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PHYTOCHEMICAL INVESTIGATION OF *Aloe pulcherrium* ROOTS AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

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PHYTOCHEMICAL INVESTIGATION OF *Aloe pulcherrium* ROOTS AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ORGANIC CHEMISTRY

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Phytochemical Investigation of *Aloe pulcherrium* Roots and Evaluation of Its Antibacterial Activities

By

Dele Abdissa Keneni

A Thesis Submitted to School of Graduate Studies Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Organic Chemistry

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DECLARATION

I, the undersigned declare that this thesis is my original work and has not been presented for research in any other university.

_____

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List of abbreviations

ATCC	American Type Culture Collection
CC	Column chromatography
COSY	Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple-Bond Correlation
IR	Infrared
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
STDs	Sexually transmitted diseases
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
WHO	World Health Organization

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Abstract

Natural products isolated from plants have been providing noble and clinically active drugs. This plant-based traditional medicinal system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Thus, the objective of this study was to isolate and characterizes bioactive natural products from the roots of *Aloe pulcherrium.* The air dried was extracted successively with n-hexane, chloroform, acetone and methanol solvents by maceration. The extracts were evaluated for their antibacterial activity against different bacterial strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis* ATCC 6633). The MIC were conducted using concentration ranging from 200 to 12.5 mg. mL^{-1} by serial dilution. The acetone extract has shown superior activities against all the strains. The extract has shown the inhibition zone diameter 15, 16, 12 and 24 mm against *B.subtilis*, *E.coli, S.aureus* and *P.aeruginosa,* respectively. Likewise, the corresponding inhibition zone of Gentamycin was found to be 20, 20, 23 and 25 mm against *B.subtilis, E.coli, S.aureus* and *P.aeruginosa,* respectively. The MIC results has also indicated that the lowest concentration (12.5 $mgmL^{-1}$) have shown a significant influence on the four strains. Then, the acetone extract was subjected to fractionation using Column Chromatography (CC) over oxalic acid impregnated silica gel eluted with n-hexane containing increasing amounts of ethyl acetate and has resulted two compounds (**AP-8G** and **AP-11D**). Meanwhile, the antibacterial activities of the isolated compounds were carried out. The corresponding inhibition zone diameter for **AP-11D** was 22, 21, 18 and 23 mm, and **AP-8G** was 27, 22, 18 and 21 mm on *B.subtilis, E.coli, S.aureus,* and *P. aeruginosa*, respectively. Then, the pure compounds were characterized by using a combination of spectroscopic techniques such as IR, UV, ^{1}H-NMR, ^{13}C-NMR and, 2D NMR, so as to establish the structure of the isolated compounds. Finally, **AP-8G** was identified as 1, 6-dihydroxy-8-methyl-anthracene-7-methyl ester-9, 10-dione, (trivial name aloesaponarin I) and **AP-11D** as 1, 6-dihydroxy-8-methylanthracene-9, 10-dione, (trivial name aloesaponarin II) with melting points of 207-209 ℃ and 190-192 ℃, respectively. Devising alternative method of extraction as well as carrying the antifungal and antiplasmodium activities of this plant are recommended for further researchers.

1. Introduction

1.1 Background of the study

The history of medicine is an account of mankind's effort to deal with human illnesses. In prehistoric times, people obtained medicine for their ailments from their environments; particularly from plants [1]. There is comprehensive evidence that medicinal plants represent the oldest and most widespread form of medication [2, 3]. The most primitive accounts of the therapeutic use of plants are recorded in the Rig-Veda (4500-1600 BC) and Ayurveda (2500-600 BC) [4]. The Buddhist period boosted the use of medicinal plants and gave considerable attention to the cultivation of these plants in a scientific manner [5]. Plant synthesizes a wide variety of chemical compounds, which can be stored by their chemical class, biosynthetic origin and functional groups in to primary and secondary metabolites. Secondary metabolites do not seem to be vital to the immediate survival of the organism that produces them and are not an essential part of the process of building and maintaining living cells [6].

The advent of phytochemistry and pharmaceutical chemistry during the early twentieth century developed the ability to isolate and subsequently synthesize an enormous variety of medicinal drug molecules and allowed the treatment of previously incurable and/or life-threatening diseases. From then, chemically synthesized drugs gained popularity and became the basis of growth of the pharmaceutical industry. Beginning in the 1990s, natural products drug discovery was nearly eliminated in most of the big pharmaceutical companies. This was primarily due to the promise of the then-emerging field of combinatorial chemistry [7], where by huge libraries of man-made small molecules could be rapidly synthesized and evaluated as drug candidates. However, synthetic drugs have been plagued by unwanted side-effects, toxicity, and inefficiency, among other problems. Furthermore, there was a decrease in the number of new drugs introduced into the world market during this era [8]. Over all, the search for new drugs against a variety of illnesses through combinatorial chemistry and other modern approaches has not been encouraging. These factors, as well as the emergence of new infectious diseases, the proliferation of disorders and growing multi-drug resistant pathogenic micro-organisms, have prompted renewed interest in the discovery of potential drug molecules from medicinal plants [9].

This has led only recently to a new found respect for the privileged structures inherent within natural products. Plants consist of a number of biologically active ingredients such as alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds [10].

These phytochemicals are synthesized in all parts of the plant body and are mainly attributed to the pharmacological actions. Medicinal plants are usually screened for phytochemicals that may lead to its further isolation, purification and characterization of active principle. The active compound can be then used as the basis for a new pharmaceutical product [11, 12]. The development of useful and widely used drugs like Digoxin (**1**) and Digitoxin (**2**), from *Digitalis* leaves; quinine (**3**) from the *cinchona bark*; reserpine (**4**) from *Rauwolfia serpentine*; morphine (**5**) from *Papaver somniferum*; Coaine (**6**) from *Erythroxzion coca* and the anticancer, Vincristine (**7**) and Viblastine (**8**) from *Cartharathus roseus* of Madagascar, and anticancer, Bruceatin (**9**), from *Brucea antidysentrica* (Figure 1) of Ethiopian plant are a pointer that medicinal plants remain a good source of novel and effective drugs [13].

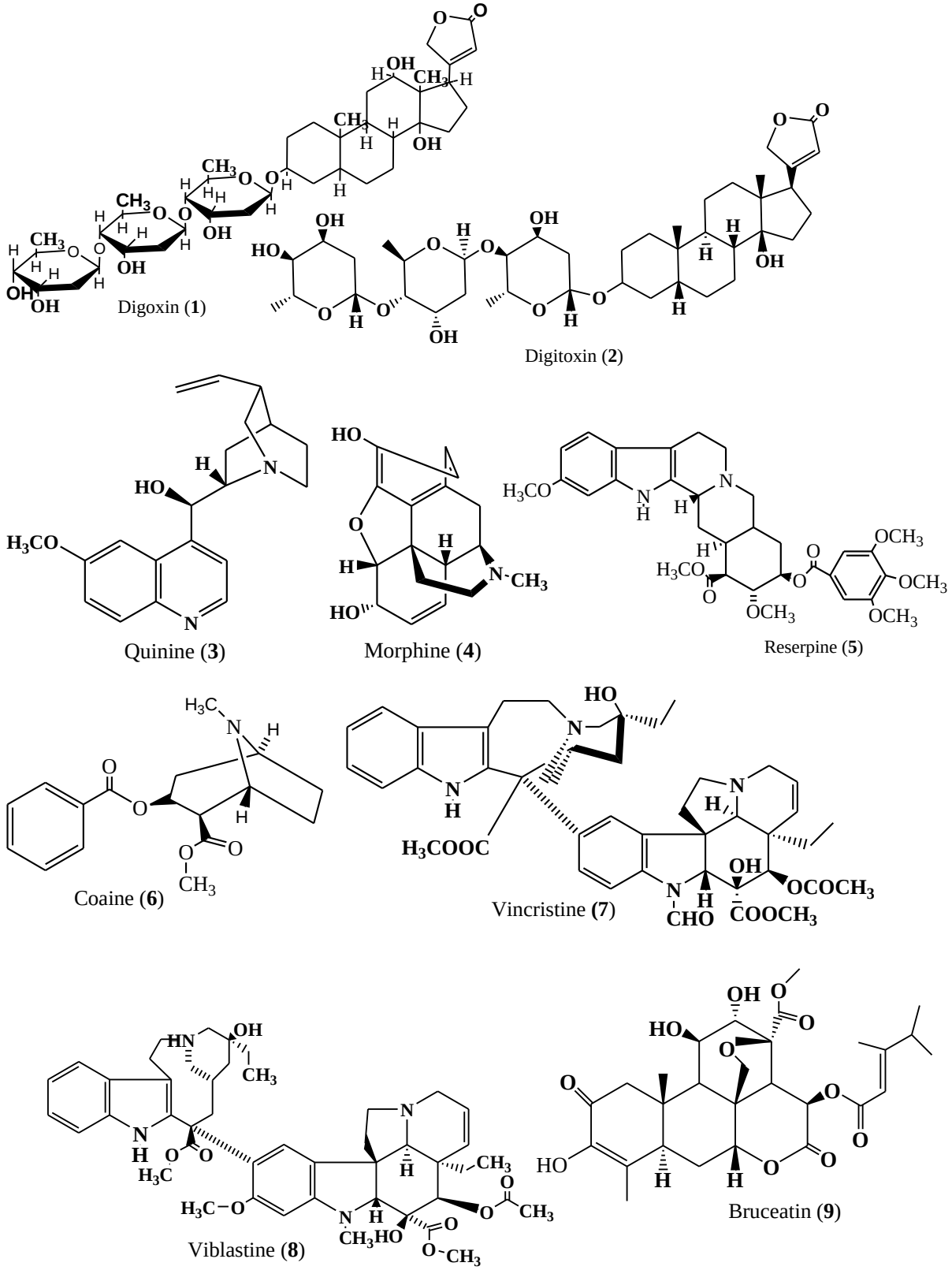


Figure 1. Some Example of Drugs obtained from Medicinal plants

Historically pharmaceutical companies utilized plant extracts to produce relatively crude therapeutic formulations, but with the advancement of antibiotics in the mid-twentieth century, drug formulations of fairly purified compounds have become more typical. Natural products have been the major sources of chemical diversity for starting materials for driving pharmaceutical discovery over the past century. Many natural products and synthetically modified natural product derivatives have been successfully developed for clinical use to treat human diseases in almost all therapeutic areas [14]. With corollary to this, over 40% of all modern clinical drugs are of natural origin **[**15]. Medicinal plants are continue providing valuable leads to new drugs. This is because the degree of chemical diversity and novelty found among natural products is broader than that from any other source [16,17]. Africa has an immensely rich biodiversity and knowledge in using plants to treat various ailments. In fact, WHO estimates that 80% of the population in Sub-Saharan Africa depend solely on traditional medicine from plants for their primary health care needs because of their ready availability, cheapness and socio-cultural background [18].

In Ethiopia, it is estimated that about 80% of the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants [18]. Despite their wide use in the traditional health care, the work that has been done to evaluate the efficacy of Ethiopian traditional medicinal plants is not extensive andresources, however, are hardly scientifically investigated [19]. Due to the ability of plants to bio-synthesize a plethora of compounds, whose structural diversity is often beyond the dreams of even the most imaginative chemists, plants remain important in the treatment of various diseases, including bacteria. Infectious bacterial diseases are one of the major causes of severe number of death of children and young adults in developing countries [19]. Besides, the emergence of various resistant organisms limited therapeutic efficacy of many of currently available drugs. The main reason for this serious situation is the limitation in both number and structural variety of anti-bacterial drugs in clinical use.

Therefore, there is a need to develop effective and structurally diverse anti-bacterial drugs with new modes of action from medicinal plant, including *Aloe pulcherrium* roots to replace the current drugs that are becoming ineffective.

1.2 Statement of the problem

Several plant species have been studied and yielded thousands of secondary metabolites among which those in the classes; anthraquinones, polyphenols, alkaloids and glycosides exhibited promising activity against a wide range of bacterial species. Therefore, a continued investigation of medicinal plants and screening against their biological activities is still needed today specifically on those plants which have recognized traditional uses.

Aloe pulcherrium is one of the endemic *Aloe* species of Ethiopia, which is traditionally used for the treatment of various infectious diseases. It has been commonly used for the treatment of various ailments; particularly for wound healing [52]. A study conducted by Haile and co-workers, [67] has revealed that *Aloe pulcherrium* gel is used by the 'Gomma' and 'Seka Chokorsa' community for wound healing and its decoction has been taken by the same community for the treatment of malaria. However, there is no scientific data pertaining to the phytochemical isolation of the natural product and pharmacological activities of the plant. Thus, the present work has focused on isolation, characterization and the evaluation of the bioactive molecules for their antibacterial activity from the roots of the *Aloe pulcherrium*.

1.3. Objectives of the study

1.3. 1 General objective

- The main objective of this study was to identify the antibacterial constituents from *Aloe pulcherrium* roots.

1.3.2 Specific objectives

- To evaluate the antibacterial activities of the crude extracts and isolated compounds from the roots of *Aloe pulcherrium* against four pathogenic bacteria strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilus*).
- To isolate the bioactive molecule(s) from the roots of *Aloe pulcherrium*.
- To characterize the isolated bioactive compound(s), using spectroscopic techniques such as IR, UV-Vis, ^{1}H and ^{13}C-NMR, 2D spectra.

1.4 Significance of the study

The rich variety of bioactive compounds from plants directed civilization to use them for traditional healing, and many of them have complained their own pharmacopoeia fabricated from plants. The bioactive phytochemical constituents present in the plant plays a significant role in the development of medicines and drug discovery.

The phytochemical information and bioactive molecules from *Aloe pulcherrium*, which has been widely practiced by the community for its medicinal role has not been reported.

Therefore, findings of this thesis would,

- help to isolate the promising antibacterial from the roots of *Aloe pulcherrium* that can be used as a lead compounds in the discovery of antimicrobial agents.
- provide important information on the chemical profile of the plant and can be used as guideline for further isolation and purification of the active principles of a similar work to be carried on other pathogens to confirm broad activity of the isolated compounds.

2. Review of Related Literature

2.1 Botanical Information of the genus *Aloe*

The botanical genus of *Aloe* has been classified in the family of *Liliaceae*, because it germinates from an original bulb in the same way as *lilies* [20]. However, the plant family *Liliaceae* was conceived in 1789 with its recognized 300 genera and 4500 species, widely distributed in tropical and sub-tropical areas of the world [21]. It was later noted that it embraced a heterogeneous assortment of genera. Consequently, revision was made by Dahlgren and Clifford (1982) [22], it was subdivided into six homogeneous families; *Asparagaceae, Alliaceae, Asphodelaceae, Dracaenaceae, Eriospermaceae* and *Hyacinthaceae*. According to the new classification, the family *Asphodelaceae* comprised three sub-families; namely *Astelioideae*, *Anthericodeae* and *Asphodeloideae* with the genera placed in these families as shown below: *Asphodeloideae: Aloe, Alectorurus, Aprica, Asphodeline, Asphodelus, Kniphofia, Bilbine, Bulbinella, Bulbinopsis, Chortolirion, Eremurus, Gasteria, Glyphosperma, Haworthia, Arachyandra and verinea. Astelioideae: Astelia, Cohnia, Cordyline and Milligania.Anthericodeae: Agrostocrinum, Alania, Anthericum, Arnocrinum, Dasystachys, Debesia, Dichopogon, Diuranthera, Paradisia, Simethis, Tricoryne and Verdickia.*

In 1985, the family *Asphodelaceae* was again revised and the sub-families *Astelioideae* and *Anthericodeae* were raised to families referred to as *Asteliaceae* and *Anthericaceae* respectively [23]. Whereas, the sub-family *Asphodeloideae* was kept in the *Asphodelaceae* family with two defined new sub-families *Alooideae* (with the genera *Aloe, Astroloba, ChamaeAloe, Gasteria, Haworthia, Lomathophyllum*, and *Poellnitzia*) and the *Asphodeloideae* (with the genera: *Asphodeline, Asphodelus, Bulbine, Bulbinella, Kniphofia, Smiethis*, and *Trachyandra*) [21].

Aloe is a perennial evergreen shrub with succulent leaves having flowers of an elongated tubular form varying in color according to the species, from orange to bright scarlet red. The name *Aloe* is from the Greek alsos and refers to the bitter juice from the leaves of these plants. It is probably derived from the earlier Arabic word alloeh or the Hebrew word allal, both meaning bitter [24]. *Aloes* are native to sub Saharan Africa, the Saudi Arabian Peninsula, and to many islands of the western Indian Ocean, including Madagascar.

The genus *Aloe* is the largest genus in the family *Asphodelaceae*, which is represented by 600 species and subspecies [25]. About 46 species of *Aloe* are known so far in Ethiopia and Eritrea with a high proportion of endemics. In Ethiopia, there are about 38 species of *Aloe* with many of them occurring in the drier parts of the country. The members of the genus are perennial, with or without a woody trunk. They have thick, usually bright yellow roots (due to the high content of anthraquinone, a yellow pigment characteristic for the families *Aloaceae* and *Asphodelaceae*). Most representatives have succulent leaves in a basal rosette, or on the trunk. The flowers are large and showy, usually fleshy, glabrous or hairy, bisexual, and nearly radially symmetric. The perianth is situated below the ovary and consists of six tepals. Septal glands producing nectar are found in the walls that separate the three rooms of the ovary. The ovary develops into a capsule which opens by three slits between these walls. In each room there are two to numerous seeds [26].

2. 2 *Aloe pulcherrium*

Aloe pulcherrium is one of the most beautiful of the *Aloe* taxa found in Ethiopia. It is known as "*Sete-Eret*" in some localities in Shewa province of Ethiopia and is mainly used to accelerate wound healing. Chemical analysis of its leaves revealed the noticeable absence of Barbaloin (**34**) but showed instead Nataloin (**43**) and 7-hydroxyaloin (**60**) as its major constituents [32]. It is one of the endemic *Aloe* species of Ethiopia which is traditionally used for the treatment of various infectious diseases in central, southern and northern part of Ethiopia, such as Wollo, Gojam, Jimma, Shewa and around Debre Libanos Monastery [33]. It is used in treatment of skin disorder and specifically, as topical medication for the treatment of burns. The slow growing stemless to short stemmed rosettes with the dark green leaves are the distinct morphological features of the plant. The leaves are mostly flattened with often prickly or spiny margins. When a fresh cut is made into any part of the leaf, slimy, clear or yellowish fluid oozes out along the cut area [34].

Figure 2. *Aloe pulcherrium* species (Photography taken from study area, 'Buuyyoo')

2. 3 Ethnomedicinal uses of the genus *Aloe*

Primary metabolites are the key metabolites, which mainly involved in plant growth, development and reproduction. Later, plants utilize these primary metabolites as precursors for synthesis of a large number of structurally diverse secondary metabolites. These secondary metabolites have numerous medicinal importance, they are not essential for growth and tend to be strain specific. They have a wide range of chemical structures and biological activities. They are derived by unique biosynthetic pathways from primary metabolites and intermediates [27].

These phytoconstituents employed by plants to protect them against pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine [28]. The genus *Aloe* has a long medicinal history since 1500 BC. *Aloe* gel, which is found in the interior of the leaves, has been used for treatment of a variety of skin ailments. Four species, specifically *Aloe barbadensis* Miller (commonly known as *Aloe vera*), *Aloe ferox* (*Cape Aloe*), *Aloe arborescens (Candelabra Aloe)* and *Aloe perryi* baker (*Perry's Aloe*), have had their ethno-pharmacological usage particularly well documented. Of these, *A. barbadensis* has been the most extensively studied. The *Aloe* gel works by hydrating and protecting the wound until the body can repair itself. It is important to note that some *Aloe* species are poisonous, so correct identification of the species must be made before treatment. *Aloe vera* and some others, such as *A. ferox* have been used for a long time in folk medicine for the treatment of constipation, burns and dermatitis [29].

Recently, some species of *Aloe* such as, *A. trichosantha*, *A. pubescens*, *A. citrine*, *A. bertemariae, A. eumassawana* and *A. scholleri*, have been used in a wide range of skin and hair care products, and also form the basis of health drinks and tonics. The slimy gel inside the leaves consists of a complex mixture of polysaccharides, amino acids, minerals, trace elements and other biologically active substances, such as enzymes. *Aloe* species have been the source of laxative drugs, the main purgative principle being an anthrone-C-glycoside, aloin, which occurs at levels of between 18 and 30% of the dried product [30]. Balcha, 2014 [31] has cited that as *Aloe* root is used to overcome the Anthrax disease. The community prepares by pounding its root and mix with cold water and local alcohol (tella) and take orally.

2. 4 Phytochemistry of the genus *Aloe*

The genus *Aloe* is the most extensively investigated genus among the genera of the family *Asphodelaceae*. This may be due to its well documented medicinal uses and chemotaxonomic interests. The first real analyses oriented towards gaining knowledge of the phytochemical content of *Aloe* was conducted in 1940. The two primary compounds identified and named as the first constituent, known today as aloe-emodin (**13**) and aloin (**33**). Compound **33** is found in *Aloe* leaf latex whereas Aloe-emodin is found in the gel, sap or leaves of *Aloe vera.* The extracts of **13** and **33** are cited in the British Pharmacopoeia Codex of 1907, with indications for its use mainly as a purgative. Compound **33** contained in the plant, an anthraquinone, has numerous actions, i.e., laxative, blood purifying, diuretic, cytoprotective and anti-tumoural effects [20].

However, the first scientific study to discover the active constituents responsible for the extraordinary effects of *Aloe* was carried out in 1851. The constituents of *Aloe* are derived from the leaf or, more precisely, from its three main sections. The first section is the external cuticle, made up of pericyclic plant cells, xylemes, and pholemes which allow the passage of sap and where photosynthesis takes place. Next is the mucilaginous stratum. Finally, there is the internal parenchyma which is its famous gel [20]. With corollary to this, most of the compounds so far reported from *Aloe* are quinine derivatives, mainly pre-anthraquinones, anthraquinones, and anthrones. Other compounds including chromones, phenylpyrones, flavonoids, alkaloids and steroids have also been reported [32].

2. 4.1 Anthraquinone and its physical properties

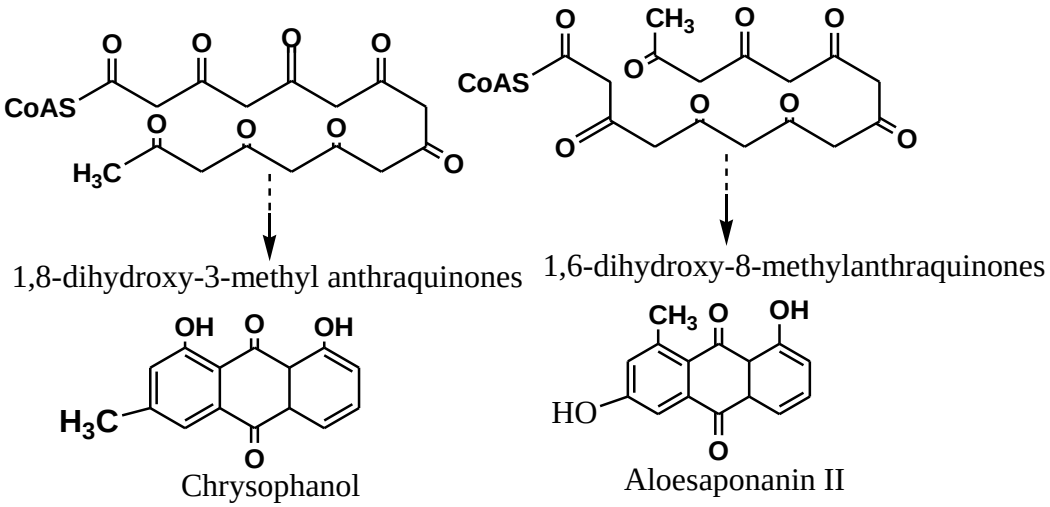
Anthraquinones are the largest and most important group of naturally occurring quinones. Numerous anthraquinones have a linear tricyclic ring framework, with various number of phenolic functional groups. They are used as natural and synthetic color and are constituents of plants used for dyeing materials. Many plants contain anthraquinones are used as pharmaceutical drugs [35]. They are group of functionally diverse aromatic chemicals, structurally related to anthracene, with parent structure 9, 10-dioxoanthracene. It has the appearance of yellow or light gray to gray-green solid crystalline powder [36]. A wide range of biological and pharmacological activities of anthraquinones, such as antimicrobial, antiviral, anti-inflammatory, anti-cancer,

antioxidant, and antifungal depend on their tricyclic scaffold, and on the nature and/or positions of substituents [35].

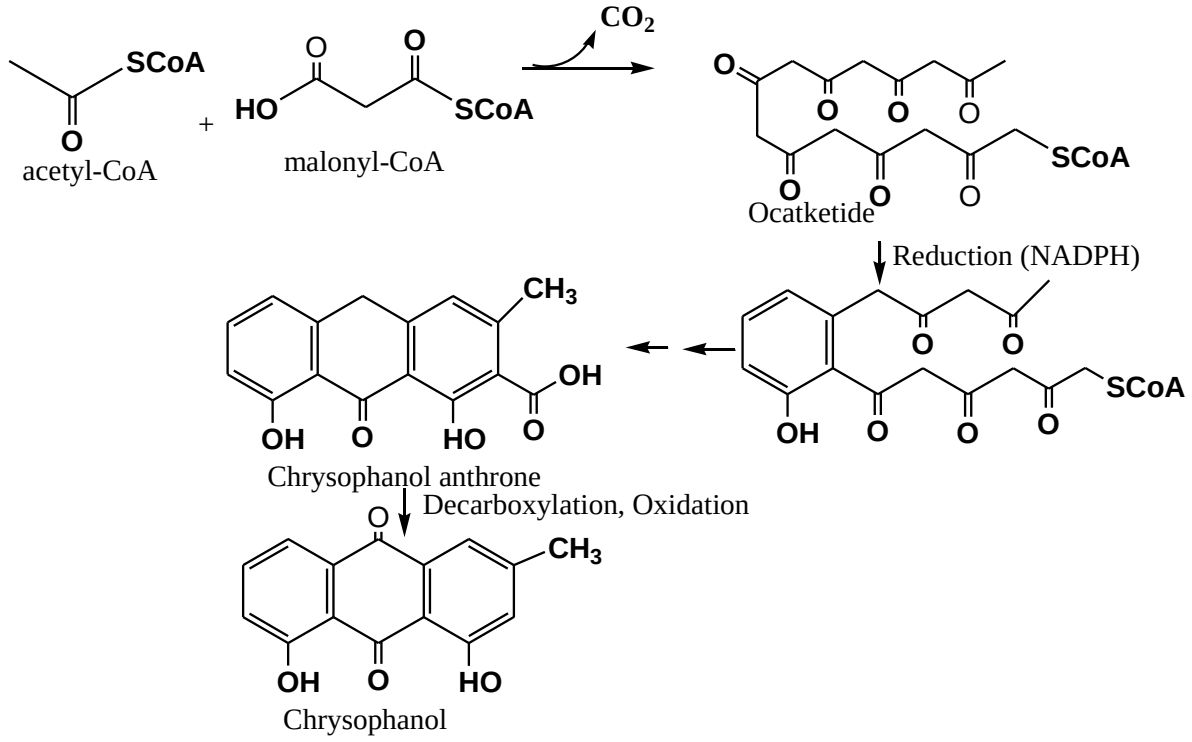
The exact mechanism for this substance's ability to have indirect and so far potent effects on the body is not yet known. However, the strong eradication action is closely related to the chemical structure of the molecule. Indeed, the anthraquinones present in *Aloe* are many and the effects differ to some extent between them. Some of these compounds are aloe-emodin, aloeitic acid, anthranol, crysophanic acid, an ester of cinnamic acid, and resistannol. The typical bitter taste of *Aloe* is due to these compounds [20].

Botanically, the anthraquinones are found in high concentrations in the sap that runs through the trunk of the plant, with a smaller amount of sap evidenced in the outer layer of the cuticle or skin of the *Aloe* leaves. Similar anthraquinone substances have been found in *Senna*, *Rhubarb*, *Cascara,* and in *Polyganum cuspidatum* plants, all of which have a laxative and digestive use in the herbal pharmacopoeia [20].

However, the anthraquinone derivatives found in *Aloe* are generally of two types. These are, 1, 8-dihydroxy-3-methyl anthraquinone and 3, 8-dihydroxy-1-methyl anthraquinone derivatives. These two groups of anthraquinone derivatives appear to have been formed by two parallel biosynthetic routes differing by the way the octaketide chain is folded [37,38].

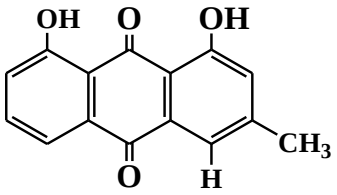


Scheme1: The two different folding of the octaketide chain in the roots of *Aloe* [39]

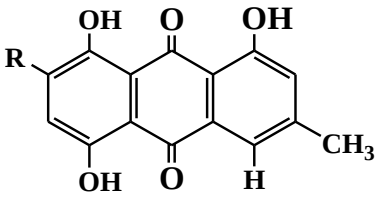


Scheme 2: Biosynthetic pathway of Anthraquinones [79]

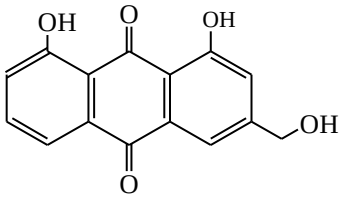
The 1, 8-dihydroxy-3-methyl anthraquinone derivatives are chrysophanol-based where the cyclization of an octaketide chain has folded in the common way. Anthraquinones of this-type are known to occur both in leaves and roots of *Aloe* species. Chrysophanol (**10**) and related anthraquinone derivatives such as helminthosporin (**11**) and isoxanthorin (**12**) are detected in both roots and leaves of most of the *Aloe* species surveyed [40-42]. Whereas, aloe-emodin (**13**), nataloe-emodin (**14**), nataloe-emodin-8-methyl ether (**15**), including its *O*-glycosides, *O*-α-L-rhamnopyranosylaloe-emodin (**16**) and nataloe-emodin-2-*O*-β-D-glucopyranoside (**17**) (Figure 2) are the main constituents of the leaves of *Aloe* species [43-45]. A dimeric anthraquionone, Asphodeline(**18**) (Figure 3) has been reported from subterranean stem of *A. saponaria* [40].



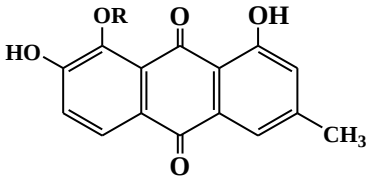
Chrysophanol (**10**)



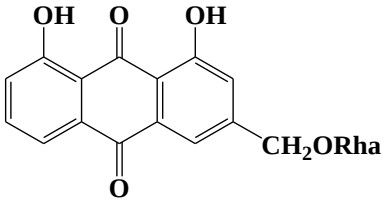
Helminthosporin (**11**) R=H
Isox anthorin (**12**)R=OCH_3



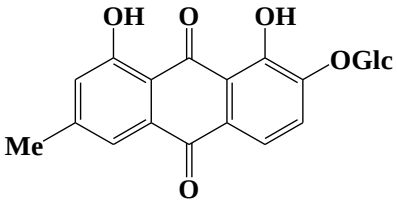
Aloe-emodin (**13**)



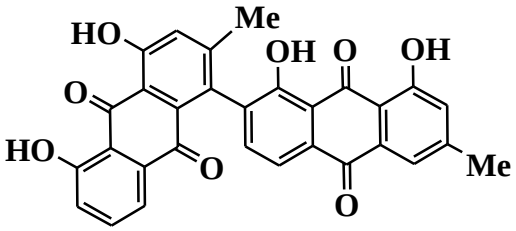
Nataloe-emodin (**14**)R=H
Natae-emodin-8-methylether(**15**)R=CH_3



O-α-L-Rhamnopyranosylaloe-emodin (**16**)



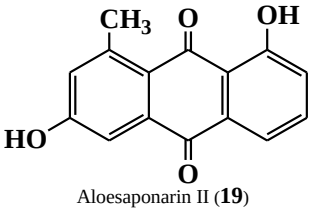
Nataloe-emodin-2-*O*-β-D-Glucopyranoside (**17**)



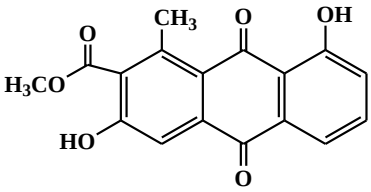
Asphodeline (**18**)

Figure 3. Chrysophanol (1, 8-dihydroxy-3-methyl anthraquinone) derivatives

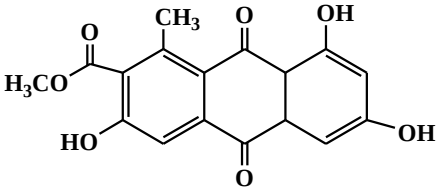
On the other hand, the rare 3, 8-dihydroxy-1-methylanthraquinone derivatives where the cyclization of an octaketide chain has folded in an unusual way, as in aloesaponarin II (**19**), are confined to roots of these plants [36]. Aloesaponarin I (**20**), Laccaic acid D methyl ester (**21**) and deoxyerythrolaccin (**22**) (Figure 4) were reported to have anti-microbial activities [37] and occur in the roots of many *Aloe* species including *A.* saponaria [38]. It is worth noting that, apart from genus *Aloe* and the related genus *Lomathophyllum* [39], there is no report of such anthraquinone derivatives from any other sources.



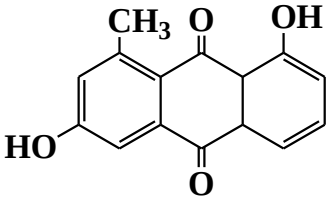
Aloesaponarin II (**19**)



Aloesaponarin I (**20**)



Laccaic acid D methyl ester (**21**)

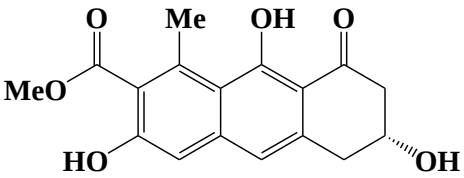


Deoxyerythrolaccin (**22**)

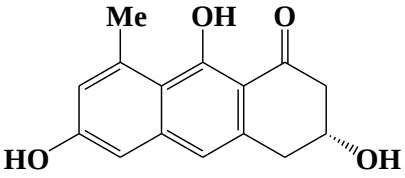
Figure 4. Derivatives of 3, 8-dihydroxy-1-methyl anthraquinone

2. 4. 2 Pre-anthraquinones

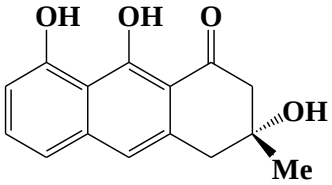
Pre-anthraquinones are considered to be the precursors (pre-stage of the anthraquinone derivatives) and they readily convert to the corresponding anthraquinones upon treatment with bases [46, 47]. Aloesaponol I (**23**) and aloesaponol II (**24**) occur in the roots and subterranean parts of many *Aloe* species [48]. The precursor of chrysophanol, pre-chrysophanol (**25**) was reported from the subterranean parts of *Aloe graminicola* [49]. Whereas aloesaponol III (**26**), aloesaponol IV (**27**), aloesaponol I-6-*O*-glucopyranoside (**28**), aloesaponol II-6-*O*-glucopyranoside (**29**), aloesaponol III-8-*O*-glucopyranoside (**30**) were isolated from subterranean parts of *A. saponaria* [50]; aloesaponol III (**26**) has also been reported from the roots of *K. foliosa*[42]. From the dark brown callus tissue of *A. barbadensis* leaves, two pre-anthraquinone glucosides; aloesaponol- III-4-*O*-glucopyranoside (**31**) and aloesaponol IV-4-*O*-glucopyranoside (**32**) (Figure 5) were isolated [51]. The wide presence of pre-anthraquinone derivatives in particular, aloesaponol II (**19**) and aloesaponol I (**20**) (Figure 4) only in the genus *Aloe*, indicated that these compounds are characteristic constituents of the genus. Chemotaxonomic studies have also shown that these types of compounds are clearly absent from the roots of closely related genera including *Kniphofia* and *Bulbine* [39].



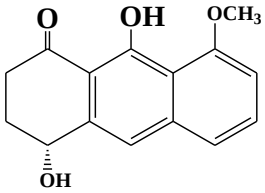
Aloesaponol I (**23**)



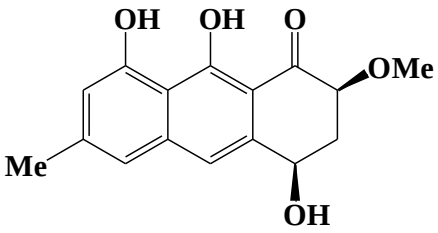
Aloesaponol II (**24**)



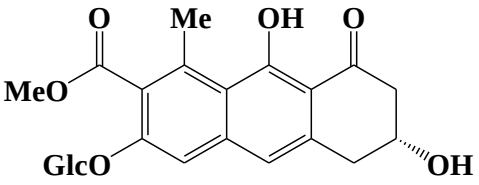
Pre-chrysophanol (**25**)



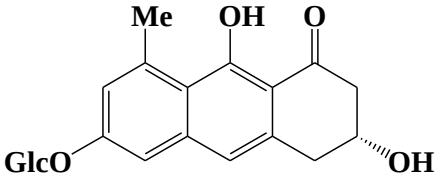
Aloesaponol III **(26)**



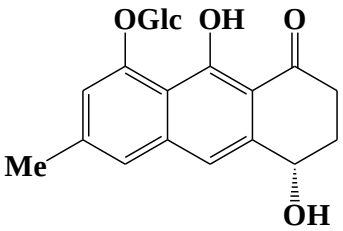
Aloesaponol IV (**27**)



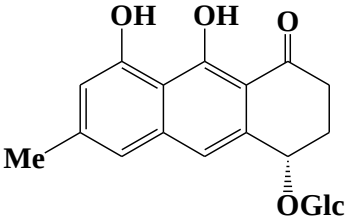
Aloesaponol I-6-*O*-glucopyranoside (**28**)



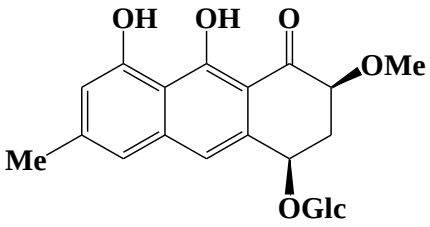
Aloesaponol II-6-*O*-glucopyranoside (**29**)



Aloesaponol III-8-*O*-glucopyranoside (**30**)



Aloesaponol III-4-*O*-glucopyranoside (**31**)

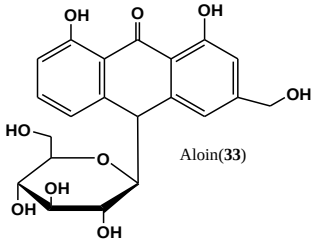


Aloesaponol IV-4-*O*-glucopyranoside (**32**)

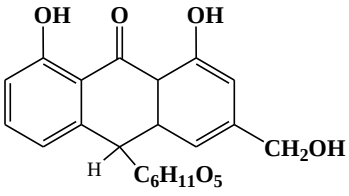
Figure 5. Pre-anthraquinones Derivatives

2. 4.3 Anthrones

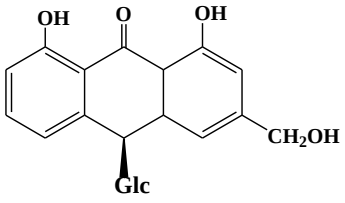
Anthrones are considered to be the biosynthetic precursors of anthraquinones *via* simple oxidation [52]. The *C*-glycosylated anthrones (e.g. Barbaloin (**34**) (Figure 6)) are the most important classes of compounds found in *Aloe* species and is believed to be mainly responsible for the bitter and purgative properties of the well-known commercial *Aloe* drug. Barbaloin (**34**) was first isolated from *A. barbadensis* [40,51], and is a collective name of aloin A (**35**), and B (**36**),(Figure 6) two diastereomeric *C*-glycosides that differ in the configuration at C-10 of the anthrone moiety. The study on *A. arborescens* showed that compound **34** is concentrated on the top of each leaf (near the apex) than those at the lower parts of the branch [53]. In general, the anthrones with *C*-glycosides were reported as the major constituents of the leaves of *Aloe* species [54]. Aloinoside A (**37**),and B (**38**), 10-hydroxyaloin A (**39**), 10-hydroxyaloin B (**40**), chrysophanol anthrone (**41**), aloe-emodin anthrone (**42**), nataloin(**43**), homonataloin (**44**), 8-*O*-methyl-7-hydroxyaloin A (**45**) and 8-*O*-methyl-7-hydroxyaloin B (**46**) are among some of the anthrones reported from *Aloe* species [55]. The rare anthraquinone-anthrone dimer, bianthracene II (**47**) and bianthracene IV (**48**) are also reported from subterranean parts of *A. saponaria* [50].The glycosylated anthraquinone/anthrone derivatives, elgonicardine (**49**) (Figure 6) were reported from the roots of *A. elgonica* [56].



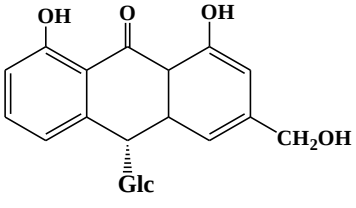
Aloin(**33**)



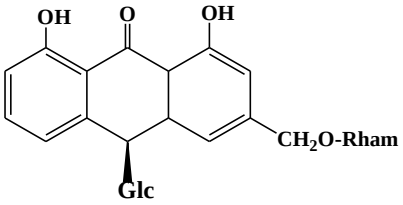
Barbaloin **(34)**



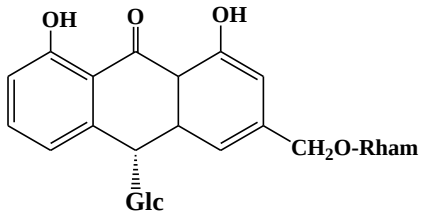
Aloin A (**35**)



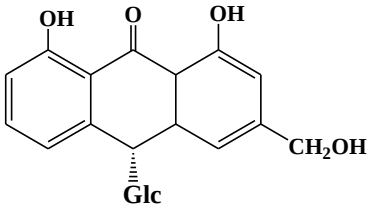
Aloin B (**36**)



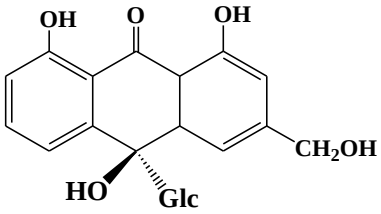
Aloinoside A (**37**)



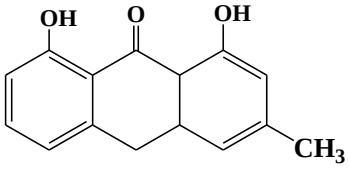
Aloinoside B (**38**)



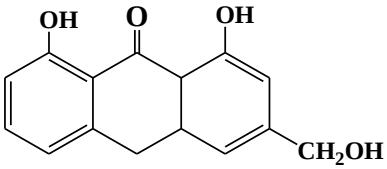
10-Hydroxyaloin A (**39**)



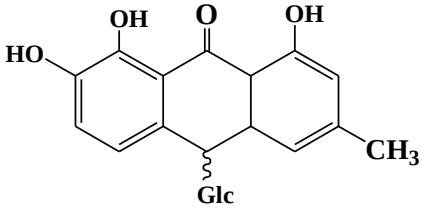
10-Hyroxyaloin B (**40**)



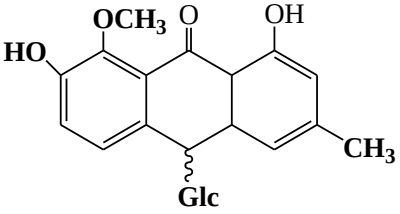
Chrysophanol anthrone (**41**)



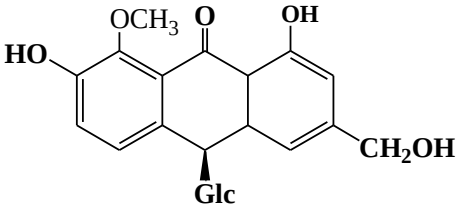
Aloe-emodinanthrone (**42)**



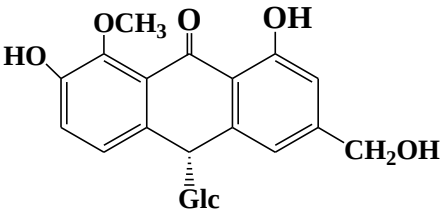
Nataloin (**43**)



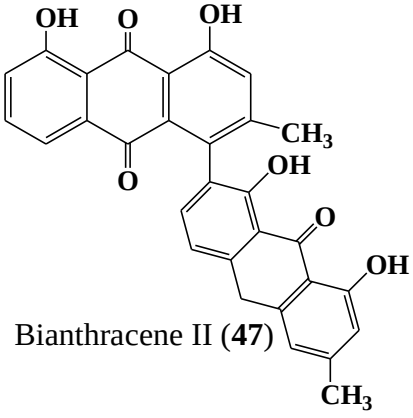
Homonataloin(**44)**



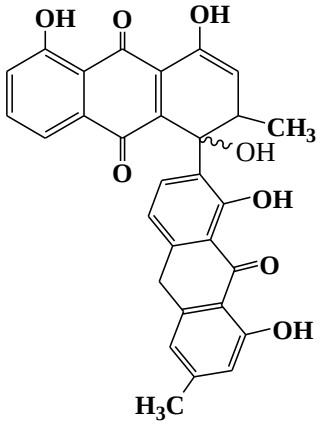
O-methyl-7-hydroxyaloin A (**45**)



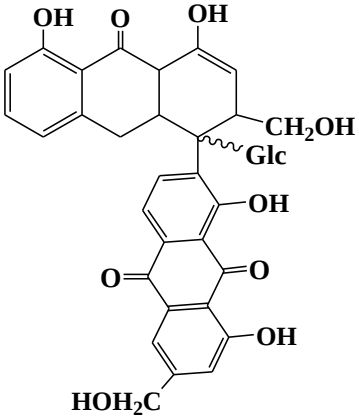
8-O-methyl-7-hydroxyaloin B (**46)**



Bianthracene II (**47**)



Bianthracene IV (**48**)

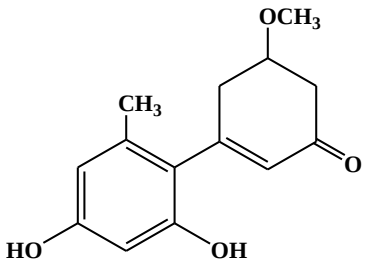


Elgonicardine(**49**)

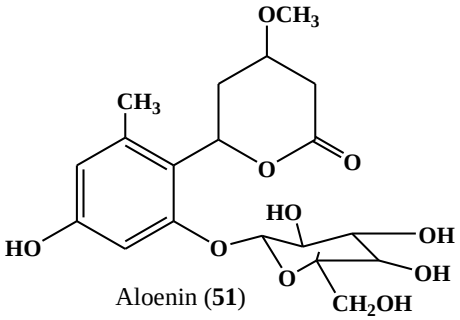
Figure 6. Anthrones C-glycosides and Dimeric compounds

2.4.4 Other secondary metabolites isolated from *Aloe*

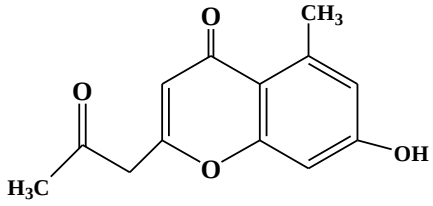
Some pyrones (**50-51)**, chromones (**52-53**), flavonoids (**54-55**), steroids (**56-57**) and alkaloids (**58-59**) (Figure 7) are known to occur in the genus *Aloe*.



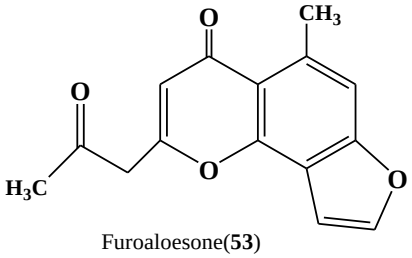
Aloenin aglcone **(50**)



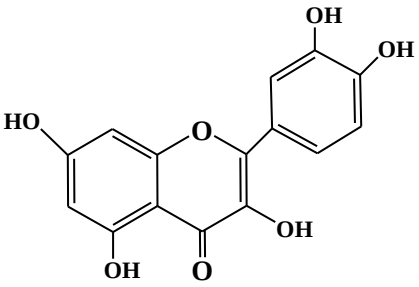
Aloenin (**51**)



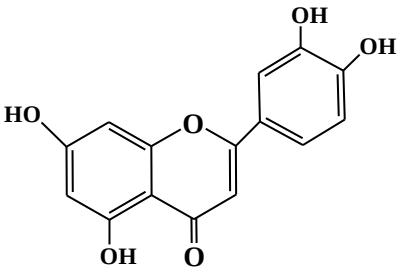
Aloesone (**52**)



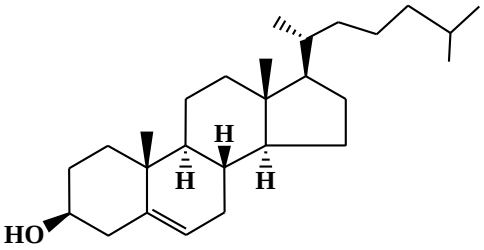
Furoaloesone(**53**)



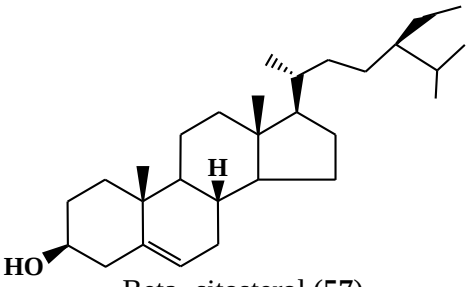
Queretin (**54**)



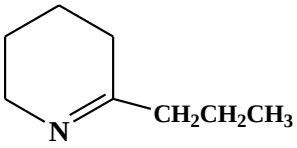
Luteolin (**55**)



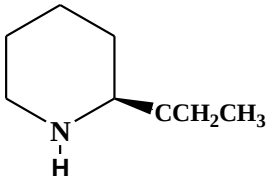
Cholesterol (**56)**



Beta -sitosterol (**57**)



Coniceine (**58**)



Coniine (**59**)

Figure 7. Metabolites isolated from *Aloe* other than anthraquinones and anthrones

2.5 Antimicrobial activity

The spread of multiple antimicrobial-resistant pathogenic bacteria has been recognized by the World Organization for Animal Health, the Food and Agriculture Organization and WHO as a serious global human and animal health problem. Hence, antimicrobial resistance is a natural biological phenomenon which was recognized first as a scientific curiosity and then as a threat to the effective treatment of most microbial infections [57]. Infectious diseases are the second leading cause of death worldwide, and the third leading cause of death in developed countries in both children and adults. The burden of infectious diseases falls most heavily on people in developing countries by resulting in high morbidity and mortality [58, 59]. These diseases are caused by viruses, bacteria, fungus, and parasites which results in respiratory infections,

HIV/AIDS, diarrhoeal diseases, tuberculosis, malaria, STDs (other than HIV, such as Gonorrhoea), meningitis and, tropical parasitic diseases [60].

The challenge associated with fighting these diseases has become an increasingly complex one, because of the fast development of resistance to the classic antibiotics; the evolution of multiple drug resistant human pathogenic microorganisms and the changing nature of the infections observed in the elderly and other immune-compromised patients. This demands the search for new sources of antimicrobial substances mainly from medicinal plants. Because, plants constitute a potentially useful resource for new and safe drugs for the treatment of microbial infections and other diseases [61].

So, to prevent and control infectious diseases it is of vital interest and concern in both developed and developing countries to come up with new remedies for such diseases [60]. To overcome this, a new antimicrobial agents with potent action against bacterial, fungal, parasite and viral diseases are urgently needed [52].

2.6 Bacterial infections and antibacterial agent

Bacteria are widely distributed and the most abundant group of unicellular organisms on earth, capable of adapting in a diverse range of environments such as in soil, water and air. They are both useful and harmful to humans. Many parasitic bacteria do not harm their hosts. Some cause disease through different mechanisms including production of toxins and/or hydrolytic enzymes. They have a prokaryotic cell type with a rigid wall which protects the cell against osmotic damage. The structure of the cell wall differs in Gram-positive and Gram-negative bacteria [60]. Gram-negative bacteria differ from Gram-positive by the presence of an outer membrane composed of lipopolysaccharides. It also contains specific proteins for transporting hydrophilic molecules. Other proteins are receptor sites for phages and bacteriacins. The outer membrane in Gram-negative bacteria covers the peptidoglycan layer, which is attached to the outer membrane by lipoproteins. The layer is separated by periplasm from the cytoplasmic membrane. Gram-positive bacteria have thicker peptidoglycan layers with no periplasm [53].

Typical Gram-positive bacteria include *Bacillus species* and *Staphylococcus species* while Gram-negatives could be best represented by *Escherichia coli*, *Salmonella* and *Shigella*. *Bacillus subtilis* is a rod shape endospore-forming Gram-positive bacterium that occurs in chain-like formations.

Its primary habitat is the soil and is responsible for food poisoning [54]. *Staphylococcus aureus* is a spherical Gram-positive parasitic bacterium that causes illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis and septicemia [55].

Antibiotics are the first preference for the treatment of infections. The development of resistance against antibiotics is another dark face of the antibiotic treatment against infections. Some bacterial strains already developed resistance to the commonly used antibiotics. For example, *Bacillus subtilis* are resistant to ampicillin, *Escherichia coli* resistant to, amikacin, ampicillin, cephalothin, cefpirome, chloramphenicol, carbenicillin, gentamicin, netilmicin, piperacillin, sulfamethoxazole, tobramycin and tetracycline [62]. It is a Gram-negative bacterium normally present in the intestinal tract of humans and other animals. It can sometimes be pathogenic, thus posing a threat to food safety, causing diarrhea, wound and urinary infections. So, there is an urgent need for new, affordable and accessible antibacterial agents from nature with different mechanism of action. The emergence and potential spread of strains of bacteria which are resistant to currently available drugs, has actually prompted the search for new drugs through the use of high-throughput screening and combinatorial chemistry, genomics, and vaccine development. However, this effort has yet to deliver a single drug despite the enormous resources expended during the past many years. Natural products will continue to serve as lead structures for the development of antibacterial drugs. This is in fact a pointer that new antibacterial leads may emerge from plants, especially from those with recognized traditional uses [20].

With parallel to this idea, *Aloe* has an extraordinary natural antibiotic capacity. This effect is accomplished *via* the different anthraquinoic glycosides, such as aloin and aloetic acid. Its antibiotic activity can be appreciated through the use of *Aloe*-based disinfecting and antibiotic creams for topical use, as well as an *Aloe* juice drink regularly targeted to the body's problems. For instance, the bacteria that attack our body are rapidly combated with the use of *Aloe* gel or juice. The combined antibacterial effects of the lignin, cinnamic acid, and Chrysophanic acid are immediate. The cytotoxic characteristics owed to their anthracenic structure respond quickly on superficial cells, liberating these cells from unwanted guests. Regarding its antiseptic properties, there are three antiseptic centers present in *Aloe*; these are saponins, cinnamic acid, and salicylic acid. The saponins are glycosides and are very particular. Unlike the saponins of some plants

which exhibit irritant and hemolytic actions, especially in the digestive tract, the saponins in *Aloe* have purifying, antiseptic, and antimicrobial actions which are not destructive to surrounding cellular tissues. Cinnamic acid, present in several spices like cinnamon, is an organic acid with outstanding antiseptic and germicidal activity [20].

With topical *Aloe* gel use, its anti-inflammatory actions are complemented by its capacity to heal wounds and regenerate skin cells in affected areas. The mechanisms for this action are quite different. If the anti-inflammatory response displays a tendency towards inhibitory reactions, then its wound-healing response shows the opposite phenomenon, that is, the stimulation of cells whose job is the formation and repair of tissues. The reabsorption of a wound is promoted by at least two factors. The first factor includes several active principles and a high molecular weight composed of long chains of a sugar called mannose, a glucomannan monomer which stimulates macrophage activity. The second factor in the remargination of a wound is found in the plant hormones' actions the giberelline and auxine present in *Aloe* which stimulates cell reproduction [20].

Itching is an allergic response to a harmful substance coming in contact with the body and can easily be sedated by the use of an *Aloe*-based cream or gel. Apply the product topically a few times a day until the symptom disappears. This product can also be useful in all areas affected by this symptom to include conditions like vaginal itch, inguinal itch, and general itchiness [20].

With similar Pharmacological aspects, recent research has indicated that *Aloe* might kill the bacteria responsible for tuberculosis, *Mycobacterium tuberculosis*, and also the herpes virus causing *herpes genitalis*. Research has further shown that *Aloe* inhibits growth of many common organisms such as yeasts, fungi, and bacteria infecting wounds [51].

Along with anti-inflammatory, immunomodulatory and redox-state maintenance bioactivities, it has also been reported to have good antimicrobial bioactivity. *A. ferox* and *A.arborescens* have also been shown to inhibit the growth of various bacteria associated with wound infections and thus enhance wound healing. Similarly, *A. perryi* baker has been shown to inhibit the growth of *Staphylococcus aureus* and *Enterococcus faecalis* in *vitro*. Although much of the scientific investigation into the antibacterial properties of *Aloe* species is dominated by *A. barbadensis*, *A. ferox*, *A. arborescens* and *A. perryi* baker, there have been a number of other fruitful investigations

into the antibacterial properties of other *Aloe* species. *Aloe chinensis* and *Aloe succotrina* have been reported to be effective against *Mycobacterium tuberculosis*. *Aloe secondi flora* has been shown to inhibit *Salmonella gallinarum* growth and *Aloe* excels inhibits the growth of various bacteria in *vitro Aloe ferox* and *Aloe vera* belong to this family and are used to treat poultry diseases. The methanol extract and petroleum ether extract of *Aloe* species has been found to have activity against *Bacillus subtilis* [29].

To consolidate this scientific finding, a study conducted by Tekleab and co-workers 2014,[33] on the antimicrobial constituents from the leaf latex of *Aloe pulcherrium*, in which the latex is subjected to the isolation of two major anthrones identified as **42** and **60**, and has shown, broad spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria. Their activity was potent particularly against *E. coli* and *Vibrio cholera* strains, which was comparable to that of ciprofloxacin. It is interesting to note that the zones of inhibition displayed by **42** on two strains of *E. coli* were equal to that of the standard drug. The effect of compound **42** against the resistant Gram-positive bacterium, *S. aureus* was also very high. In general, the activity patterns of the latex and isolated compounds appear to be similar in that they are more active against the Gram negative bacteria rather than the Gram positive ones, indicating that the antibacterial effect of the latex is largely due to the presence of these compounds [65].

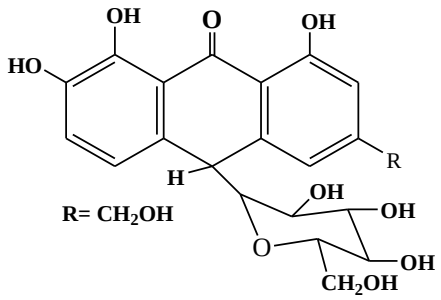


Figure 8. Structure of 7-hydroxyaloin (**60**)

Antibiotic resistance has been an issue of debate since the introduction of antibiotics into clinical practice in the 1940s. At the beginning, it was demonstrated that antibiotics could inhibit bacterial growth *in vitro* in specific, minimal concentrations (MICs); since then, this value has been used to denote susceptibility *in vivo* and to guide clinical practice. Moreover, the acquisition of resistant mechanisms either by mutations or through interbacterial communication has rendered bacteria more tolerant to antibiotics and more difficult to treat [63**]**. In case, the MICs method allows

comparisons between the microorganisms exposed to the same chemical agents, but does not allow analog comparisons between the activities of different chemical agents [64]. So, MIC is defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation, and minimum bactericidal concentration the lowest concentration of antimicrobial that will prevent the growth of an organism after sub-culture on to antibiotic free media [65]. The zone of inhibition correlates inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms [66].

3. Materials and methods

3.1 Chemicals

Chemicals that were used for this study include acetone (99.5%), chloroform (99%), ethyl acetate (99%), are all from (Blulux Laboratories Ltd.), n-hexane (95%), and methanol (99.99%), are from (Fisher Scientific, UK) for gradient column elution and extraction; Silica gel 60-120 mesh size, Oxalic acid (Riedel-de Haen,99.5%), distilled water, dimethyl sulfoxide (DMSO), Mueller Hinton agar and nutrient broth as culture media were used for antibacterial activity test in this study. Deuterated chloroform ($CDCl_3$, 99.8%) and acetone (DMSO, 99.96%) solvents were used for recording NMR spectra. All the chemicals and reagents of analytical grade were used.

3.2 Apparatus and equipment

Apparatus that were used for this study includes rotary evaporator (Labo Rota 4000, Heidolph Instrument), TLC plates, round bottom flask (50, 100 and 250 mL), measuring cylinders, Erlemnery flasks, pestle and mortar, filter papers, weighing balances, oven for drying purpose, ,Melting point apparatus (Griffin 590), glass columns for CC and UV-254 and 365 nm chamber (UV-Tec) for detection of spots on TLC were used for the study. Spectral recording was done using Bruker 500 MHz advance NMR spectrometer with TMS as internal standard. The FT-IR spectrum was recorded using a Perkin Elmer FT-IR Spectrometer with KBr pellets. UV-VIS spectra were obtained using JENWAY UV/VIS 6705 spectrophotometer (Bibby Scientific).

3.3 Collection and preparation of the plant sample

The plant materials, *Aloe pulcherium* vernacular name '*Hargisa dhala*' (Afaan Oromo) and '*Sete Eret'* (Amharic) root was collected from their natural habitat, uncultivated land in Jimma zone South western, 'Saka Chokorsa' district, and '*Qachamaa'* Kebele specifically in a place called '*Buuyyoo*' in April, 2015. Identification of the plant species was made by a plant botanist and a voucher specimen was deposited as AP001 at botanical science laboratory Herbarium, Biology Department Jimma University. The roots of the plant was collected, air-dried under shade and powdered to suitable size to improve the subsequent extraction by rendering the sample more homogenous, increasing the surface area, and facilitating the penetration of solvent into the cells by using mechanical grinder. Sequentially extraction method was used since; plants are complex

matrices, producing a range of secondary metabolites with different functional groups and polarities ranges [71].

3.4 Extraction and isolation

3.4.1 Extraction

An 810 g of the powdered root of the plant was subjected to sequentially extraction with of *n*-hexane (2x2L), chloroform (2x2L), acetone (3x2L) and methanol (2x2L) at room temperature in 24 hr. by maceration method. The extracts of each solvents were filtered first using cotton plugged followed by Whatman No 1 filter paper. The filtrates were concentrated using rotary evaporator (Laborota 4000) at a temperature of 40 °C. The results of each extracts were stored in desiccator until dry and used for antibacterial activity tests (Bioassays). The resulting dry crude extracts were weighed and its percentage yield was calculated as 2.0 g (0.25%), 3.2 g (0.39%), 4.9 (0.61%) and 4 g (0.49%) of hexane, chloroform, acetone and methanol, respectively.

Then, the choice of solvent used for isolation of compounds was made after carrying out the TLC analyses in various combinations of solvent systems with different polarity. Finally, ethyl acetate in n-hexane has displayed an isolated spots on TLC plate of the extract.

3.4.2 Isolation of compounds by column chromatography

The acetone crude extract (4 g) was adsorbed on silica gel and subjected to CC on oxalic acid impregnated silica gel, which was then eluted with *n*-hexane containing increasing amounts of ethyl acetate 100:0, 99:1, 98:2, and 97:3 up to 0:100% respectively. The fractions were initially collected, 100 mL up to the colored compound approaches to elute and then constantly it was collected 20 mL. From CC thirty two fractions were eluted with n-hexane: ethyl acetate combination. Based on TLC profile, fraction five (F-5, 1.4 g) was mixed with silica gel and subjected to CC for further isolation then it was collected and reduced to twenty nine fractions. Among, fraction nineteen (F-19, 10 mg) with an R_f value 0.54, named as **AP-3D** pure compounds was obtained with 25 % ethyl acetate in n-hexane. Then, based on their TLC similarities six fractions (F7-12) were combined together and dissolved in acetone, at the same time concentrated using Rota vapor and dry application was made to the already packed smaller column. It was

reduced to fifty-one fractions, among fraction twenty-eight (F-28, 20 mg) has an R_f value of 0.70, **AP-4D** pure compound was obtained with 10%.ethyl acetate in n-hexane.

Likewise, based on their TLC resemblances again six fractions (F 13-18) were combined and subjected to CC for further purification, F-14 (20 mg, **AP-2B)** has an R_f value of 0.64 and F-23 (27mg, **AP-8G**) has an R_f of 0.50 yellow pure compounds were fractionated with n-hexane: ethyl acetate at 90:10 and 80:20% respectively. Similarly, on the bases of TLC correspondence five fractions (F-26, 28, 29, 30 and 31) were combined, applied into small column and eluted with n-hexane: ethyl acetate combination. Then, fraction forty was (F-40, 33 mg) **AP-11D**, has an R_f of 0.37 was eluted with 50% ethyl acetate in n-hexane. Repetitive CC and melting point determination was made for further purification. The level of separation was monitored by TLC analyses. At the same time, detection of the spot was done using UV (254 and 366 nm) and Iodine chamber.

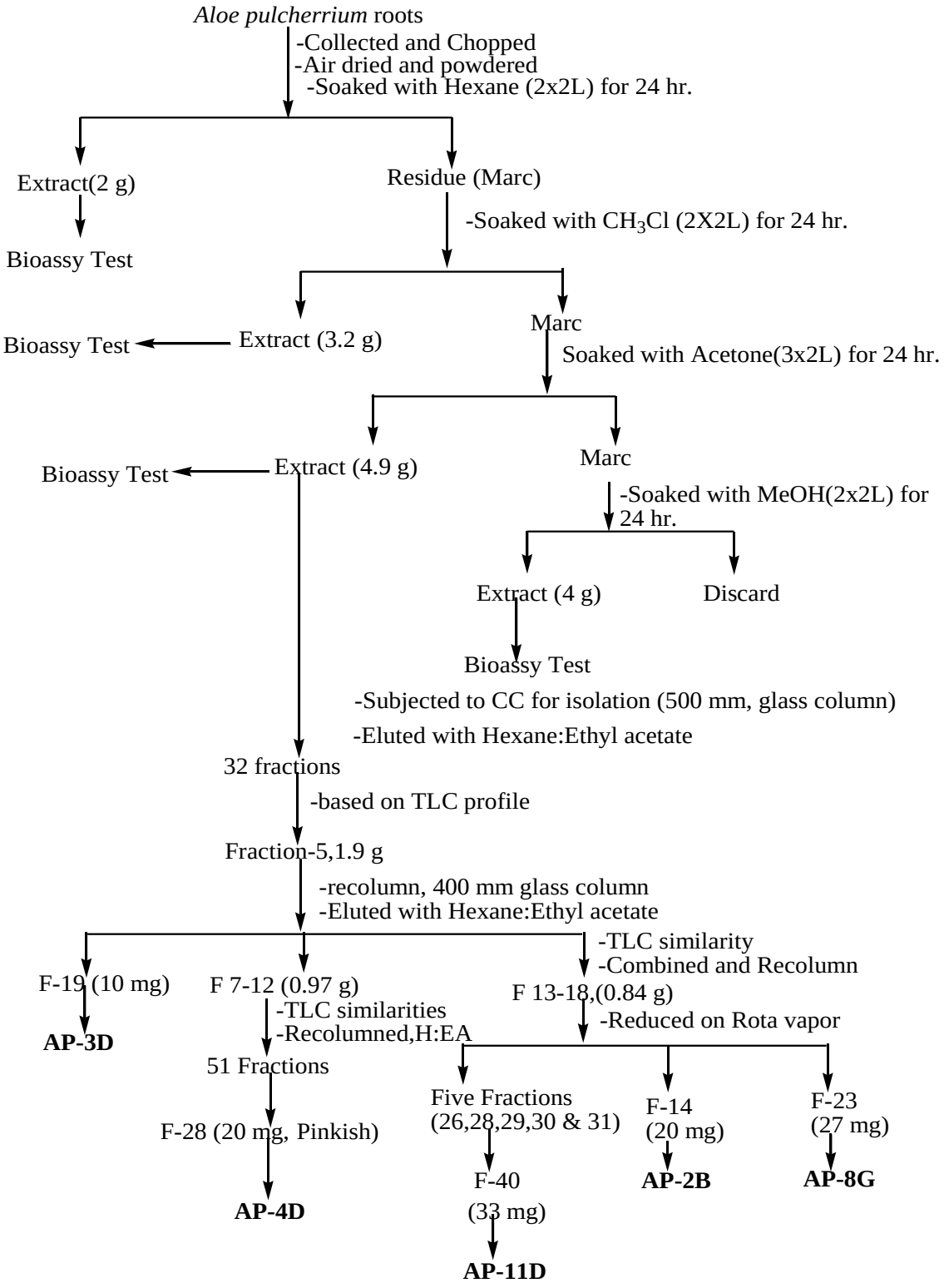


Figure 9. Bioassay guided isolation of *Aloe pulcherrium* root

3.5 Culture media

The study of microorganisms requires basic microbiological methods for isolation and characterization of microorganisms collected from their natural habitat. Cultivation of microorganisms under laboratory conditions requires the use of synthetic media. Thus, microbial culture media are used to isolate and identify bacteria, reveal their metabolic properties, and allow long-term storage of pure cultures. In this study, Muller Hinton Agar, nutrient broth and nutrient Agar were used. Nutrient broth was used for determination of minimum inhibitory concentration, while nutrient Agar was also used for routine handling or storage of stock cultures and sub culturing [69].

3.5.1 Test strains

Microorganisms used for determination of antibacterial activities of the crude plant extracts and fractions were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* A TCC 27853 and *Bacillus subtilis* ATCC 6633. The standard strains were obtained from Biology Department, Microbiology Research Laboratory, Jimma University, originally obtained from Professor Dr. Ruth Anne Schmitz-Streit's Laboratory, Kiel, Germany. Meanwhile, the bacterial strains were reactivated by sub culturing in nutrient broth at 37 ºC and maintained on nutrient agar slant at 4 ºC for further activity.

3.5.2 Standardization of inoculum

To standardize the inoculum density for a susceptibility test, a Barium sulphate ($BaSO_4$) turbidity standard, equivalent to a 0.5 McFarland standard, should be used. Accordingly, $BaSO_4$ 0.5 McFarland standard was prepared, by adding 0.5 mL of 0.048 $molL^{-1}$ barium chloride ($BaCl_2$) 1.175% w/v dihydrated barium chloride ($BaCl_2.2H_2O$) in to 99.5 mL of 0.18 mol/L Sulphuric acid (H_2SO_4, 1% v/v) with constant stirring to maintain a suspension. This mixture was considered to be equivalent to cell density of 1 to $2x10^8$ $CFUmL^{-1}$. The turbidity standard is then aliquot into test tubes identical to those used to prepare the inoculum suspension. McFarland turbidity standard tubes were sealed with para film to prevent evaporation. Barium sulphate turbidity was compared with identical tubes containing inoculum 0.85% NaCl saline solution [60]. For each bacterial strain, Gentamycin was taken as positive control and pure solvent (DMSO) as the negative control [70].

3.6 Antibacterial activity test

3.6. 1 Agar disc diffusion method

Agar Disc diffusion method was used to evaluate the antibacterial activities of medicinal plant. Accordingly, an overnight cultures of the test strains whose cell densities were adjusted to 0.5 McFarland standards (equivalent to 1 to $2x10^8$ $CFUmL^{-1}$ bacterial suspensions) were uniformly spread on pre-prepared sterile Muller Hinton Agar plates to form lawn cultures. Then, 0.2 g crude extracts of n-hexane, chloroform, acetone and methanol were dissolved separately in DMSO. The stock solution was prepared with concentrations of 200 $mgmL^{-1}$ for each solvent extracts. The blotting paper discs (6 mm) was soaked in various dilute solvent extracts and dried for 5 min. to avoid flow of extracts in the test media. The discs were placed on a plate with standardized culture suspension at equidistance to one another to avoid overlap of zones of growth inhibitions.

Antibacterial activity of potential plant extract against bacterial pathogens was determined after incubation of the plates for 24 hr. at 37 ºC, by measuring the diameter of zone of inhibition of growth. The diameter of zone of growth inhibition surrounding the discs was measured and expressed in millimeter (mm) using transparent ruler [59]

3.6.2 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extract was determined using broth dilution method. Nutrient broth was prepared according to the manufacturer's instruction. Ten milliliter of the broth was dispersed in to sets of five test tubes and capped. It was sterilized at 121 ℃ for 15 min. using autoclave. McFarland's turbidity standard scale of 0.5 (standardized inoculums) was prepared to give a turbid solution. Sterile normal saline was used to make a turbid suspension of the microbes; serial dilution of the micro-organism was done continuously in the normal saline until the turbidity matched that of McFarland's scale by visual comparison. The concentration at which the turbidity of McFarland standard and cell suspension approximately matches, the micro-organisms have a concentration of 1×10^8 $CFUmL^{-1}$, serial dilution of the extracts at the initial concentration of 200 $mgmL^{-1}$ was constituted in the sterilized nutrient broth in the tubes.

The initial concentration was obtained by dissolving 0.2 g of the acetone extract in 1 mL DMSO. The dilution was carried out with the aid of sterilized syringe; having obtained the different

concentrations from 200 to 12.5 mgmL^{-1} through dilution, the suspension of the test microbes was transferred into the tubes. The tubes were incubated at 37 ℃ for 24 hr., and finally examined for turbidity [59, 71].

3.6.3 Characterization procedure for the Isolated compounds

During this study the electronic absorption spectra of isolated compounds **AP-11D** and **AP-8G** was studied using UV-Vis spectrophotometry in quartz glass by preparing 1 mg of the isolated compounds in 10 mL MeOH at 298 K and then the colored solution was diluted by half serially and MeOH was also used as a blank solution, the scan rate was in between 200 to 500 nm the data were recorded with 20 nm difference. The maximum wavelength for the possible transition was assigned by plotting the absorbance vs. wavelength in nm.

Melting points were determined using a Griffin 590 melting point apparatus equipped with a thermometer by adsorbing the fine powdered sample in to the capillary tube; meanwhile, the temperature it begins to melt and ends was recorded in range.

With respect to the IR and NMR spectroscopy, its IR was recorded using a Perkin-Elmer 1600 series FTIR spectrometer with KBr pellets, displayed as transmittance vs. wavelength in cm^{-1}. ^{1}H, ^{13}C-NMR and 2D spectra were run on a Bruker 500 Ultra shield NMR at 500 and 125 MHz, respectively, using $CDCl_3$ and DMSO as solvent. Chemical shifts are reported in ppm and δ scale with the coupling constants given in Hz.

4. Result and Discussion

4.1 Percentage yields of the crude extracts

The yield of extracts were calculated on dry weight basis using the formula,

$$\% \text{ extract yield} = \frac{\text{weight of dry extract}}{\text{weight taken for extraction}} \times 100\%$$

Relatively higher yield of extract (0.61%, 4.9 g) was obtained with acetone as solvent and the least with n-hexane (0.25%, 2.0 g).

Table 1.Amounts of the crude extracts obtained from sequential extraction

Types of Extracts	Mass of the crude extract(g)	%yield
Hexane	2.0	0.25
Chloroform	3.2	0.39
Acetone	4.9	0.61
Methanol	4.0	0.49

4.2 Antibacterial activities of extracts from root of *Aloe pulcherrium*

The zone of inhibition for all the crude extracts against the reference strains is as displayed in Table 2 (and Appendix 1). It was observed that the zones of inhibition for different extracts fall in the range of 8-24 mm as compared to 20-25 mm for the positive reference standard Gentamycin.

When the zone of growth inhibition values of the crude extracts are compared with each other, the activities of acetone extracts was found to be superior against all the bacterial species. The data show that the zone of growth inhibition values are 15, 16, 12 and 24 mm against *B.subtilis, E.coli, S.aureus* and *P.aeruginosa*, respectively (Table2, Appendix 1). The corresponding bacterial growth inhibition zone for Gentamycin was found to be 20, 20, 23 and 25 mm against *B.subtilis, E.coli, S.aureus* and *P.aeruginosa*, respectively (Table 2, Appendix 1). From this result it can be said that acetone extract has shown almost equal inhibition potency with the reference standard in case of *P. aeruginosa* strain. However, no antibacterial activities were observed for the DMSO that was used as negative control.

Table 2. Antibacterial Activity of *Aloe pulcherrium* roots crude extracts (200 $mgmL^{-1}$)

Bacterial strain	Gram	Diameter of zone of Growth Inhibition (mm)					
		Hexane extract	Chloroform extract	Acetone extract	Methanol extract	G	DMSO
B. subtilis	+ve	9	20	15	9	20	NI
E.coli	-ve	10	9	16	8	20	NI
S.aureus	+ve	10	8	12	9	23	NI
P. aeruginosa	-ve	13	15	24	11	25	NI

Key: NI=Not inhibitory,–ve=Gram negative, +ve =Gram positive, G=Gentamycin B.subtilis=*Bacillus subtilis*, *E.coli=Escherichia coli*, S.aureus=Staphylococcus aureus, *P .aeruginosa=Pseudomonas aeruginosa*

In agreement with the above observation, Tekleab and co-workers, 2014 [33], has reported that the leaf latex of *Aloe pulcherrium* has the broad antibacterial activity against both Gram-positive and Gram-negative bacteria. Equivalently, the results of this study also indicated that the root of *Aloe pulcherrium* crude extract has similar potency on the bacterial strains of both Gram-positive and Gram-negative bacteria. As it can be observed, chloroform and acetone crude extracts have significant effects on *B.subtilis* and *P.aeruginosa* (Table 2, Appendix 1).

4.3 MIC results for acetone crude extracts

Supplementary to the above antibacterial activity tests, the potency of the acetone crude extract was further confirmed by carrying out the MIC on the four bacterial strains. It perhaps used to judge the performance of the above method, i.e., Agar disk diffusion method to determine the susceptibility testing of microorganisms to the acetone crude extract of *Aloe pulcherrium* root which has shown superior activities against the four bacterial strains. Comparatively, the acetone crude extract has displayed good to significant antibacterial properties on the tested bacteria. The MIC results are depicted in the following Table 3, Appendix 2).

As it can be seen from the data Table 3, the *in vitro* antibacterial activities tests were carried out against four bacterial strains using different concentration of the crude extract. The serially diluted extract has shown a good antibacterial activities against both the Gram negative bacteria (*E. coli and P. aeruginosa*) and Gram positive *(B. subtilis* and *S. aureus),* but with reference to the positive

standard (Gentamycin) it has shown significant activities on the Gram negative bacteria (*E.coli* and *P.aeruginosa*) as compared to Gram positive (*B. subtilis* and *S.aureus*) down the column.

Across the Table 3, the zone of growth inhibition by 200 $mgmL^{-1}$ concentrated extract on *B.subtilis, E. coli, S.aureus, and P.aeruginosa* was 10, 20, 13 and 22 mm respectively. And for the lowest concentration 12.5 $mgmL^{-1}$ its activities on *B.subtilis, E. coli, S.aureus, and P. aeruginosa* was 11, 17, 14 and 18 mm, respectively. The analogous bacterial growth inhibition zone result for Gentamycin was found to be 20, 20, 23 and 25 mm against *B.subtilis, E. coli, S. aureus and P. aeruginosa*, respectively (Table 3, Appendix 2).

Table 3. Antibacterial activities of different concentrations of acetone extracts

Concentration (in $mgmL^{-1}$)	Diameter of zone of Growth Inhibition (mm)			
	Bacterial strain			
	B.subtilis	*E.coli*	*S.aureus*	*P. aeruginosa*
200	10	20	13	22
100	10	14	14	20
50	10	14	17	17
25	11	16	11	16
12.5	11	17	14	18
G	20	20	23	25
DMSO	NI	NI	NI	NI

Key: NI= No inhibition, DMSO = Dimethyl sulfoxide G= Gentamycin

As reported by Kahlmeter and Poulsen 2012, [66] the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. Relatively the MIC results of this study has shown that as the lowest concentration (12.5 $mgmL^{-1}$) was potent than 100, 50 and 25 $mgmL^{-1}$ on bacterial species. But, the potency of the extract on Gram negative bacteria was greater than Gram positive bacteria when compared with positive reference standard.

4.4 Antibacterial activities of isolated compounds

For *in vitro* antibacterial activity tests for the isolated compounds similar procedures used above were followed to evaluate the antibacterial activities of the pure compounds. **AP-2B**, **AP-3D**, **AP-4D**, **AP-8G** and **AP-11D** are the isolated compounds tested against bacterial strains. Of these lists,

only **AP-8G** and **AP-11D** were demonstrated a good antibacterial against the four strains (Table 4). **AP-2B** and **AP-4D** were also not sufficient for characterization.

Table 4.Antibacterial Activity tests of isolated compounds

S.No	Isolated compounds	Diameter of zone of Growth Inhibition (mm)			
		Bacterial strain			
		B.subtilis	*E.coli*	*S.aureus*	*P. aeruginosa*
1	AP-8G	27	22	18	21
2	AP-2B	23	17	17	25
3	AP-4D	20	17	16	24
4	AP-11D	22	21	18	23
5	AP-3D	10	NI	10	12
6	G	25	23	22	22
7	DMSO	NI	NI	NI	NI

Key: NI= No inhibition, DMSO = Dimethyl sulfoxide G= Gentamycin

Several chemical components of the *Aloe* root are thought to be responsible for its medicinal properties. Table 4 (Appendix 3) illustrates that isolated compounds (labeled as **AP-2B, AP-3D, AP-4D, AP-8G** and **AP-11D**) had some activities against the bacterial test strains. The diameter of growth inhibition zone displayed on both Gram negative and Gram positive were good with variable degree of potency among the tested compounds.

However, **AP-8G** and **AP-11D** have shown significant anti-bacterial activity on *B. subtilis* and *P. aeruginosa.* As compared to the standard positive control (Gentamycin) **AP-2B**, **AP-4D** and **AP-11D** compounds have displayed almost greater activity on *P. aeruginosa.* Comparatively, **AP-8G** and **AP-11D** have shown a significant antibacterial activity against the four bacterial strains. Both structures **AP-8G** (Aloesaponarin I) and **AP-11D** (Aloesaponarin II) are dihyroxy with one methyl group on the region of the aromatic ring structure. Comparatively, the C-7 methyl ester **AP-8G** (Figure 11) has displayed slightly better antibacterial activities against the four strains.

According to Van den Berg and co-workers, 1988 [69] this compound (Aloesaponarin I) was reported to have anti-microbial activities and Dagne and co-workers, 2000 [51] reported this compound occurs in the roots of many *Aloe* species including *A. saponaria*.

4.5 Structural Elucidation

During this study **AP-8G** and **AP-11D** pure compounds were chosen for characterization among the isolated compounds from the roots of *Aloe pulcherrium* on the basis of bacterial activities and characterized for structural determination by spectroscopic methods including IR, UV-VIS, ^{1}H , ^{13}C, 2D NMR and other physical data such as the melting point. The collected data were analyzed and compared with review of related literature values for the reported compounds.

4.5.1. Characterization of compound AP-11D

Compound **AP-11D** was isolated as a yellow crystal (26 mg) has an R_f value of 0.37 (50% ethyl acetate in n-hexane). Its molecular formula was predicted as $C_{15}H_{10}O_4$ on the basis of its ^{1}H and ^{13}C NMR spectral data which is consistent with eleven degrees of unsaturation. And its melting point was determined to be 190-192 ℃.

Its UV-VIS spectrum (MeOH) has showed four λ_{max} at 226, 272, and 285 nm in the UV region and 427 nm in the visible region of electromagnetic radiation. In case, the 427 nm is the assignment with respect to the n to $\pi*$ transition for C=O in the visible region and the transition from π to $\pi*$ represents the λ_{max} at 285 nm which is for the conjugated aromatic system. The λ_{max} at 272 nm could be for the n to $\sigma*$ transition occurred due to the hydroxyl (-OH) and the fourth transition is from the aromatic π-bond to the methyl group is consistent with the anthraquinone nature of the compound (Appendix 9).

The ^{13}C NMR spectrum showed fifteen carbon signals (Table 5); five aromatic methines, seven aromatic quaternary carbons, two carbonyl carbons and a methyl group. The ^{1}H NMR and COSY spectrum, showed the presence of three mutually coupled aromatic protons of ABX spin pattern centered at δ_H 7.59 (*dd*, *J* =8.3 Hz, 1H), 7.67 (*t*, *J* =7.9 Hz, 1H) and 7.28 (*dd*, *J* =7.6 Hz, 1H) and two *meta*-coupled protons resonating at δ_H 7.59 (*dd*, *J* = 2.1 Hz, 1H) and 7.28 (*dd*, *J* =2.1 Hz, 1H). The up-field shifted (δ_H 2.66) signal for a methyl group in this compound indicating its *peri*-position to carbonyl group. Furthermore, the presence of only one chelated hydroxyl group (at δ_H 12.93), allowed the unequivocal placement of the methyl group at C-8. The HMBC $^{4}J_{C,H}$ correlation observed between methyl protons with the carbonyl carbon, C-9 (δ_C 189.4) further confirmed the assignment.

Therefore, the structure of compound **AP-11D** was characterized as 1,6-dihydroxy-8-methylanthracene-9, 10-dione, trivial name aloesaponarin II (**AP-11D**). According to Van Wyk and co-workers, 1995b [43] and Yagi and co-workers, 1974 [73] this compound has been reported from the roots of several *Aloe* species. As a result the proposed structure of **AP-11D** was compared with reported chemical shifts.

Table 5.^{1}H NMR Spectroscopic Data for Compound **AP-11D** δ (in ppm) and Multiplicity

Observed(DMSO-d_6,500 MHz)				Reported($CDCl_3$, 500 MHz) [74, 75]			
δ^1H	J_{HH} (in Hz)	m	Description	δ^1H	J_{HH} (in Hz)	m	Description
2.66	-	*s*	-CH_3 (3H)	2.47	-	*s*	CH_3(3H)
7.00	-	*s*	-OH (H)	12.03	-	*s*	-OH (H)
7.28	7.6, 2.1	*dd*	H-4	7.30	8.5	*dd*	H-7
7.41	-	*s*	H-5	7.11	1	*d*	H-2
7.58	8.5	*d*	H-7	7.66	1	*d*	H-4
7.59	8.3, 2.1	*dd*	H-2	7.83	8.5	*dd*	H-5
7.67	7.9	*t*	H-3	7.67	8.5	*t*	H-6
12.93	-	*s*	Chelated(OH)	12.13	-	*s*	Chelated(OH)

The 2D NMR correlation was used for further confirmation of the proposed structure of **AP-11D**, its COSY, HSQC and HMBC together with ^{1}H and ^{13}C NMR Spectroscopic data as depicted in Table 6.

Table 6. ^{1}H NMR, ^{13}C NMR and HMBC Spectroscopic data for **AP-11D**

Protons	δ_H	m	δ_C	HMBC
H-2	7.59	*dd*	118.3	C-3,C-4,C-1a
H-3	7.67	*t*	136.9	C-1, C-2, C-4,C-1a, C-4a
H-4	7.28	*dd*	122.5	C-2,C-3,C-10,C-1a,C-4a
H-5	7.41	*s*	112.1	C-6,C-7,C-10,C-10a,C-9a
H-7	7.58	*d*	136.1	C-5,C-8,C-9a,C-11
H-1'(OH)	12.93	*s*	162.4	C-1,C-2,C-1a
H-6'(OH)	7.00	*s*	124.6	C-5, C-6, C-7
H-8'(CH_3)	2.66	*s*	23.6	C-7,C-8, C-9,C-9a

Key: m-multiplicity, *dd*-double-doublet, *t*-triplet, *s*-singlet,δ-chemical shift in ppm

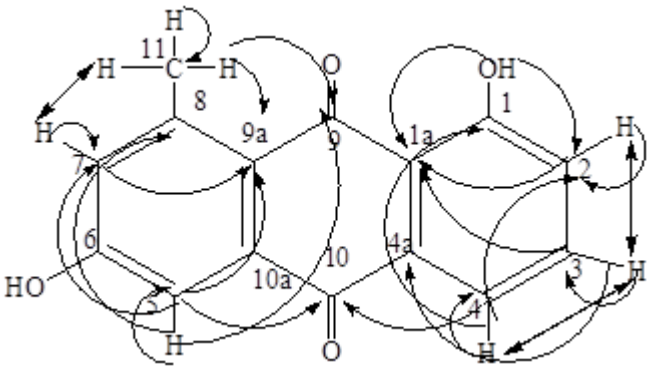


Figure 10. Pertinent COSY (↔), HSQC (→) and HMBC (→) correlations for proposed structure of **AP-11D** (Aloesaponarin II)

4.5.2 Characterization of compound AP-8G

Compound **AP-8G** (20 mg) was isolated as yellow crystals with an R_f value of 0.50 (20% ethyl acetate in *n*-hexane). Its molecular formula was established as $C_{17}H_{12}O_6$ on the basis of its ^1H and ^{13}C NMR spectral data which is consistent with twelve degrees of unsaturation. It melting point was 207-209 ℃.

Its UV-VIS spectra (MeOH) was carried using spectrophotometer. The compound has displayed four λ_{max} at 220, 272, 310 and 420 nm. The corresponding transitions with respect to the λ_{max} was assigned as n to $\pi*$ is at 420 nm due to C=O chromophore, from π to $\pi*$ represents the λ_{max} at 310 nm which is for the conjugated aromatic system. The λ_{max} at 272 nm could be for the n to $\sigma*$ which the transition occurred due to the hydroxyl (-OH) chromophore and the fourth transition is from the aromatic π to $\sigma*$ at 220 nm which is to the lower λ_{max} due to the electron withdrawing acetyl group. This data are reliable with anthraquinone derivative nature of the compounds (Appendix 13).

Its IR spectra (KBr) indicated an absorption bands belonging to the characteristic vibration frequency of its C-H (CH_3), Ar-C=O (C-9), Ar-C=O (C-10) stretching were detected at 2951, 1629, 1671, cm^{-1},respectively and aromatic skeletal vibrations for C=C and C-C were also observed at 1580 and 1460 cm^{-1}, respectively. Two absorption bands at 1739 and 1311 cm^{-1} were attributed to ester C=O and C-O, respectively.

The ^1H NMR has showed highly deshielded signal at δ_H 12.93 due to chelation of the hydroxyl proton at C-1 (δ_C 163.6), a three-proton singlet at δ_H 4.06 (δ_C 53.3) and a shielded carbonyl (δ_C 170.7), indicating the presence of a methyl ester. The up field shifted methyl signal observed at δ_H 2.98 (δ_C 22.0) due to the *peri*-effect allowed its placement at C-8 (δ_C 148.1) as in aloesaponarin I. In addition, the ^1H NMR spectrum further showed the presence of three mutually coupled aromatic protons with an ABX pattern centered at δ_H 7.30 (*dd*, J = 8.2 Hz, H-2), 7.62 (*t*, J = 7.6 Hz, H-3) and 7.33 (*dd*, J = 7.6 Hz ,H-4) of ring A as in aloesaponarin I . In ring C, the singlet aromatic proton at δ_H 7.80 showed correlation with C-6, C-7, C-10 and C-10a which is in agreement with its placement at C-5 of trisubstituted (C-6, C-7, C-8) ring C. The substituents in this ring, a methyl group (δ_H 2.98 at C-8), a hydroxyl group (at C-6) and methyl ester (at C-7) are evident from the NMR spectra (Table 7).

The ^{13}C NMR spectral data (Table 8) revealed the presence of seventeen carbon atoms; three carbonyls (δ_C 189.8, 182.1 and 170.7), four aromatic methine carbons (δ_C 135.9, 124.6, 119.1, and 115.2), two oxygenated aromatic quaternary carbons (δ_C 163.6 and 162.6), six non-oxygenated aromatic quaternary carbons (δ_C 148.1, 138.9, 132.8, 125.2, 121.2 and 115.2), a methoxy group (δ_C 53.3) and a methyl carbon (δ_C 22.0). Based on the above spectral data, compound **AP-8G** was identified as 1, 6-dihydroxy-8-methyl-anthracene-7- methyl ester-9, 10-dione, trivial name Aloesaponarin I (**AP-8G**). According to Yagi and co-workers, 1974 [73], this compound was previously reported from the stem of *Aloe saponaria*.

Table 7.^1H NMR Spectroscopic Data for Compound **AP-8G** δ (in ppm) and Multiplicity

Observed ($CDCl_3$-d_6,500 MHZ)				Reported ($CDCl_3$, 500 MHZ) [76,77]]			
δ^1H	J_{HH} (in Hz)	m	Description	δ^1H	J_{HH} (in Hz)	m	Description
2.98	-	*s*	CH_3 (3H)	2.88	-	*s*	1-CH_3 (3H)
4.06	-	*s*	OCH_3 (3H)	4.02	-	*s*	OCH_3 (3H)
7.30	8.2	*dd*	H-2 (1H)	7.31	1.3,8.0	*dd*	H-7 (1H)
7.33	7.6	*dd*	H-4 (1H)	7.63	1.3, 8.0	*dd*	H-5 (1H)
7.62	7.6	*t*	H-3 (1H)	7.70	8.0	*t*	H-6 (1H)
7.76	8.0	*d*	OH-6 (1H)	7.06	2.6	*d*	OH-3 (1H)
7.80	8.0	*d*	H-5 (H)	7.48	2.6	*d*	H-4 (1H)
12.93	-	*s*	Chelated (OH)	-	-	-	-

Therefore, on the basis of spectroscopic data analysis the likely the structure of **AP-8G** has anthraquinone derivative known as Aloesaponarin I.

Table 8. ^{1}H and ^{13}C NMR Spectroscopic Data for AP-8G δ in ppm and Multiplicity

	Observed ($CDCl_3$-d_6)			Reported (DMSO-d_6) [76-78]		
Carbons	$\delta^{13}C$	$\delta^{1}H$	m	$\delta^{13}C$	$\delta^{1}H$	m
C-1	163.6	-	-	161.4	-	-
C-2	124.6	-	-	124.6	-	-
C-3	135.9	7.76	*d*	135.8	7.06	*d*
C-4	119.1	7.80	*d*	112.0	7.48	*d*
C-5	115.2	7.33	*dd*	118.1	7.63	*dd*
C-6	162.6	7.62	*t*	161.4	7.70	*t*
C-7	125.2	7.30	*dd*	124.1	7.31	*dd*
C-8	148.1	12.93	*s*	145.2	-	-
C-9	189.8	-	-	189.2	-	-
C-10	182.3	-	-	182.1	-	-
C-10a	138.9	-	-	136.8	-	-
C-9a	115.2	-	-	116.4	-	-
C-4a	132.8	-	-	132.5	-	-
C-1a	121.2	-	-	122.2	-	-
C-11	22.0	2.98	*s*	23.4	2.88	*s*
C-12	170.7	-	-	169.9	-	-
C-13	53.3	4.06	*s*	53.2	4.02	*s*

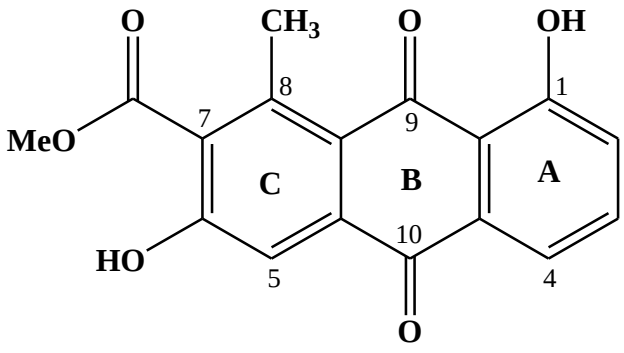


Figure 11. The proposed Structure of Compound **AP-8G** (Aloesaponarin I)

4.6 Summary of the spectral and physical data of the isolated compounds

AP-11D (Aloesaponarin II)

It was a yellow crystal (26 mg) with R_f value 0.37 (in 50% ethyl acetate in n-hexane) and melting point 190-192 ℃. UV-VIS (MeOH) λ_{max} 226, 272, 285 and 427 nm. ^{1}H NMR (500 MHz, DMSO-*d6*) δ in ppm :7.67 (1H, *t* ,H-3), 7.59 (1H, *dd*, H-2) 7.28 (1H, *dd*, H-4), 7.41 (1H, *s*, H-5), 7.00 (1H, *s*,H-6), 12.93 (1H, *s*,H-1) and 2.66 (3H, *s*,H-8) (Appendix 4). ^{13}C-NMR (DMSO-*d*, 125 MHz): C-11 (23.6), C-10 (182.3), C-10a (116.5), C-9 (189.4), C-9a (145.5), C-1 (162.4), C-1a (132.6), C-2 (118.3), C-3 (136.9), C-4 (122.5), C-4a (162.5), C-5(112.1), C-6 (124.6), C-7 (136.1), C-8 (161.5) (Appendix 5). Based on these spectral data the compound was proposed as 1, 6-dihydroxy-8-methylanthracene-9, 10-dione, trivial name aloesaponarin II. And its molecular formula was predicted as $C_{15}H_{10}O_4$. Its corresponding inhibition zone on bacterial strains was 22, 21, 18 and 23 mm for *B.subtilis, E.coli, S.aureus,* and *P. aeruginosa,* respectively (see Table 4, Appendix 3).

AP-8G (Aloesaponarin I)

It was a yellow crystal (20 mg) with an R_f 0.5 (in 20% ethyl acetate in n-hexane) and its melting point was 207-209 ℃. UV-VIS (MeOH) λ_{max} observed at 220, 272, 310 and 420 nm**.** IR spectra (KBr) υ in cm^{-1}: C-H (CH_3) at 2951, Ar-C=O (C-9) at 1629, Ar-C=O (C-10) at 1671, C=C at 1580 and C-C at 1460. Two absorption bands for ester C=O at 1739 and C-O at 1311 (Appendix 12).

The ^{1}H NMR has showed highly deshielded signal at δ_H 12.93 at C-1 (δ_C 163.6), a three-proton singlet at δ_H 4.06 (δ_C 53.3) and a shielded carbonyl (δ_C 170.7) is for methyl ester. The up field at δ_H 2.98 (δ_C 22.0) due to the *peri*-effect allowed its placement at C-8 (δ_C 148.1) as in aloesaponarin I. In addition, δ_H 7.30 (*dd, J* =8.2 Hz, H-2), 7.62 (*t, J* =7.6 Hz, H-3) and 7.33 (*dd, J* =7.6 Hz, H-4) of ring A as in aloesaponarin I. In ring C, at δ_H 7.80 showed correlation with C-6, C-7, C-10 and C-10a which is in agreement with its placement at C-5 of trisubstituted (C-6, C-7, C-8) ring C. In this ring, a methyl group (δ_H 2.98 at C-8), a hydroxyl group (at C-6) and methyl ester (at C-7) are evident from the NMR spectra (Table 7)

The ^{13}C NMR spectral data (Table 8) revealed carbonyls (δ_C 189.8, 182.1 and 170.7), four aromatic methine carbons (δ_C 135.9, 124.6, 119.1 and 115.2), two oxygenated aromatic quaternary carbons (δ_C 163.6 and 162.6), six non-oxygenated aromatic quaternary carbons (δ_C148.1, 138.9, 132.8, 125.2, 121.2 and 115.2), a methoxy group (δ_C 53.3) and a methyl carbon (δ_C 22.0).

Based on the above spectral data, compound **AP-8G** was identified as 1, 6-dihydroxy-8-methyl-anthracene-7- methyl ester-9, 10-dione. Its resultant inhibition zone on bacterial strains was 27, 22, 18 and 21 mm for *B.subtilis, E.coli, S.aureus,* and *P. aeruginos*a, respectively (Table 4, Appendix 3).

5. Conclusion and Recommendation

In the present study two compounds **AP-8G** and **AP-11D** were chosen for characterization based on the bioassay guided isolation of compounds from the acetone extract of *Aloe pulcherrium* roots. Which was fractionated and purified by CC on oxalic acid impregnated silica gel G (60–120 mesh size) with increasing gradient of ethyl acetate in n-hexane after being dry adsorbed on the packed column, the progress of isolation was followed by TLC analysis. The antibacterial activities of both the extract and isolated compounds were tested against four bacterial strains *B.subtilis, E.coli, S.aureus and P.aeruginosa* using Agar disc diffusion method; meanwhile, the inhibition zone was measured. Among the pure compounds displayed a significant bacterial activity was submitted to spectroscopic for characterization and there was analyzed. However, based on structural comparison the C-7-methyl ester **AP-8G** (Aloesaponarin I, Figure 11) has displayed slightly better bacterial activities against the four strains than **AP-11D** (Aloesaponarin II, Figure 10).

Therefore, the *in vitro* antibacterial activity tests has revealed that antibacterial activity of the isolated compounds from the roots *Aloe pulcherrium* to be the promising source of bioactive compounds that could be used as lead compounds in search new clinically effective antibacterial compounds.

The characterized compounds were identified as 1, 6-dihydroxy-8-methylanthracene-9, 10-dione, trivial name aloesaponarin II and 1, 6-dihydroxy-8-methyl-anthracene-7-methyl ester-9, 10-dione, trivial name aloesaponarin I, their occurrence in the roots of *Aloe* was confirmed from the biosynthetic pathway. However, these two compounds were not reported so far from the *Aloe pulcherrium* root.

Based on the current finding, further study on the same plant is recommended for further investigation of its bioactive compounds for its antifungal and anti-plasmodium activities.

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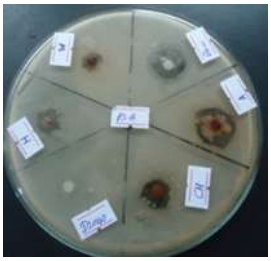
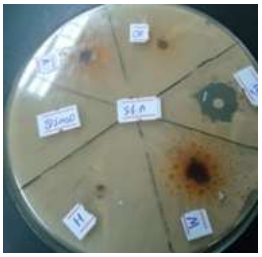
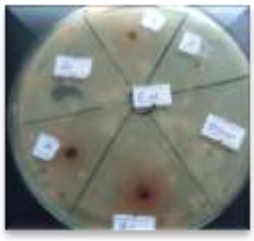
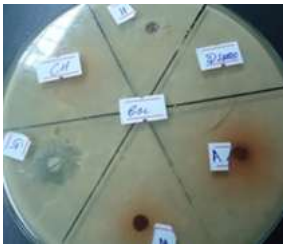
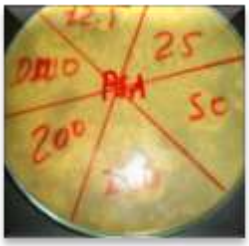
Appendices

Appendix 1. Bioassays Tests of Crude Extracts Zone of Growth Inhibition

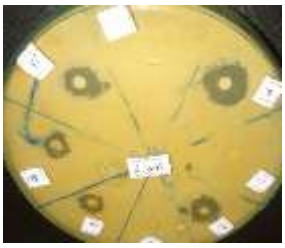
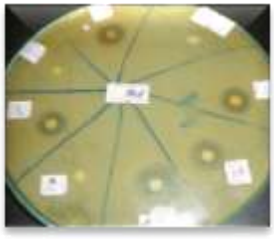
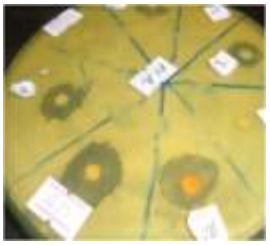
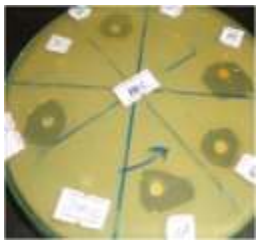
P.aeruginosa *S. aureus* *E. coli* *B. subtilus*

Appendix 2. Antibacterial activities of different concentrations of acetone extracts



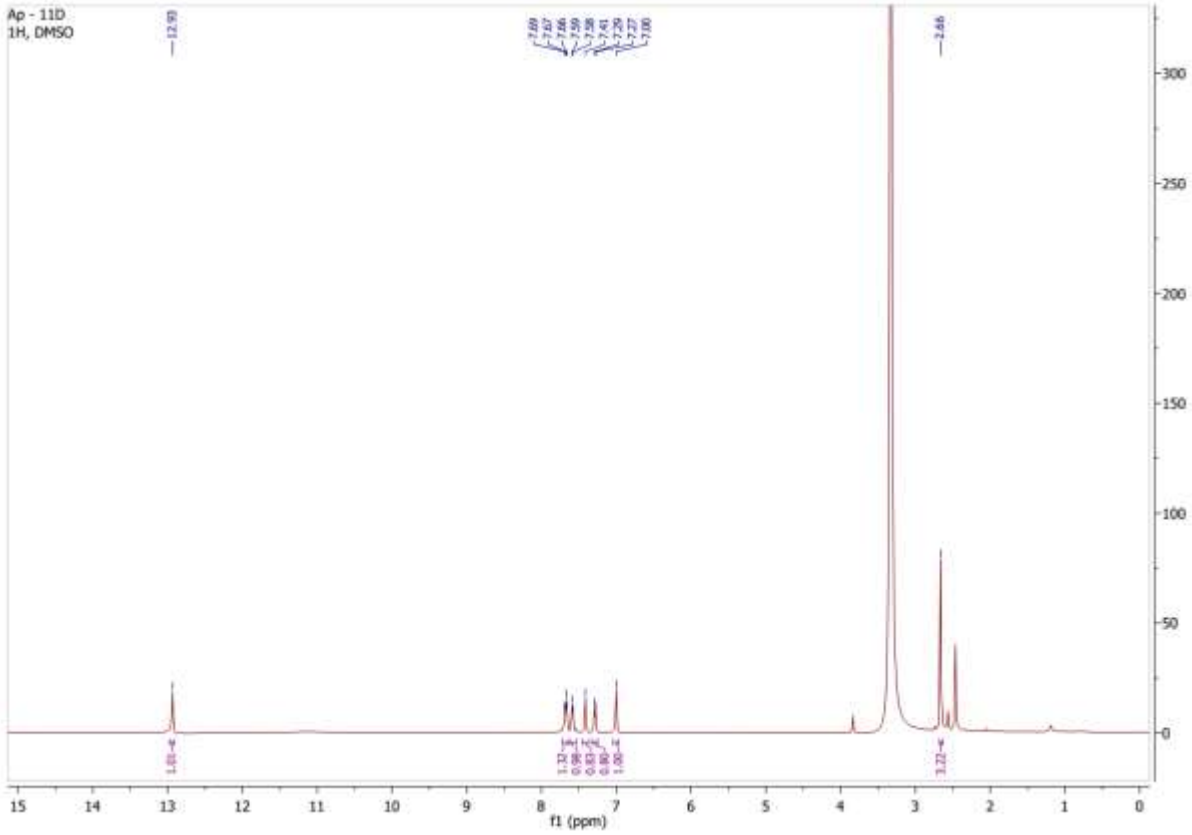
P.aeruginosa *S. aureus* *E.coli* *B.subtilus*

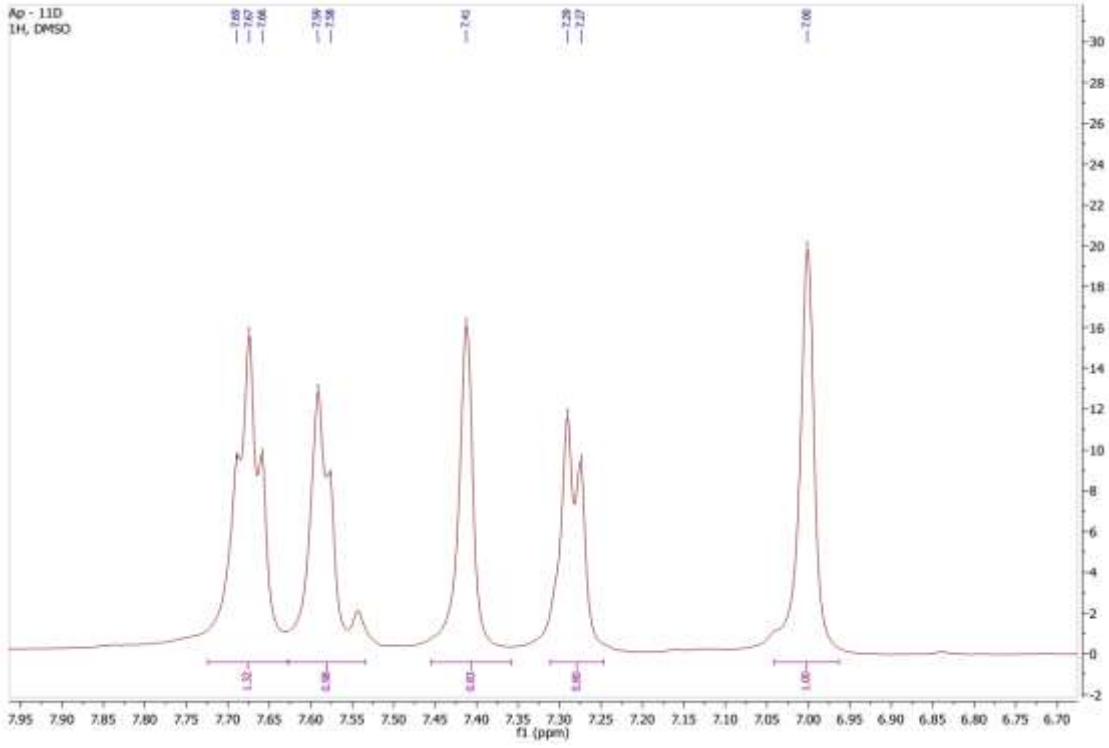
Appendix 3. Bioassays Tests of Isolated Compounds' Zone of Growth Inhibition

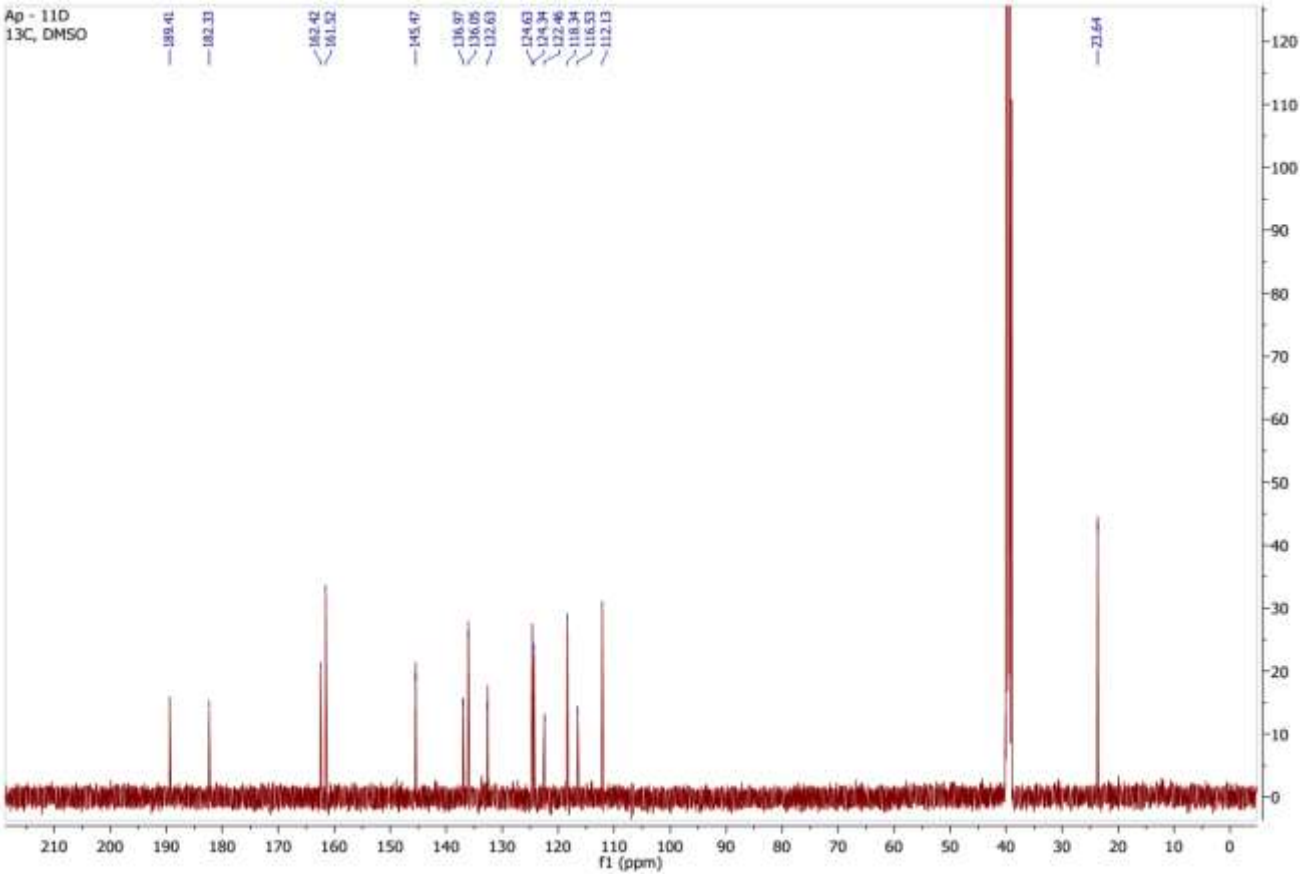
E.coli *S. aureus* *P.aeruginosa* *B.subtilus*

Appendix 4. ^{1}H-NMR Spectrum of **AP-11D** in DMSO

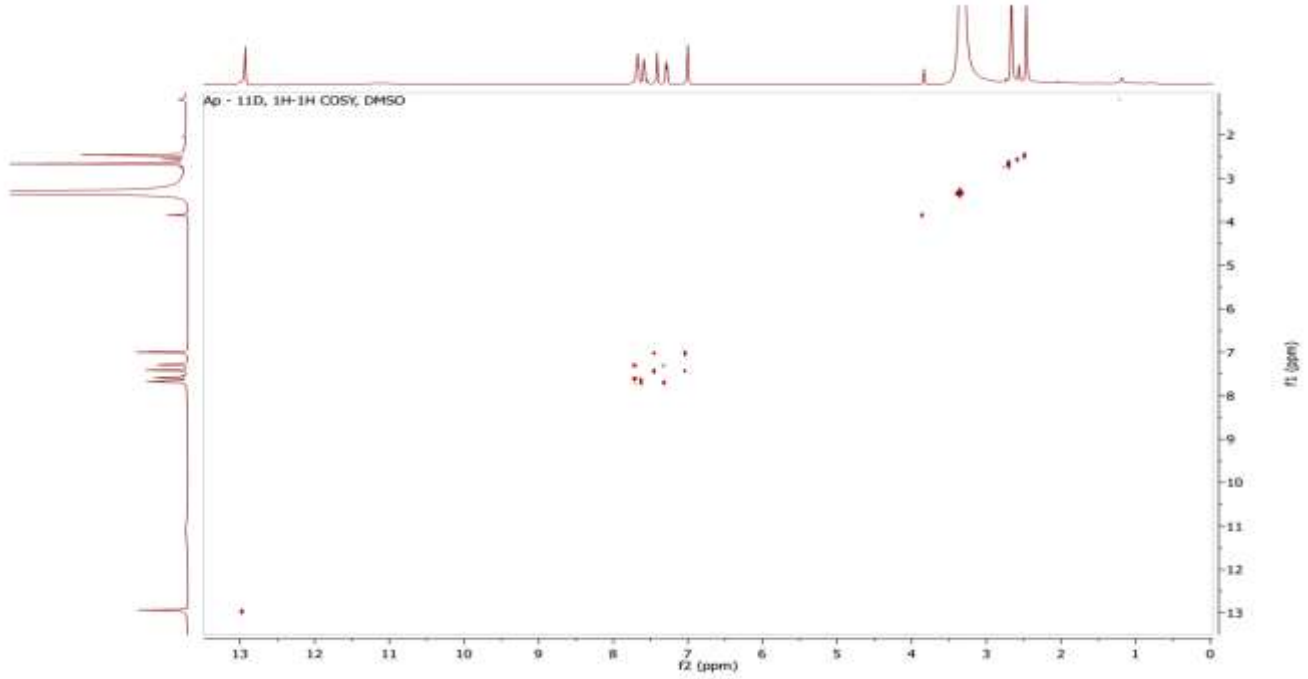




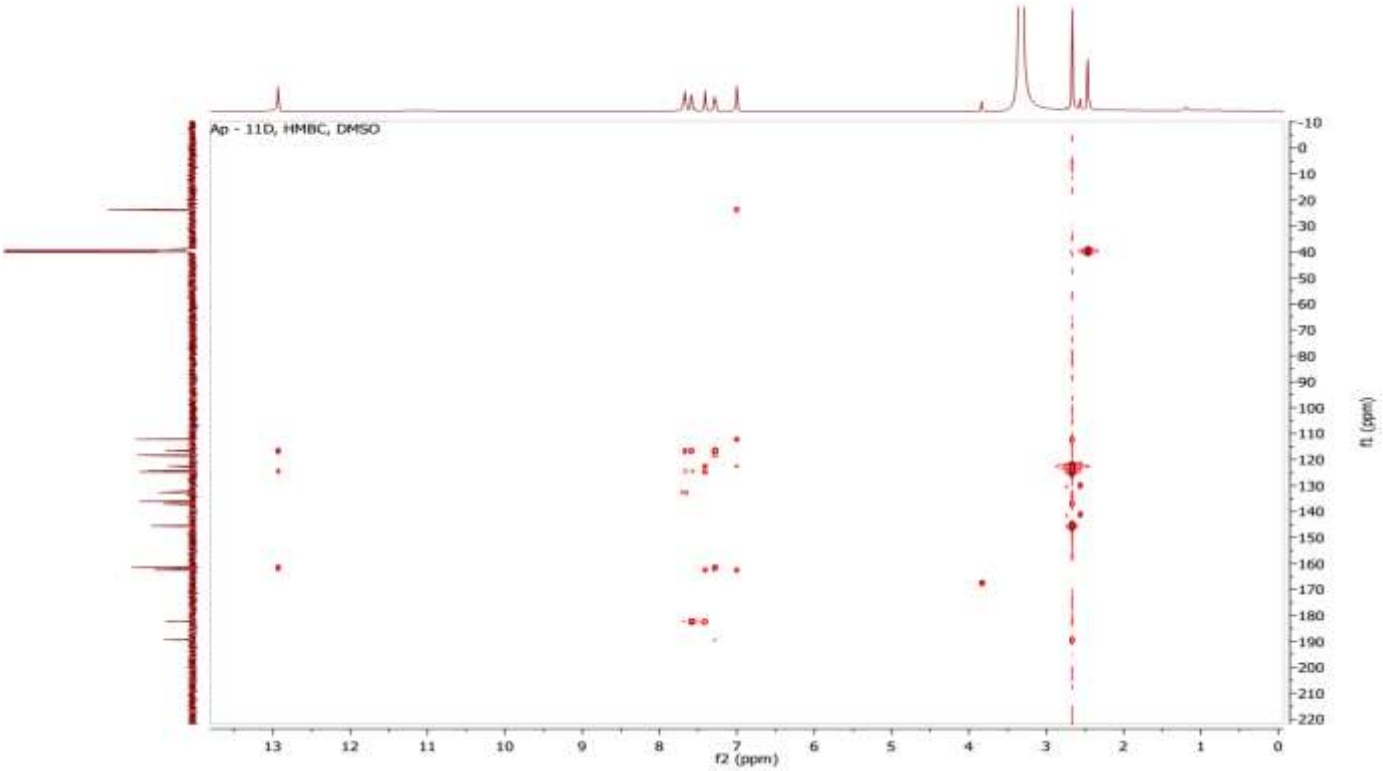
Appendix 5. ^{13}C-NMR Spectrum of **AP-11D** in DMSO



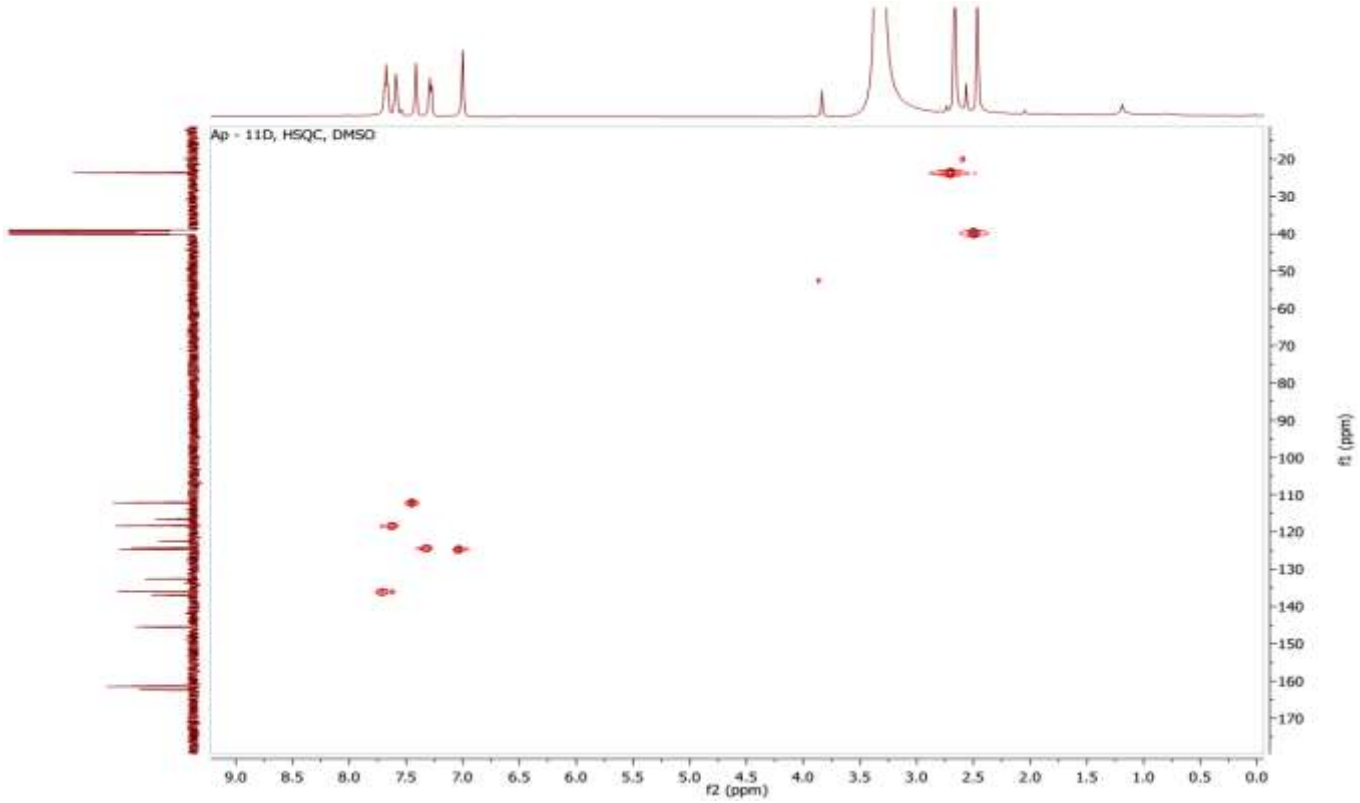
Appendix 6. COSY Spectrum of **AP-11D** in DMSO



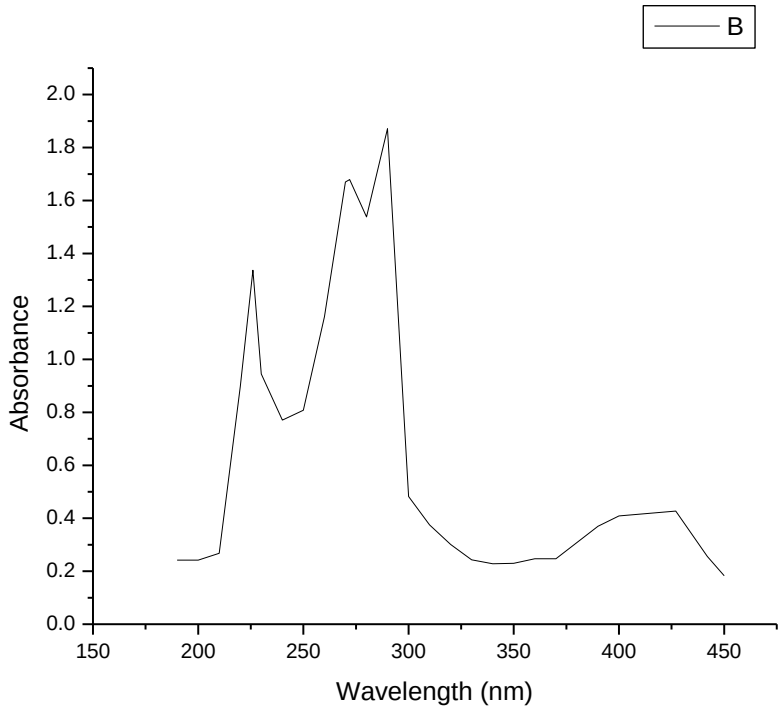
Appendix 7. HMBC Spectrum of **AP-11D** in DMSO



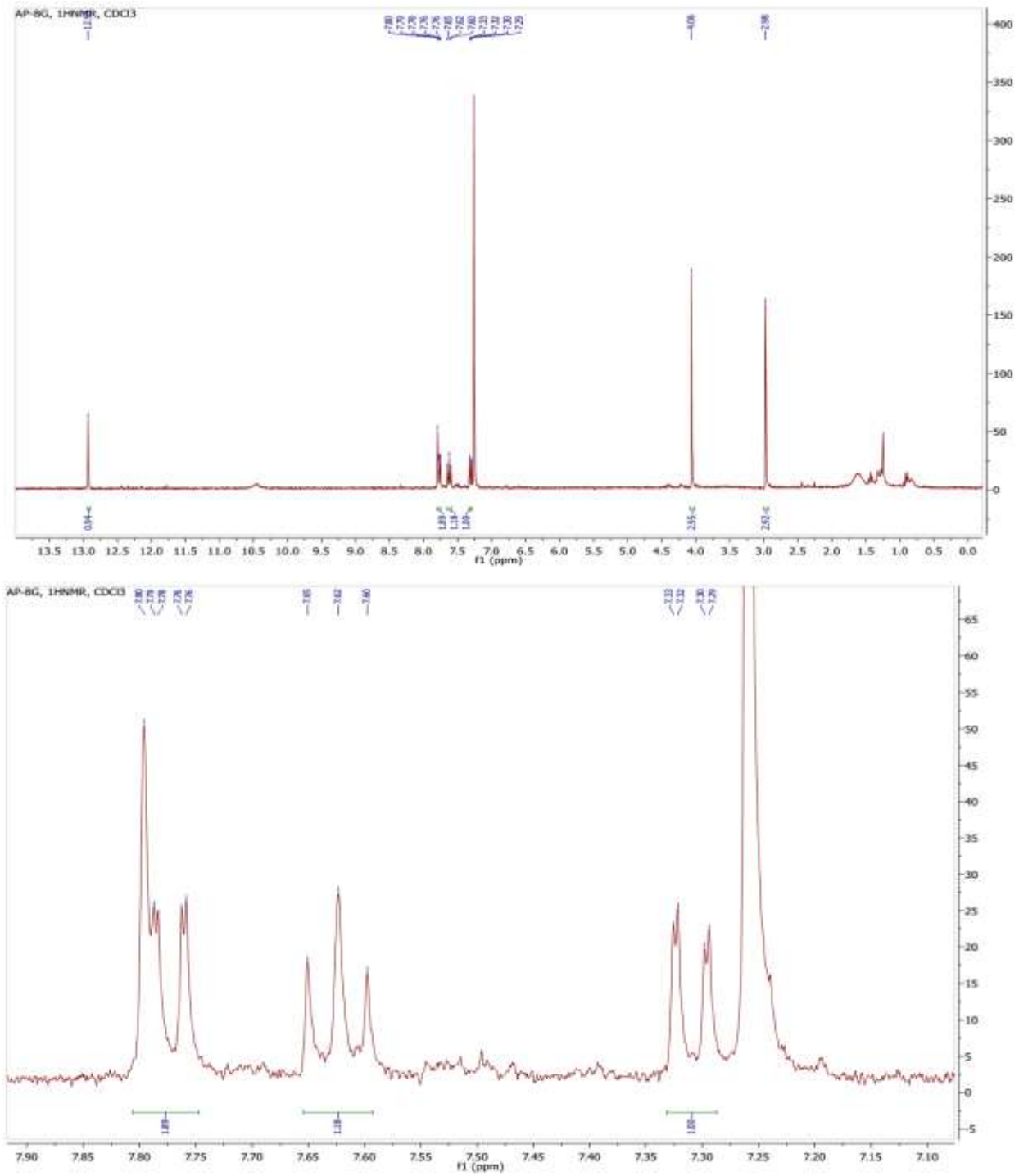
Appendix 8. HSQC Spectrum of **AP-11D** in DMSO



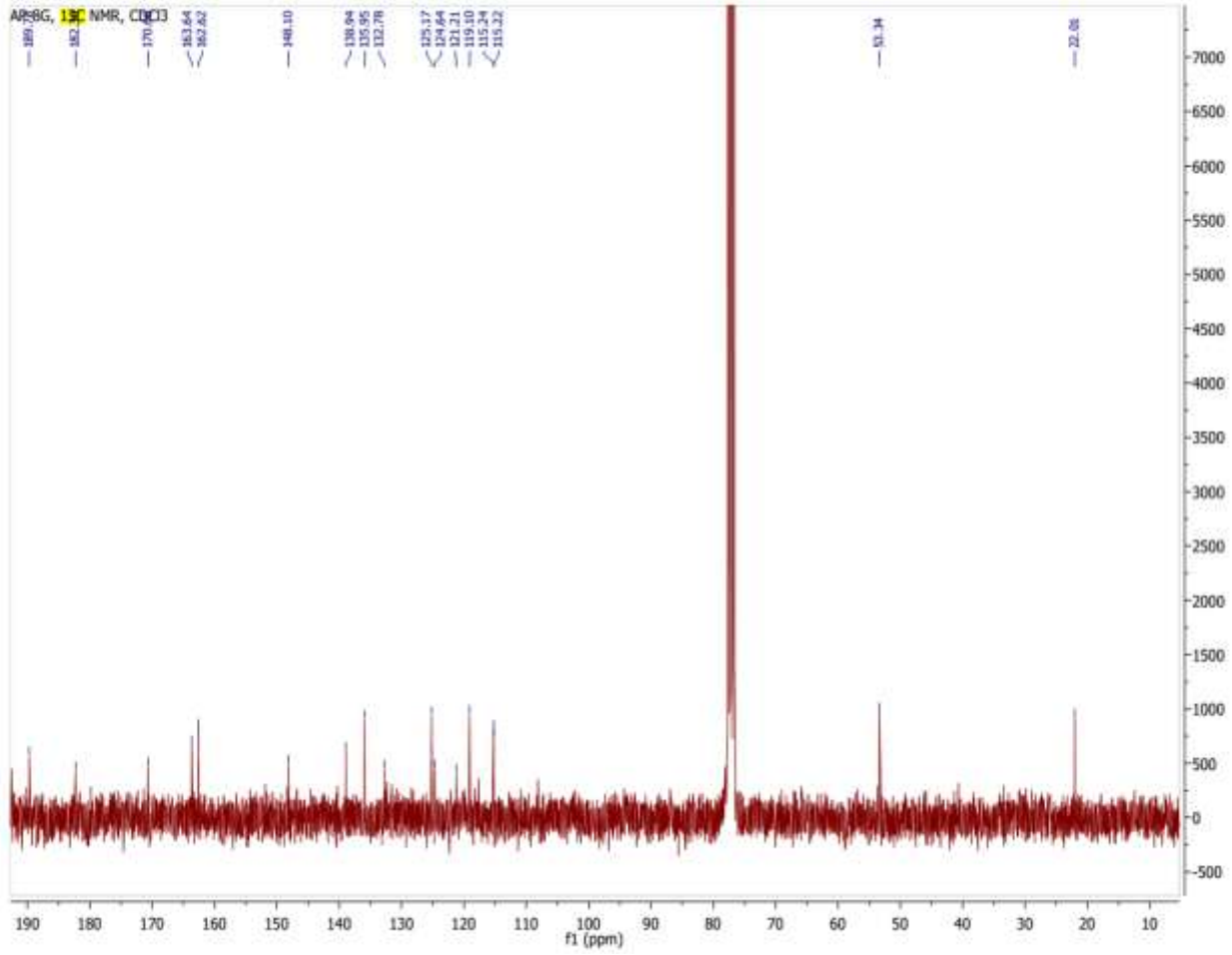
Appendix 9. UV-VIS Spectra of **AP-11D**



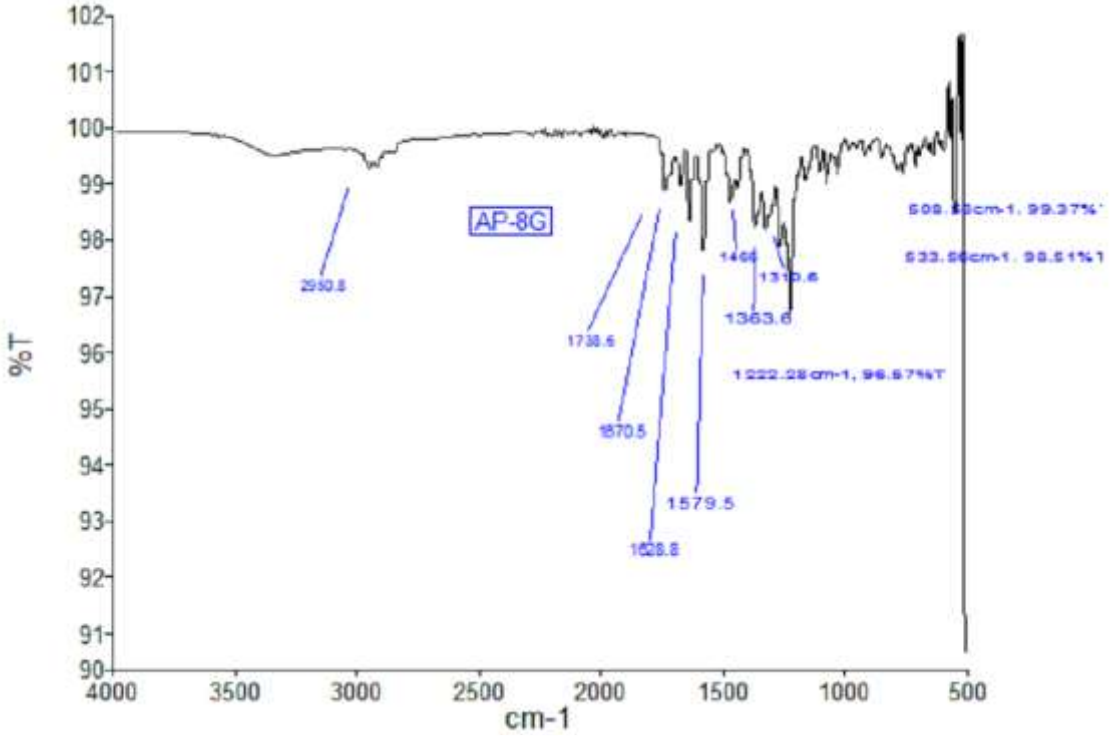
Appendix 10. ^{1}H-NMR Spectrum of AP-8G in $CDCl_3$



Appendix 11. ^{13}C-NMR Spectrum of AP-8G in $CDCl_3$



Appendix 12. .IR Spectra of Compound AP-8G



Appendix 13. UV-VIS Spectra of AP-8G