56].

2.4.1 Uses of *Osyris quadripartite*

The roots of *O.quadripartita* are used for the treatment of cancer in Tanzania. The powder fresh / dry fruit of *O.quadripartita* , mixed with water , is given orally for 3 days and applied to topically on infected body part of livestock in Wonga Wereda of Ethiopia. Leaf of *O.quadripartita* has anti-ulcer pharmacological activity. It reduces capillary permeability associated with inflammation and has antioxidant, antibacterial and antifungal as well as anti malarial activity [57].The leaf of *O.quadripartita* was active aganest Gram-negative bacteria such as *E.coli* and *Pseudomonas aeruginosa* as well as Gram-negative like *Staphylococcus aureus*. Though its effect was relatively lesser [58]. The bark is boiled in water, stressed and boiled again to form a gelatinous mass that is applied in the eyes to relive inflammation. The powdered fresh dry fruit of *O. quadripartite*, mixed with water, is given orally and applied on the infected body part [59].

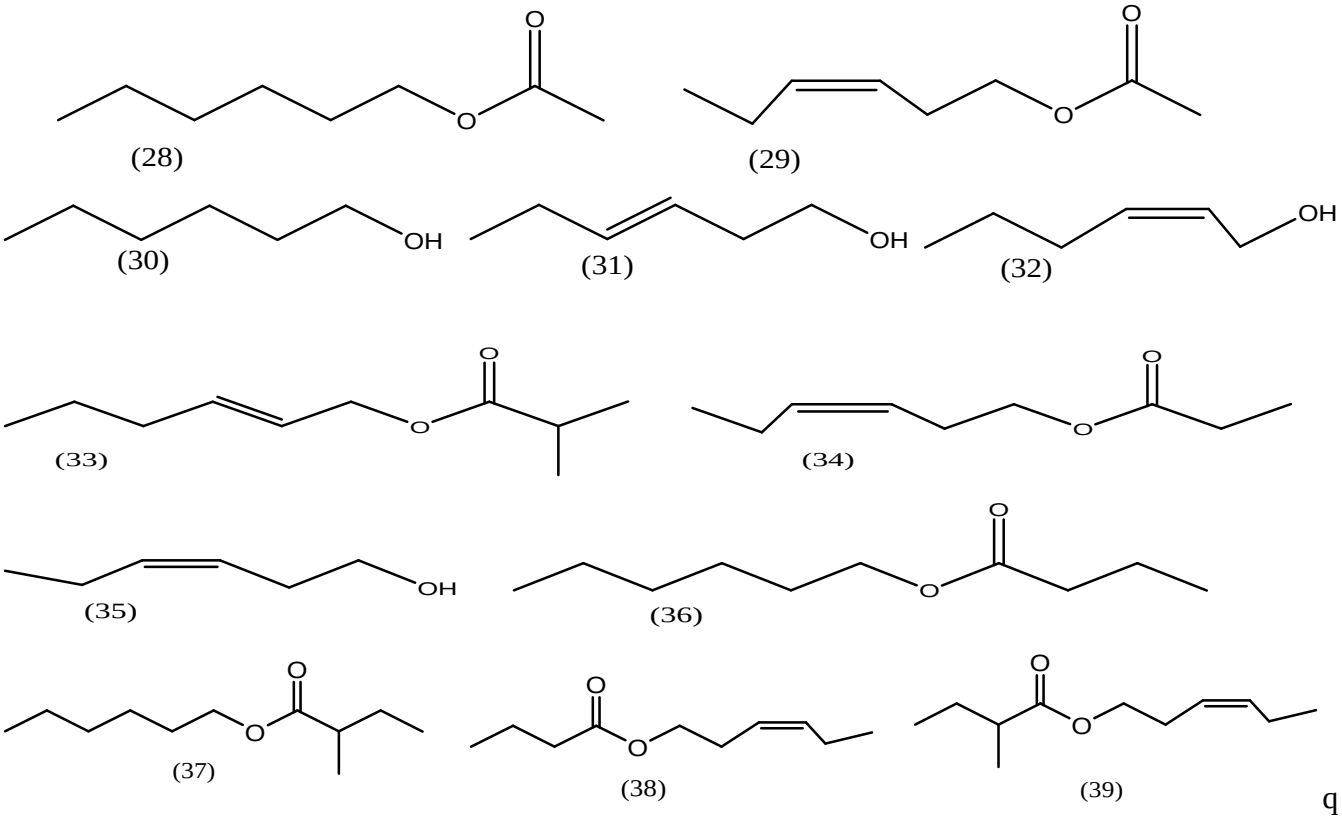
(A) Root

(B) Stem bark

(C) Leaf

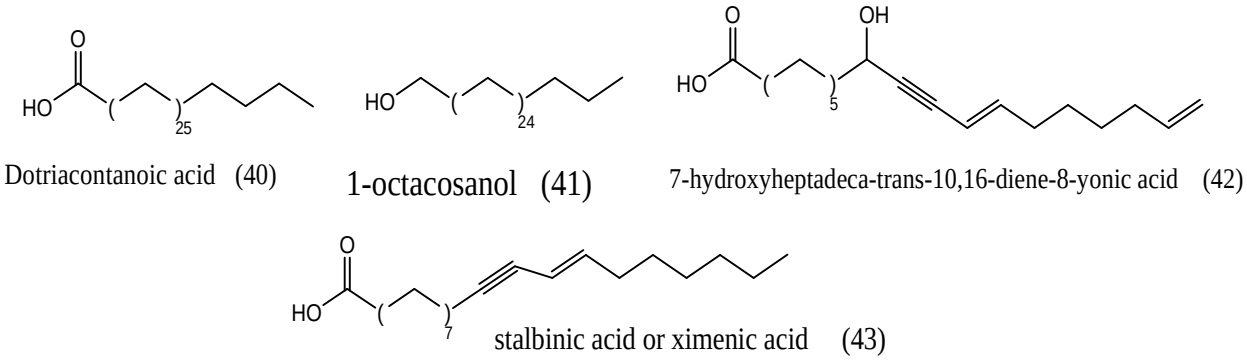
2.4.2 Chemical constituents in Genus Osiris

The genus osyris includes 34 species. A few of them are Osyris alba, osyris lanceolata, Osyris wigntiana, Osyris quadripartita, Osyris teniufolia, Osyris compressa, and Osyris abyssinica. Phytochemical investigation of genus Osyris has resulted in 108 compounds with varying structural patterns. Volatile constituents of genus contain hexyl and hexenyl derivative, sesquiterpenes, long chain hydrocarbons and fatty acids. triterpenes, dihydro-β-agarofuransesquiterpenes, flavonoids, phenolic acids and phenyl propanoids, pyrrolizidine and quinolizidine alkaloids, norisoprenoids, lignans, and β-carboline alkaloids are also present [60].The phytochemical study leaf of *O.quadripartita* resulted in the presence of alkaloide , phenols ,terpenoids , tannins ,saponins and flavonoids but not steroids[61].



q

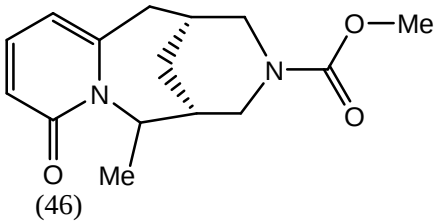
Figure 7: Hexyl and hexenyl derivatives

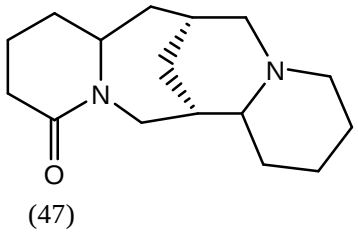


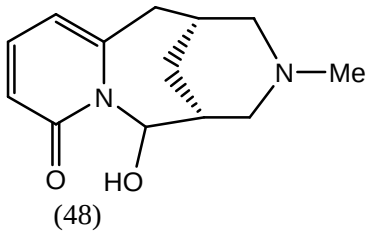
Dotriacontanoic acid (40) 1-octacosanol (41) 7-hydroxyheptadeca-trans-10,16-diene-8-yonic acid (42)

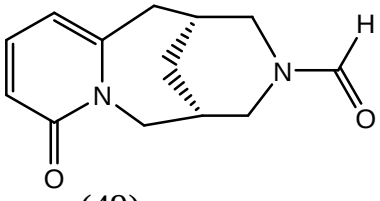
stalbinic acid or ximenic acid (43)

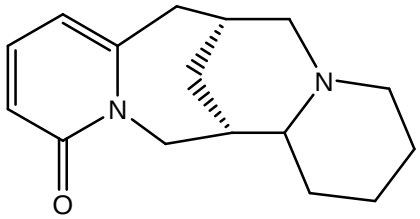
Figure 8: Long chain hydrocarbon and fatty acids

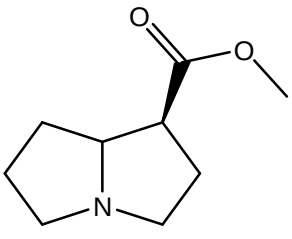












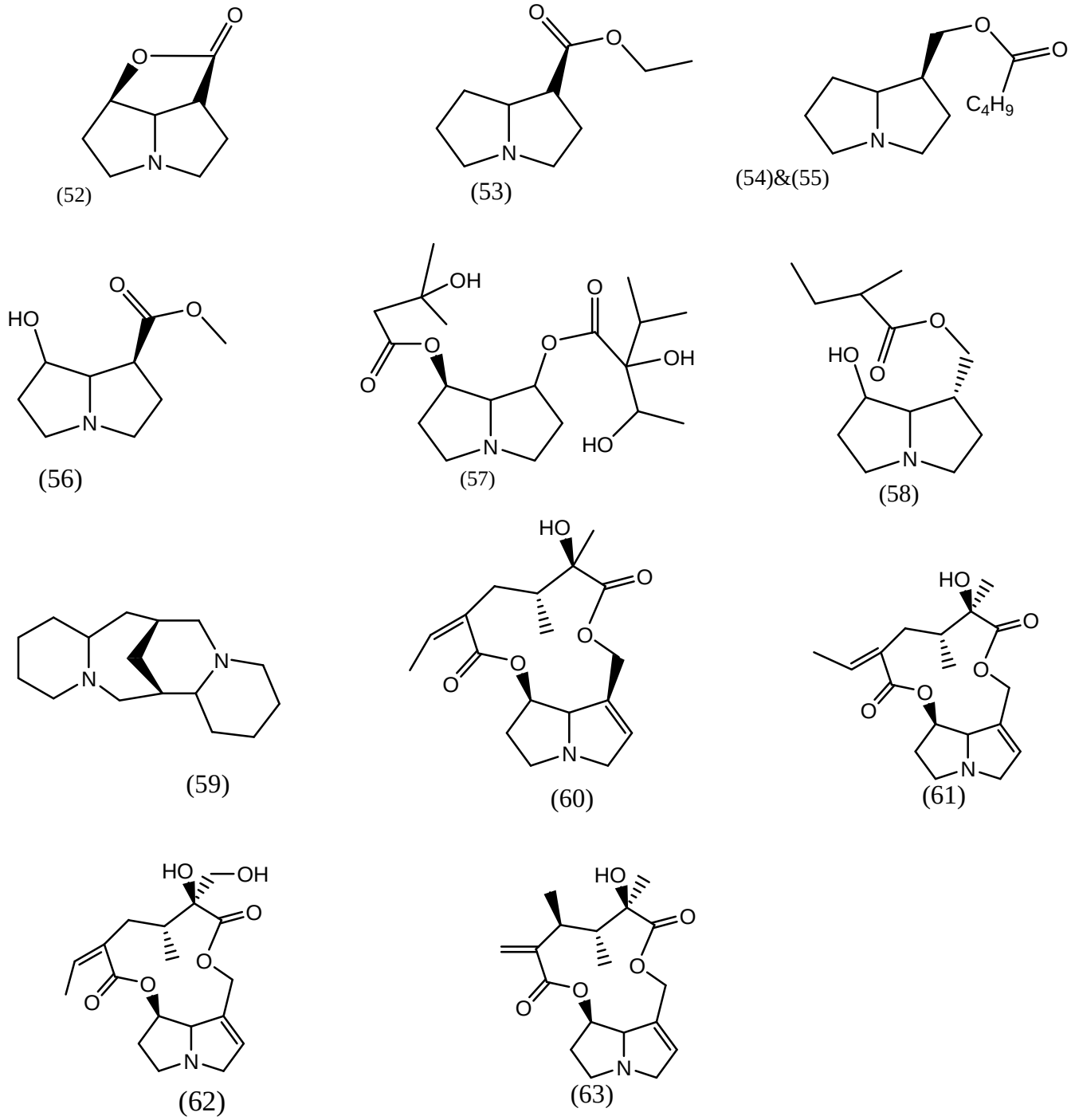


Figure 9: pyrrolizidine and quinolizidine alkaloids

The above alkaloids are extracted from *Osyris alba.*

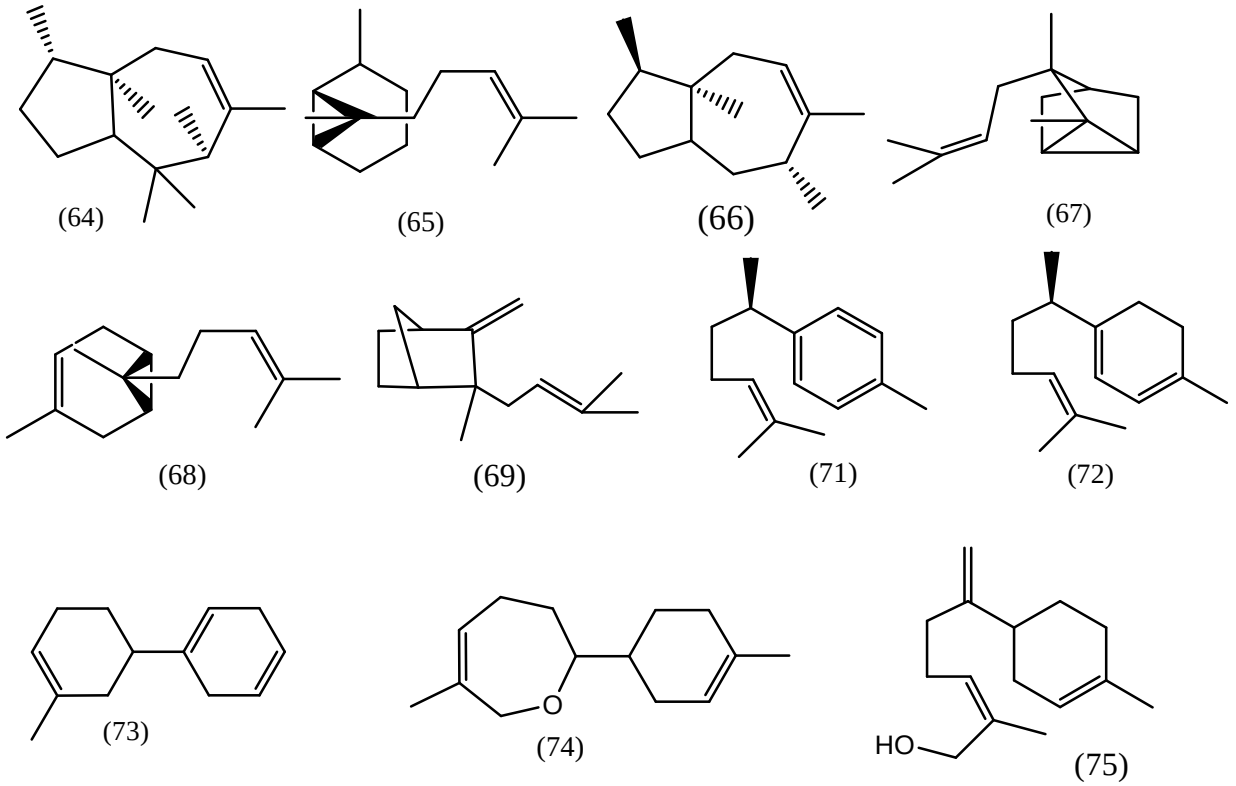


Figure 10: The structure of Sesquiterpenes

The above sesquiterpines extracted from the essential oil of *O.tenuifolia*.

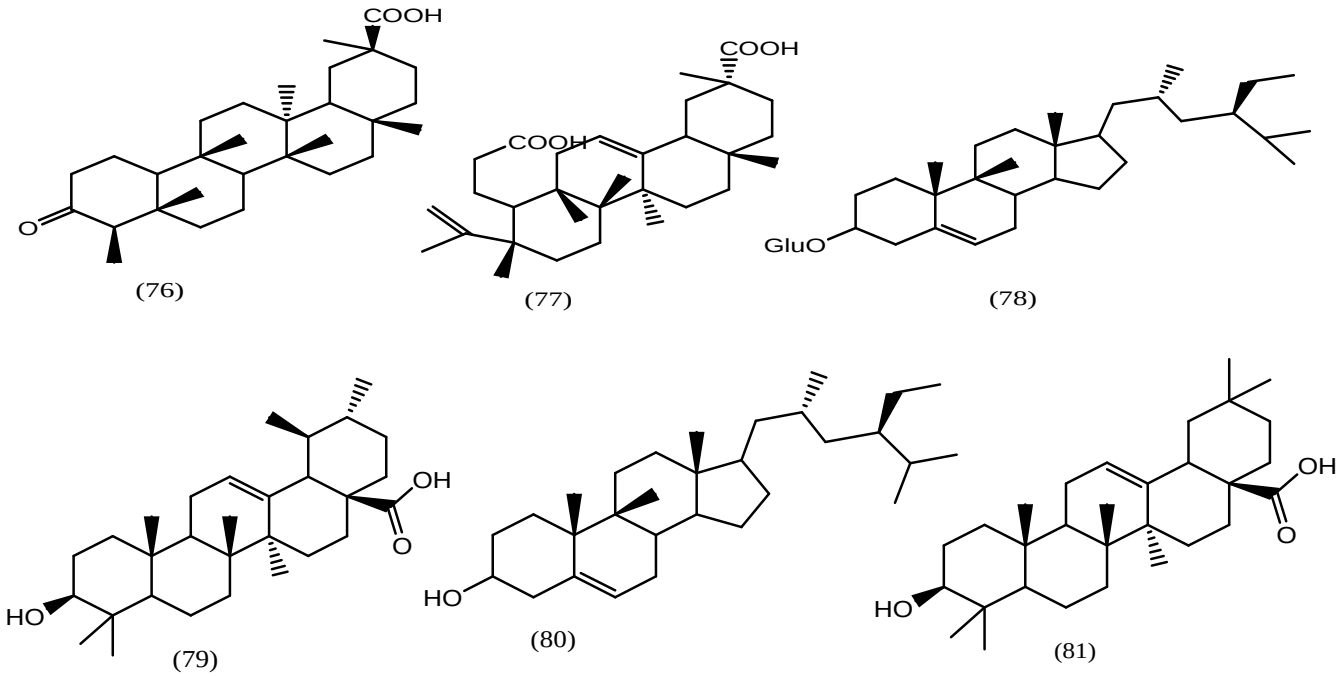
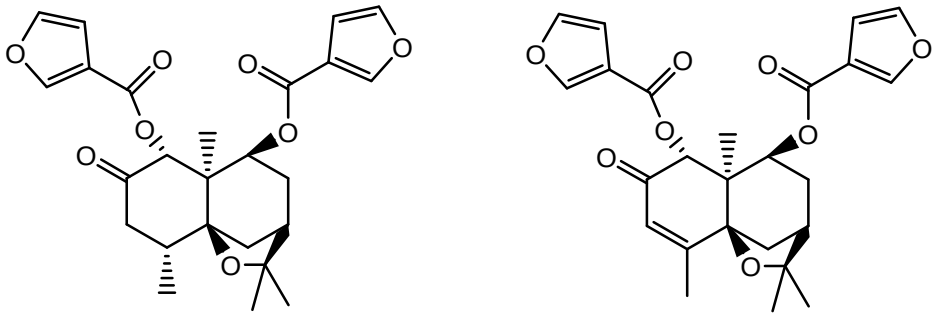


Figure 11: Structure of Triterpenes

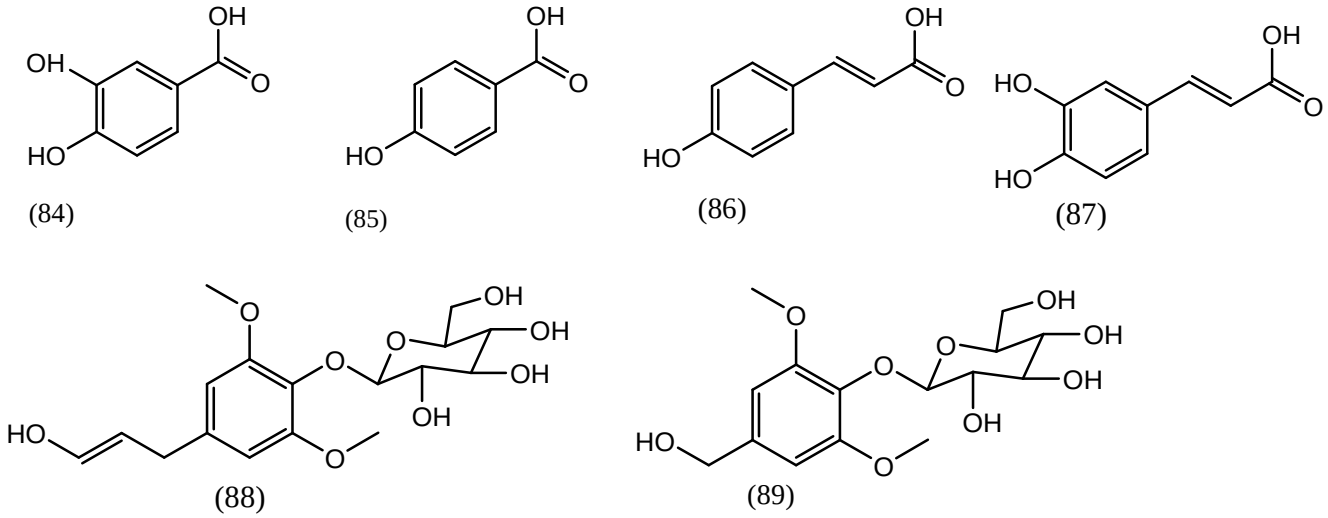
The above triterpenes are extracted from O.lanceolata.



(82) (83)

Figure 12: The Structure of Dihydro-β-agarofuran sesquiterpenes

The above compounds (Dihydro-β-agarofuran sesquiterpenes) are extracted from root bark of O.lanceolata.

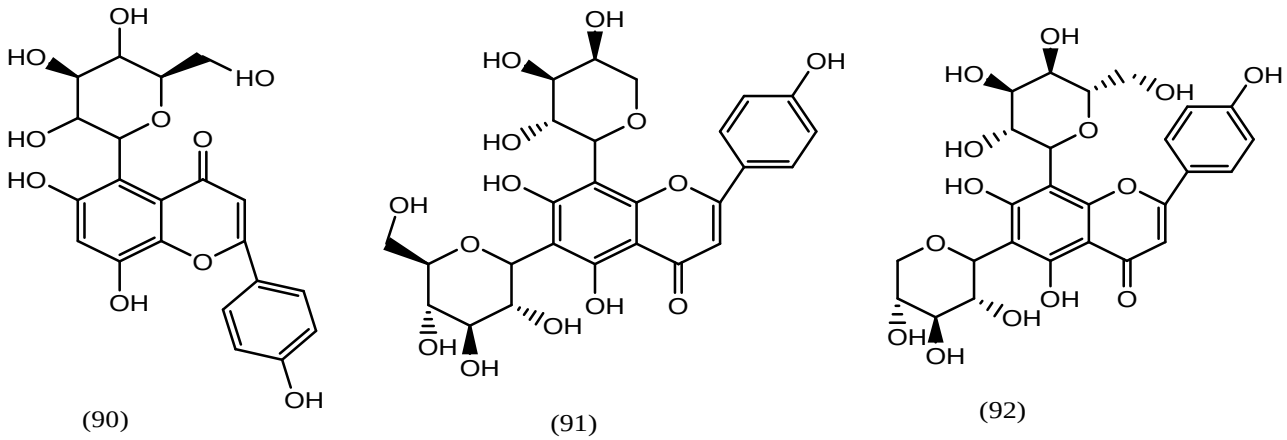


(84) (85) (86) (87)

(88) (89)

Figure 13: The structure of phenolic and phenyl propanoids

Compound 88 and 89 were isolated from O.wightiana whereas compounds 84, 85, 86, and 87 were isolated from O.alba.



(90) (91) (92)

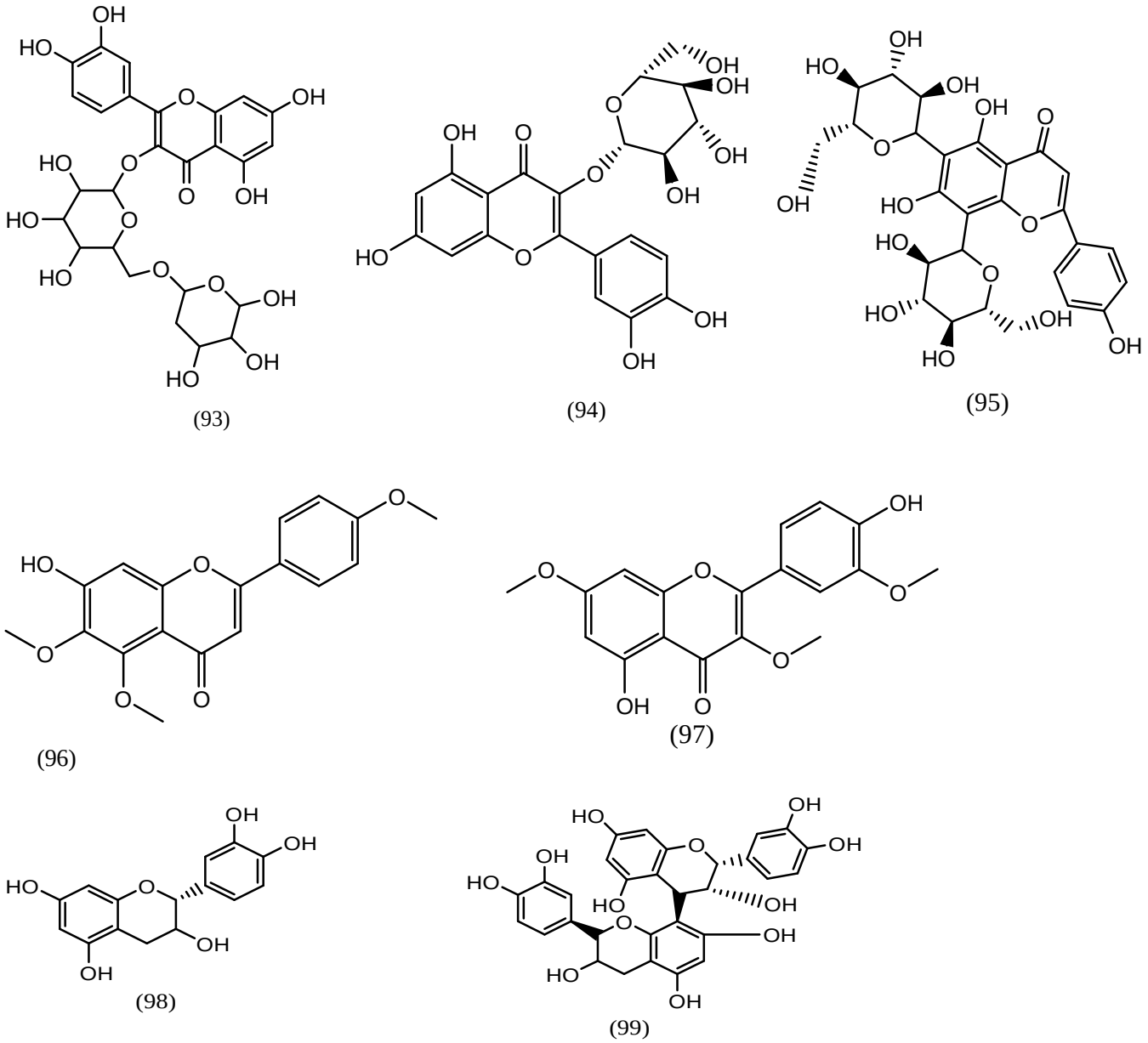


Figure 14: The Structure of Flavonoids

The above all flavonoids are extracted from the aerial parts and fruits of O.alba. Compound 93, 98 and 99 was isolated from Leaf of *O. quadripartite*.

2.5 About dyeing

Reactive dyes react directly with part of cellulose structure of the cotton fibers by forming strong covalent bond between the dye and the fiber resulting in very good to excellent wash fastness. The potential of their resistant to sunlight is variable [62]. Direct dyes are grouping of dye structures which have been used for many years. They use salt as a dyeing assistant but need very small amount compared to reactive dyes. They do not react with the fiber structure but rely on dye to fiber associations

such as H-bonding and dipole interaction to help with colour fastness after dyeing. In general they exhibit good light fastness and have poor wash fastens [63].

Vat dyes are not water soluble in their normal form. In order to dye cloth, these dyes are made water soluble by the chemical process known as reduction [64]. Disperse dyes are water insoluble dyes which are used for thermoplastic synthetic fibers, primarily polyester. They are only medium quality dyes for acrylic fibers and they are generally poor dyes for nylon [65].

2.5.1 Chemistry in dying process

Raw cotton fibers are approximately 95% cellulose. The structure of cotton cellulose is a linear polymer of β-D-glucopyranose.

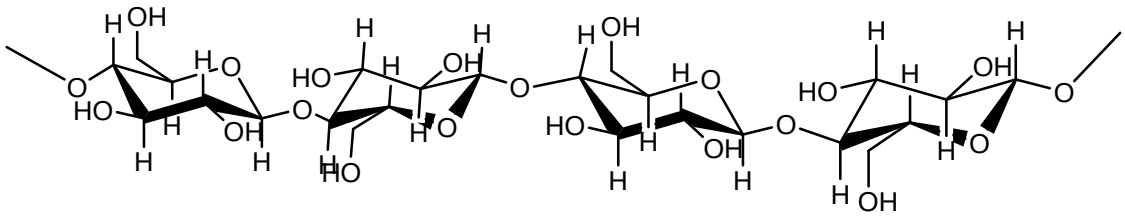


Figure 15: Four residue segment of cellulose chain

Reactive dyes contain chromophoric group attached to moieties that have functions capable of reaction with cotton cellulose by nucleophilic addition and nucleophilic substitution to form covalent bonds [66]. The acid dye family is a larger group of colorants that are attracted to polyamide and protein fibers such as nylon, wool and silk [67] which leads to electrostatics interaction between the dye and fiber when dyeing takes place under acidic condition. They include mordant dyes that are typically treated with transition metal ions which lead to the improvement in wash and light fastness [68]. A cotton fiber can be dyed by coordination bonding of cellulose molecules to natural dyes with the aid of various metal ions [69].

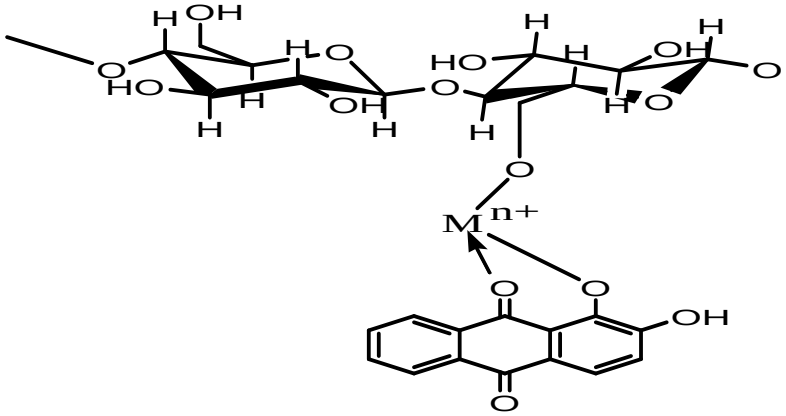


Figure 16: Bonding of a natural dye to cellulose by means of mordant (Mn+).

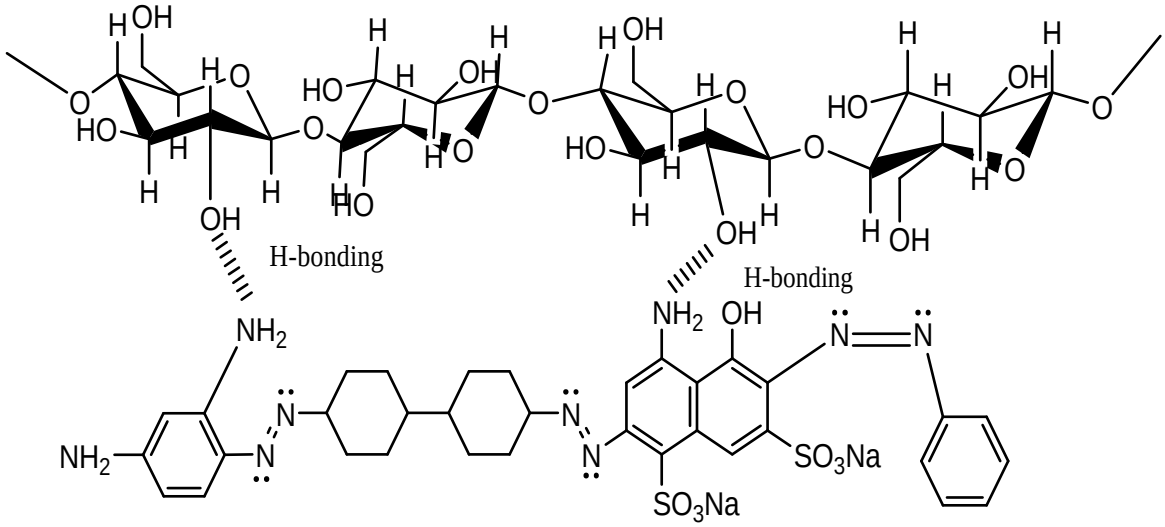


Figure 17: H-bonding between cellulose and direct dye.

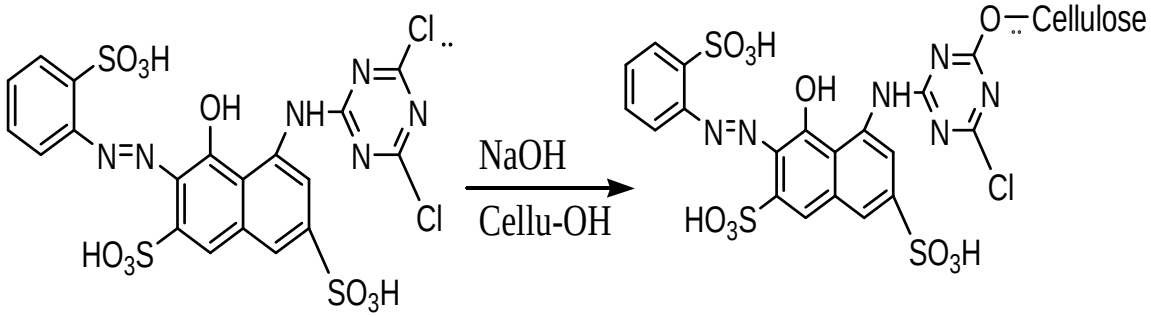


Figure 18: Dye-fiber fixation via covalent bonding

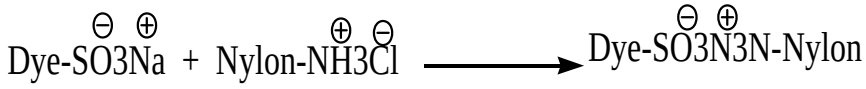


Figure 19: Schematic representation of dye-polymer binding via ionic bonding on nylon

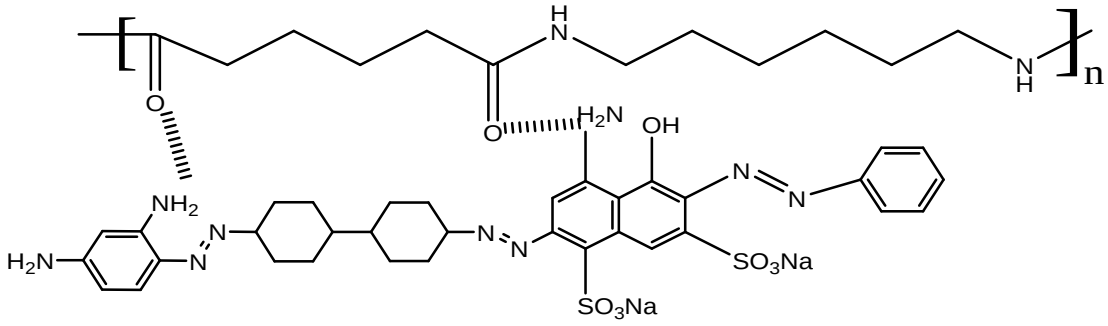


Figure-20: Schematic representation of dye-polymer binding via H- bonding on nylon.

3. MATERIALS AND METHODS

3.1 Collection and Identification of the Plant Material

The leaf, stem bark and roots of *Osyris quadripartita* were collected from Debresina town, North Showa Zone, Amhara Regional state, Ethiopia, which is located about 190 km away from the capital city, Addis Ababa, in February, 2012 E.C. The plant *Osyris quadripartite* was identified by professional botanist at Debre Berhan University (Dr. Abyou). After collection parts were dried in an open air protected from direct exposure to sun light for 2 weeks. The dried plant materials were ground using electric grinder. The resulting powders were kept in polyethylene bag to avoid from certain environmental effects (moisture, air and dusts) until used.

3.2 Apparatus and Instruments

Filter paper (Whatman No.1 filter paper), electric grinder, pipettes (different size), beakers (different size), electronic balance, conical flask, flasks (different size), measuring cylinder, water bath, rotator evaporator, polyethylene bag, test tubes, UV-spectrophotometer, separating funnel, beaker, spatula, 6mm diameter Whatman No 4 paper discs, forceps, autoclave, boiling water bath, incubator and holder. Plant cutters, paper sheets, scissors, glass rode, washing brush, funnels.

3.3 Chemicals and Reagents

The chemicals and reagents used in the study were distilled water, solvents (Petroleum ether, Methanol, dimethylsulfoxide (DMSO)), ethyl acetate and ethanol), Muller |Hinter agar media for bacteria, concentrated sulfuric acid, chloramphenicol, concentrated and dilute hydrochloric acid, 1% of aqueous iron chloride (10% ferric chloride solution ($FeCl_3$), concentrated sulfuric acid, Mayer reagent, acetic anhydride, NH_3, ascorbic acids, 1,1-diphenyl-2-picrylhydrazyl(DPPH).

3.4 Extraction of the Plant Materials (leaf, root, stem bark)

The extraction of plant materials were carried out following reported procedure [70]**.** The powdered (100g each) stem bark and leaf were macerated in 500ml of methanol, ethyl acetate, petroleum ether for three days (72hr). The macerated samples were filtered and concentrated to dryness by rotary evaporator to afford dried crude extracts. They were labeled as Methanol extracts of root, steam bark and leaf by 41A, 41B and 41C respectively. Ethyl acetate extract of root, steam bark and leaf labeled as 41D, 41E and 41F. Petroleum ether extract of root, steam bark and leaf labeled as 41G, 41H and 41I respectively.

3.5 List of test microorganisms and Bioassay Methods

Bacteria *Salmonella typhimurium*, gram negative, ATCC (13311), *Listeria monocytogen*, gram positive, ATCC (19115), *Escherichia coli*, gram negative, ATCC (25922), *Staphylococcus aures*, gram positive, ATCC(25923) and a fungus *Candida albican* were used in this study.

3.5.1 Preparation of microbial inoculums

One colony of each test organisms (*S. typhimurium, L. monocytogenes, E.coli, S. aureus* and *Candida albican)* grown in nutrient agar at 37^0C for 24hr was taken by clean loop and inoculated to sterilized nutrient broth to form bacterial suspension. 0.1ml of this bacterial suspension was spread on pre-dried Muller Hinton agar in Petri dish (90 mm diameter). Muller Hinton agar growth media was prepared by adding 39 g of its powder to distilled water bringing the volume to 1L and mixed thoroughly. Gently heat and bring to boiling then autoclaved at 15 Psi pressure at 121^{o}C for 15min.Finally poured in to sterile Petri dish in laminar air flow hood [71].

3.5.2 Antimicrobial activity test

Methanol, ethyl acetate and petroleum ether extracts of leaf, stem bark and the root of *Osyris quadripartita* were evaluated by using the paper disc diffusion method. The antimicrobial activities of all samples were tested against two Gram positive bacteria (*S.aureus* and *L.monocytogenes*), two gram negative bacteria (*E. coli* and *S.typhimurium*) and *one* fungus. About 20µl of each plant extract was dropped on the serialized paper disks. The paper disks, prepared from the Whatmann filter paper number 4 (6mm in diameter), were sterilized in autoclave for 1 hour and loaded with extract samples individually by handling sterile forceps, and then gently pressed down onto dried MHA. 20 µl of each extract concentration (50, 100 and 150mg/ml) were pipette to the discs in two replications. Antibiotic discs of chloramphenicol were used as positive controls. DMSO was used as negative control. Then the plate was inverted and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of 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 [71].

3.5.3 Antioxidant activity test

About 2ml of the methanol, ethyl acetate and petroleum ether extracts of the plant parts (leaf, root and steam bark) at concentration of 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml and 400µg/ml were added to 2ml solution of DPPH radicals in methanol (0.004%).

The mixture was shaken vigorously and allowed to stand for 1hr at room temperature. The sample absorbance (Ab_{sample}) of the resulting solution was measured at 517nm and converted into percentage of antioxidant activity (AA) using the following formula: AA% =100-{[Ab_{samp}-Ab_{blank}] x100/$Abs_{control}$}. A Methanol (2ml) and plant extract (2ml) solution was used as the blank (Ab_{blank}). A DPPH (2ml) and methanol (2ml) solution was used as control ($Ab_{control}$). Absorbance of Ascorbic acid (Ab_{asco}) was used as standard (reference). The radical scavenging activity was expressed in terms of amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% (IC_{50}).The IC_{50} value for each sample was determined graphically by plotting the percentage disappearance of DPPH as function of the sample concentration[72].

3.6 Phytochemical Screening Procedures

Detections of the presence of common secondary metabolites were performed for tested crude extracts from leaves, root and stem bark of *Osyris quadripartite* using the preceding analytical procedures reported elsewhere [70].

Test for alkaloid: small portion of solvent free extract was stirred with a few drops of dilute HCl and test with Mayer reagent (dissolving a mixture of 1.36g of HgCl2 and 5.00g of KI in 100ml of water) then creamy (yellow) precipitate formed.

Test for Tannins: 5ml of various solvent extract was mixed in 10 ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. Dark-green solution will appear.

Test for Phenols: To 1ml of various solvent 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 saponins (Froth Test): To 2ml of each fraction, 5 ml 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.

Test for Terpenes (Salkowski test): 2ml of crude extract will be mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 will add carefully to form a layer. Reddish-brown coloration of the interface shows the presence of terpenoid.

Test for Flavonoids: 2gm of the extract was mixed with 5ml of dilute NH_3 and a few drop of concentrated H_2SO_4 was added, it becomes yellow color, which indicates the presence of flavonoids.

Test for Sterols (Salkowski reaction): Chloroform extract of the sample was prepared and 5ml of chloroform extract was measured in to a test tube and acetic anhydride followed by concentrated H_2SO_4

(2M) were add carefully down the side of the test tube to form a layer .The presence of a bluish color at the inter face indicate the presence of sterol.

3.7 Dyeing properties of root of O.quadripartita on cotton and Nylon fibers

3.7.1 Extract preparation

Crude root extract from *O.quadripartita* was prepared by adding 10g of the dry and pulverized plant material to 250ml of distilled water in 400ml Pyrex beaker. The mixture was stirred, heated to boiling for 2hr, allowed to stand for 20min cooling and then filtered. The colored filtrate was used for dyeing cotton and nylon pieces in the presence of $FeSO_4$ and $CuSO_4$ as mordant [73].

3.7.2 Scouring of fabrics

All the tested fabrics (cotton and nylon) were cut in to 5x6 cm and washed for 20min with soap at 50^0C by keeping the material to liquor ratio 1:80. The material was then washed thoroughly with plenty of tap water and soaked in distilled water for 30 min prior to dyeing [74].

3.7.3 Mordanting

The process of mordanting was carried out by adopting pre-, simultaneous and post-mordant using 4% of mordant solution and maintaining 1:50 material to liquor ratio. The process of mordanting was carried at 60-75 ^{0}C with gentle stirring and continued for 1h [75].

3.7.4 Washing fastness

Dyed fabric sample was placed in preheated soap solution in the ratio of 1:50 in vessel for 45min.The dyed sample was removed, rinsed in cooled water and evaluated by comparison of color change [75].

3.7.5 Light fastness

The dyed fabric was exposed to sun light for 24h. The color fastness to the light was evaluated by comparison of color change of the exposed portion with the unexpected original material [75].

3.8 Data analysis

Data was analyzed both qualitatively and quantitatively. Antimicrobial activity data and antioxidant activity data analyzed quantitatively by mean ± standardivation using Microsoft office Excel 2007.Phytochemical study data and dyeing property of the plant root was analyzed qualitatively.

4. RESULTS AND DISCUSSION

In this study the antimicrobial and antioxidant activities of the extracts of the root, stem bark and leaf of Osyris quadripartita were evaluated and the phytochemical components of each solvent crude extracts were identified.

4.1 Extraction yield

Root, stem bark and leaf (100g each) of Osyris quadripartita were macerated with methanol, ethyl acetate and petroleum ether to afford crude extracts (Table 2).

Table 2: Extraction yield of plant parts

Extraction solvent	% yield		
	root	Stem bark	leaf
Methanol	10	9	8.5
Ethyl acetate	8	7	5
Petroleum ether	4	3	2

4.2 Antimicrobial activity test

Results of bacterial test for crude methanol, ethyl acetate, and petroleum ether extract of root, stem bark and leaf of *O. quadripartita* were expressed as mean ± standard Deviation (SD) of growth inhibition zone diameters obtained with two trials. The methanol, ethyl acetate and petroleum ether extract of root, stem bark, leaf of *O. quadripartita* were subjected to the antibacterial activity and the results were investigated. Chloroamphibicol was used as positive control. The results were tabulated in table below.

Table -3: Results from disc diffusion assay showing the antibacterial activity plant root bark extract against five human photogene bacteria by disc diffusion method.

Extract	Concentration (mg/ml)	Name of Microorganisms				
		Candida albican	*Listeria monocytogene*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
Methanol	150	–	–	–	–	–
	100	–	–	–	–	–
	50	–	–	–	–	–
Ethyl acetate	150	-	–	7±0.05	7±0.07	-
	100	-	–	6.5±0.05	6±0.005	-
	50	-	–	–	-	-
Petroleum ether	150	-	-	8±0.7	7.5± 0.7	7.9±0.14
	100	-	-	6.5 ±0.14	6.5± 0.7	7.3± 0.3
	50	–	–	–	–	–
Chloroamphibicol(+)control		11.5 ±0.7	10.5± 0.7	13.5 ±2	15.5± 0.7	11± 1.4

- Indicates no inhibition zone

Table -4: Results from disc diffusion assay showing the antibacterial activity plant stem bark extract against five human photogene bacteria by disc diffusion method.

Extract	Concentration (mg/ml)	Name of Microorganisms				
		Candida albican	*Listeria monocytogene*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
Methanol	150	–	–	–	–	–
	100	–	–	–	–	–
	50	–	–	–	–	–
Ethyl acetate	150	-	–	7.1±0.05	6.1±0.03	-
	100	-	–	6.5±0.05	6.1±0.03	-
	50	-	–	–	-	-
Petroleum ether	150	-	-	7.5±0.7	8± 0.07	7.5±0.07
	100	-	-	7 ±0.14	8± 0.07	7± 0.07
	50	-	-	-	-	-
Chloroamphibicol(+)control		11.5 ±0.7	10.5± 0.7	13.5 ±2	15.5± 0.7	11± 1.4

- Indicates no inhibition zone

Table-5: Results from disc diffusion assay showing the antibacterial activity plant leaf extract against five human photogene bacteria by disc diffusion method.

Extract	Concentration (mg/ml)	Name of Microorganisms				
		Candida albican	*Listeria monocytogene*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
Methanol	150	–	–	–	–	–
	100	–	–	–	–	–
	50	–	–	–	–	–
Ethyl acetate	150	-	-	6.2±0.07	7.1±0.07	-
	100	-	-	6 ±0.07	7.1±0.07	-
	50	–	–	–	–	–
Petroleum ether	150	-	-	7.5±0.05	7.3± 0.07	7.5±0.07
	100	-	-	7.1±0.07	7.1± 0.07	7.2± 0.07
	50	-	-	-	-	-
Chloroamphibicol(+)control		11.5 ±0.7	10.5± 0.7	13.5 ±2	15.5± 0.7	11± 1.4

- Indicates no inhibition zone

The antibacterial activity of ethyl acetate and petroleum ether extract of root, steam bark and leaf of *O.quadripartita* has a slight effect on the *S.auren* and *S.typhimurium* .Additionally petroleum ether extract of all plant parts has a slight effect on *E.coli* at a concentration of 100μg/ml and 150μg/ml. Ethyl acetate extract of root and stem bark have relatively good effects on *S. aureus.* Ethyl acetate extract of root and leaf have good *result* on *S. typhimurium*.Petroleum ether extract of root has good effect on *S.*

*aureus.*Petroleum ether extract of stem bark has comparatively good result on *S.typhimurium.*Petroleum ether extract of root has good result on *E.coli.*

4.3 Antioxidants activity test

4.3.1 Absorbance of sample solution at a given concentration

Table -6: Absorbance of sample solution of methanol extract at ^{a}= 517nm.

Concentration (µg/ml)	Ascorbic acid (standard)	Methanol		
		root	stem	leaf
6.25	0.209±0.001	0.154±0.001	0.172±0.002	0.166±0.001
12.5	0.206±0.001	0.152±0.002	0.168±0.001	0.163±0.003
25	0.199±0.001	0.148±0.001	0.166±0.001	0.159±0.002
50	0.190±0.001	0.141±0.002	0.153±0.001	0.156±0.003
100	0.172±0.02	0.123±0.001	0.138±0.001	0.147±0.001
200	0.146±0.001	0.086±0.002	0.113±0.004	0.130±0.001
400	0.073±0.001	0.036±0.001	0.061±0.001	0.084±0.001

The absorbance of control and blank was 0.247 ($Ab_{control}$= 0.247) and zero (Ab_{blank}=0) respectively. The above data is mean± STDEV in triplicate.

Table -7: Absorbance of sample solution of Ethyl acetate and Petroleum ether extract at ^{a}= 517nm.

Concentration (µg/ml)	Ethyl acetate			Petroleum ether
	root	stem	leaf	root
6.25	0.209±0.002	0.215±0.001	0.211±0.002	0.223±0.001
12.5	0.205±0.001	0.213±0.002	0.202±0.003	0.223±0.001
25	0.201±0.002	0.209±0.002	0.200±0.001	0.220±0.001
50	0.198±0.002	0.206±0.001	0.193±0.003	0.219±0.001
100	0.177±0.003	0.200±0.002	0.184±0.001	0.216±0.002
200	0.156±0.002	0.191±0.001	0.167±0.001	0.212±0.001
400	0.110±0.001	0.172±0.002	0.134±0.001	0.205±0.003

The absorbance of control and blank was 0.247 ($Ab_{control}$= 0.247) and zero (Ab_{blank}=0) respectively The above data is mean± STDEV in triplicate.

4.3.2 Percent inhibition concentration of plant extract

Table-8: Antioxidant (DPPH scavenging) activity of methanol extract of O.quadripartite.

Concentration (µg/ml)	Ascorbic acid	Methanol		
		Root	Stem	Leaf
6.25	15.38±0.4	37.5±0.4	30.50±1.0	32.66±0.47
12.5	16.60±0.4	38.33±0.8	32.12±0.62	34.03±0.4
25	19.43±0.4	39.94±0.47	32.66±0.62	35.65±0.23
50	23.21±0.4	42.78±0.93	38.06±0.4	36.87±0.47
100	30.36±0.8	50.21±0.23	44.26±0.62	40.32±0.47
200	41.03±0.47	65.23±0.4	54.25±1.9	47.37±0.93
400	70.31±0.23	85.5±0.84	75.25±0.62	65.94±0.47

The above data is mean± STDEV in triplicate.

Table-9: Antioxidant (DppH scavenging) activity of Ethylacetate and Petroleumether extract of O.quadripartite.

Concentraton (µg/ml)	Ethyl acetate			Petroleum ether
	Root	Steam	Leave	Root
6.25	15.25±0.8	13.09±0.47	14.57±0.8	9.58±0.47
12.5	16.87±0.2	13.90±0.84	18.22±1.0	10.12±0.7
25	18.62±1.0	15.25±0.84	18.89±0.2	10.93±0.4
50	20.51±1.0	16.73±0.62	21.72±1.4	11.20±0.23
100	28.16±1.5	19.03±1.0	25.91±0.4	12.69±0.62
200	36.84±0.8	22.81±0.62	32.25±0.47	14.30±0.23
400	55.5±1.5	30.63±0.93	45.61±0.47	17.00±1.0

The above data is mean± STDEV in triplicate.

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract .Table 8 and 9 shows the antioxidant activity of the methanol ,ethyl acetate and petroleum ether extract of root ,steam bark and leaf of *O.quadripartite* using DPPH scavenging . 6.25µg/ml-400µg/ml of methanol extract root, steam bark and leaf has the highest DPPH scavenging activity than ethyl acetate and petroleum ether extracts of root, stem bark and leaf. Within methanol extracts root, stem bark and leaf has a decreasing order of DPPH scavenging activity respectively. Ethyl acetate extract of root has the highest antioxidant activity than ethyl acetate extract of leaf and stem bark. Generally, the antioxidant property of these extract were found to be concentration dependent. Based on the result obtained, the methanol extract was more effective antioxidant than ethyl acetate and petroleum ether extract.

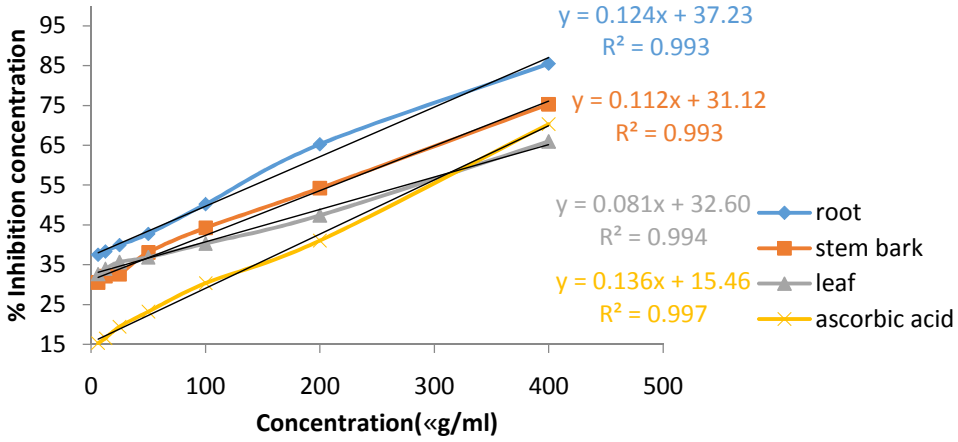


Figure-21: % inhibition concentration of methanol extracts root stem bark and leaf.

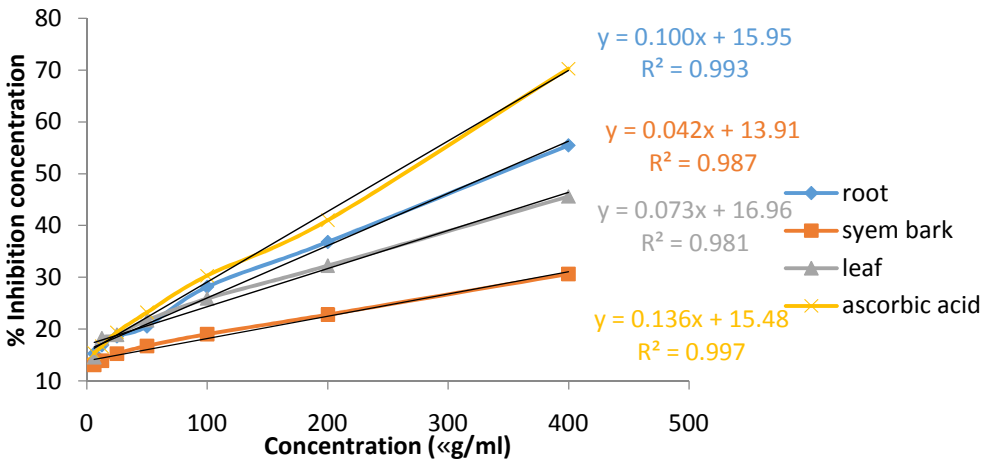


Figure-22: % inhibition concentration of ethyl acetate extracts of root stem bark and leaf.

Table-10: IC_{50} value of plant extracts.

Plant extract	Plant part	IC_{50} (µg/ml)
Methanol	root	102.9
	Stem bark	168.57
	leave	214.81
Ethyl acetate	root	340.5
	Stem bark	859.29
	leaf	452.6
Petroleum ether	root	2212.2
Ascorbic acid (standard)		253.8

Table -10, shows the comparative data of DPPH radical scavenging activity determined by the IC_{50} value of different solvent (methanol, ethyl acetate and petroleum ether) extracts. An IC_{50} is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value is inversely related to the antioxidant activity of crude extracts lowest IC_{50} value and highest anti oxidant activity was found. Methanol extract of root, steam bark and leaf has the lowest IC_{50} value than ascorbic acid (standard) , ethyl acetate and petroleum ether extract of root, steam bark and leaf of *O.quadripartita* .As a result of this methanol extract of root , steam bark and leaf have the highest antioxidant activity .Within methanolic extract of *O.quadripartita* root has the highest (IC_{50}=102.9µg/ml) antioxidant activity than steam bark (IC_{50}=168.57µg/ml) and leaf (IC_{50}=214.81µg/ml).Similarly , within ethyl acetate extract root (IC_{50}=340.5µg/ml) has the highest antioxidant activity than leaf (IC_{50}=452.6µg/ml) and steam bark (IC_{50}=859.29µg/ml).

4.4 Phytochemical screening test

Phytochemical screening test of the *Osyris qudripartita* (leaf, bark, and root) showed the presence of different phytochemicals in its parts (Table 11).

Table 11: Phytochemical constituents of ethanol ethyl acetate and petroleum ether extracts of O.quadripartite

No	Secondary metabolites	Crude extract	Plant part		
			Root	bark	Leaf
1	Alkaloids	Methanol	_	_	_
		Ethyl acetate	_	_	_
		Petroleum ether	_	_	_
2	Terpenoids	Methanol	+	+	+
		Ethyl acetate	+	_	+
		Petroleum ether	_	_	_
3	Sterol	Methanol	+	+	+
		Ethyl acetate	+	_	_
		Petroleum ether	_	_	_
4	Flavonoids	Methanol	+	+	+
		Ethyl acetate	+	+	_
		Petroleum ether	+	+	+
5	Tannins	Methanol	+	+	+
		Ethyl acetate	+	+	+
		Petroleum ether	_	_	_
6	Phenols	Methanol	+	+	+
		Ethyl acetate	+	+	+
		Petroleum ether	_	_	_
7	Saponins	Methanol	+	+	_
		Ethyl acetate	_	_	_
		Petroleum ether	_	_	_

+ = Present - = absent

As shown in Table 11 methanol extract of the root, stem bark and leaf of *O.quadripartita* contains terpenoid, sterol, flavonoid, tannin, phenol and saponin. Saponin did not found in leaf. In ethyl acetate extract of root; terpenoid, sterol, flavonoid, tannin and phenol were detected. In ethyl acetate extract of stem bark; flavonoid, tannin, and phenol were presented but not terpenoid and sterol.

In ethyl acetate extract of leaf; terpenoid, tannin, and phenol were presented but not sterol and flavonoid. Petroleum ether extract of root, stem bark and leaf of *O.quadripartita* contained only flavonoid. .

4.5 Dyeing properties

Direct dyeing of cotton and nylon with root extract of *O.quadripartita* has poor washing fastness and light fastness as a result of weak interaction between fiber and dyeing material (root).The bond exist between cotton fiber and dyeing material is H-bonding and dipole-dipole interaction which is weak bond. In order to make strong fixation, mordant technique, used between the fibers and the root extract, transition metals were involved to make coordinate bond which is relatively strong bond .Using mordant techniques, cotton and nylon has very good washing fastness and light fastness properties.

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(a) (b) (c) (d)

(e) (f) (g)

Figure-23 : (a) Direct dyeing(without mordant) of cotton fiber.(b) Pre mordant(FeSO4) dyeing of cotton fiber.(c) Simultaneous mordant($FeSO_4$) dyeing of cotton fiber.(d) Post mordant($FeSO_4$) dyeing of cotton fiber.(e) Pre mordant($CuSO_4$) dyeing of cotton fiber.(f) Simultaneous mordant($CuSO_4$) dyeing of cotton fiber.(g) Post mordant ($CuSO_4$) dyeing of cotton

(h) (i) (j) (k)

(l) (m) (n)

Figure-24 : (h) Direct dyeing(without mordant) of nylon fiber.(i) Pre mordant($FeSO_4$) dyeing of nylon fiber.(j) Simultaneous mordant($FeSO_4$) dyeing of nylon fiber.(k) Post mordant($FeSO_4$) dyeing of nylon fiber.(l) Pre mordant($CuSO_4$) dyeing of nylon fiber.(m) Simultaneous mordant($CuSO_4$) dyeing of nylon fiber.(n) Post mordant ($CuSO_4$) dyeing nylon fiber.

5. CONCLUTON AND RECOMENDATION

5.1 Conclusion

The antibacterial activity of ethyl acetate and petroleum ether extract of root, steam bark and leaf of *O.quadripartita* has a slight effect on the *S.auren* and *S.typhimurium* at a concentration of 100μg/ml and 150μg/ml .Additionally petroleum ether extract of all plant parts has a slight effect on *E.coli* at a concentration of 100μg/ml and 150μg/ml. From phytochemical result petroleum ether extract of all plant parts have flavonoid. Ethyl acetate extract of root contains terpenioids; sterol, flavonoids, tannin, and phenol .Similarly the steam bark contain flavonoids, tannins and phenol. Finally the leaf consists of terpenoid, tannin and phenol .Those the above mentioned compounds have bioactivity on microbial specifically on *S.auren*, *S.thphimurium* and *E.coli*. They inhibit enzymes responsible for the synthesis of protein and nucleic acid and also they interfere with bacterial metabolic path way then disruption of cytoplasm membrane in *S.auren*, *S.thphimurium* and *E.coli*.. Methanol extracts has no effect on the microbial .This indicated that the physicochemical properties and the stereochemistry of the compound does not interfere with the metabolic path way and inhibition of the enzymes. Methanol extract of root, steam bark and leaf of *O.qudripartita* contains terpenoid, sterol, flavonoid, tannin, phenol and saponin .Saponin did not found in leaf. Alkaloid is not present in all extracts.

Methanol extract of root has highest antioxidant capacity (IC_{50}=102.9μg/ml) than methanol extract of steam bark (IC_{50}=168.57μg/ml) and methanol extract of leaf (IC_{50}=214.81μg/ml) respectively. Ethyl acetate extract of root has the highest antioxidant activity (IC_{50}=340.5μg/ml) than ethyl acetate extract leaf (IC_{50}=452.6μg/ml) and ethyl acetate extract stem bark (IC_{50}=859.29μg/ml) respectively. The strong antioxidant activity of methanol extract is due to the presence of more phenolic compounds .phenolic compound has better hydrogen donating ability. This result indicated that the phenolic compounds were present in the plant important for prevention of disease such as cardiac, carcinogenic, DNA damage, inflammation,cancer, ulcer and other health problems. Petroleum ether extract of the plant shows weak antioxidant activities. This confirmed that there is the absence of phenolic compounds. Root of *O.qudripartita* was used as dyeing cotton and nylon fibers using mordant tequniqus.

In general the plant *O.qudripartita* was very important to prevent health problems related to microbial and internal metabolic activity in human cell. The root and steam bark of *O.qudripartita* was very important for dyeing in textile industry and coloring material in food industries.

5.2 Recommendation

- The *O. quadripartite* plant contains various bioactive compounds. So I recommended as plant of pharmaceutical importance. Further studies are needed to undertake its bioactivity and toxicity profile.
- *The O.quadripartita* has the antioxidant activity so I recommended as food additives and coloring agents specially root and the steam bark.
- The root, steam bark and leaf of *O.quadripartita* were very important to take as a form of tea.

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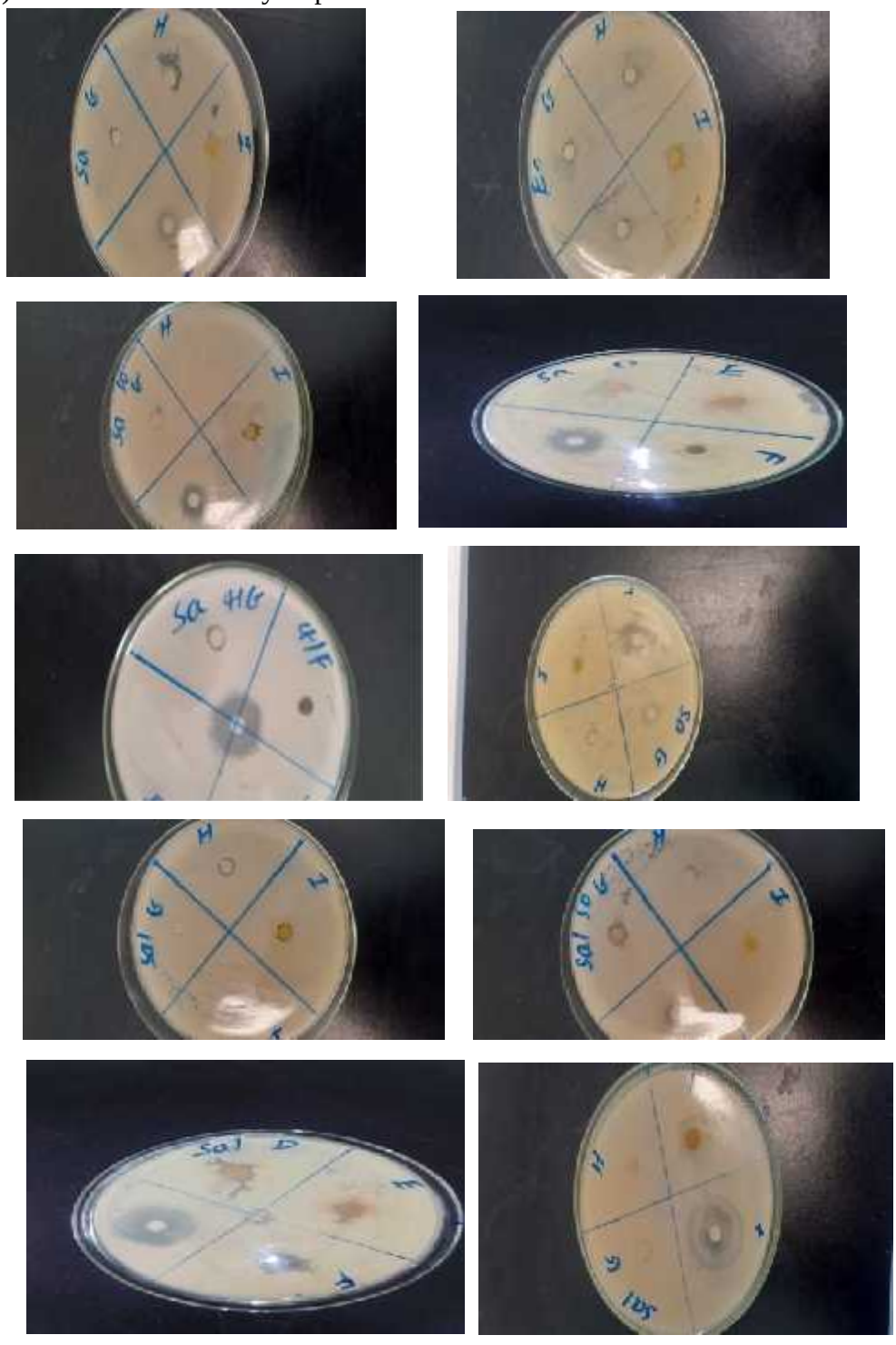
Appendix

A) Antimicrobial activity of plant extracts



B) Phytochemical identification result

I. Methanol extract of root


Sample solution


Saponin


Flavonoid


Terpenoid


Phenol


Sterol


Tannin

II. Methanol extract of stem bark


Sample solution


Saponin


Flavonoid


Terpenoid


Phenol


Sterol


Tannin

III. Methanol extract of leaf

Sample

Terpenoid

Sterol

Tannin

Flavonoid

Phenol

IV. Ethyl acetate extract of root

Sample

Flavonoid

Terpenoid

Tannin

Sterol Phenol

V. Ethyl acetate extract of stem bark


Flavonoid


Phenol


Tannins

VI. Ethyl acetate extract of leaf


Sample solution


Terpenoid


Phenol/Tannin

VII. Petroleum ether extract of root, stem bark and leaf


Sample solution (root)


Flavonoid (root)


Sample solution (stem bark)


Flavonoid (Stem bark)


Sample solution (leaf)


Flavonoid (leaf)