PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITIES OF ROOTS EXTRACTS OF *ALOE DEBRANA*

BY

BEKELE LEGESSE

A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SEPTEMBER, 2017

ADAMA,ETHIOPIA

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MAJOR ADVISOR: MILKYAS ENDALE (PH.D)

CO-ADVISOR: YADESSA MELAKU (PH.D)

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Approval Sheet of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Bekele Legesse have read and evaluated his thesis entitled "Phytochemical Investigation and Antibacterial Activities of Roots Extracts of *Aloe debrana*" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of master's in chemistry

Milkyas Endale(Ph. D) ሚልኪያስ እንዳሌ አኒሳ Milkyas Endale Anlisa (Ph.D)		02/10/2017
Major Advisor	Signature	Date
Yadessa Melaku(Ph. D),		02/10/2017
Co-Advisor	Signature	Date
Enyew Amare (Pho)		25/09/2017
Chairperson	Signature	Date
H/Michael Tesso (Ph.D)		02/10/2017
Internal Examiner	Signature	Date
Daniel Bisrat (PhD)		25/09/2017
External Examiner	Signature	Date

Advisors approval sheet

To: Applied Chemistry Program

Subject: Thesis Submission

This is to certify that the thesis entitled “Phytochemical Investigation and Antibacterial Activities of Roots Extracts of *Aloe debrana"* submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry has been carried out by Bekele Legesse (Id. No GSS/0208/05) at Applied Chemistry Program under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis for examination.

Milkyas Endale (PhD)	_____	_____
Name of major Advisor	Signature	Date
Yadessa Melaku (PhD)	_____	_____
Name of co-Advisor	Signature	Date

DECLARATION

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Bekele Legesse

Signature: _____

This MSc Thesis has been submitted for examination with our approval as thesis Advisors

Name: Milkyas Endale (PhD)

Signature: _____

Date of submission:_____

Name: Yadessa Melaku(PhD)

Signature: _____

Date of submission:_____

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LIST OF ACRONYMS

BC	Before Christ
WHO	World Health Organization
CD-RW	Compacted Disc Rewritable
R_f	Retention factor
TLC	Thin Layer Chromatography
UV-Vis	Ultra Violet visible
NMR	Nuclear Magnetic Resonance
IR	Infrared
1D-NMR	One dimensional nuclear magnetic resonance
CC	Column Chromatography
MoH	Ministry of Health
M_r	Relative molecular weight
AAU	Addis Ababa university
DEPT	Distortion less enhancement by polarization Transfer
PPm	Parts per million
TMS	Tetramethyl silane
MHA	Muller Hinton Agar

ABSTRACT

The genus Aloe (Asphodelaceae), with nearly 600 species confined mainly to Africa, has over the years proved to be one of the most important sources of biologically active compounds. There *are about 46 Aloe species in Ethiopia , of which 24 are endemic. In Ethiopian traditional medicine, the leaf latex of Aloe debrana is used for the treatment of several diseases including malaria and infectious diseases. This study focused on extraction, isolation and characterization of the chemical constituents from the roots of Aloe debrana. The roots were extracted by dissolving 500g grinded and powdered root sample in the solvent CH_2Cl_2/CH_3OH (1:1) and CH_3OH yielded 16.51g (3.3%) and 9.98g (2.0%) successively, followed by separation using repeated silica gel column chromatography which afforded two compounds. They were characterized as* isomer of aloe saponarin I (1,8-dihydroxy-3-methyl-4-anthraquinoic methyl methanoate (AD-18) and 2-ethyl-2,3-dihydro-7-hydroxychromen-4-one (AD-20). *The structures of these compounds were elucidated by using spectroscopic analysis (UV-Vis, IR and 1D- NMR). Phytochemical screening test revealed the presence of anthraquinones, saponins, and steroids whereas alkaloids, flavonoids, protiens and triterpenoides were absent. The antibacterial activity of crude extract (0.5mg/ml) as well as one of the isolated compound, AD-18 (0.5mg/ml) were conducted using disk diffusion method using gentamycine and methanol as positive and negative controls respectively on selected strains of microorganisms which were Staphylococcus aureus, Bacillus subtilis, Escherchia coli, and Proteus mirabilis. Accordingly, 11, 7 ,15 and 6 mm zone of inhibition were found to be the cut off for crude extract, AD-18 ,gentamycine (0.05mg/ml) and methanol respectively, against S. aureus. Whereas 6, 9 , 15 and 6 mm were found to be cut off for crude extract, AD-18, gentamycine and methanol respectively, against E.coli. The present study started with only 500g of the plant material and could not achieve to isolated and identify some of the minor compounds as supported by the literature reports, literature reports suggest for the genus as rich source of anthraquinones, Hence, future phytochemical work is recommended starting with higher amount of the plant material and also needs further optimization of extraction solvents for better extraction yeild.*

Key Words:-Aloe debrana , Aloe saponarin I , 2-ethyl-2,3-dihydro-7-hydroxychromen-4-one , anthraquinone and chromen.

1. INTRODUCTION

1.1. Background of the Study

Medicinal plants have been a major source of cure for human diseases since time immemorial. It is no wonder that according to the recent estimates by the WHO, more than 3.5 billion peoples in the world are dependent on traditional medicines for the treatment of various ailments, from this 80% of the population are of developing countries [1]. Plants have long history of being used for a wide variety of purposes including food, clothing, shelter, tools, weapons, and therapeutic agents. Before the advances of modern medicine, civilizations confronted with illness discovered useful therapeutic agents from within plant and fungi kingdoms. Knowledge of these medicinal preparations and their toxic potential was passed down through generations by oral tradition and sometimes recorded in herbal literature. The earliest record outlining man's usage of plant medications are more than 6000 years old. Sumerians clay tables (400 BC) detailed 1000 medicinal plants and the Pun-tsao, a Chinese record of thousands of herbal cures dates to 250 BC. The Hippocratic Corpus by Greek Hippocrates was recorded in the late fifth century of BC and the Roman writings of De Material Medica by Dioscorides documented more than 600 plant species with medicinal values [2,3]. Medicinal herbs are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals [4].

The use of plant continues to play essential roles in traditional medicine for the treatment or management of various human diseases, especially in rural Africa where infectious diseases are endemic due to poverty and poor sanitations [5]. Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs such as Anxiety, Agitation, Heart palpitation, Sweating, Restlessness , Inability to speak and Euphoria, the Western population is looking for natural remedies, which are safe and effective. It is documented that most of the World's population has taken in traditional medicine, particularly plant drug for the primary health care **[**5].

According to WHO, medicinal plants would be the best source to obtain variety of drugs [6]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body by interacting with molecular targets affecting the cells and tissues. In this respect the plant secondary products may exert their action by resembling endogenous

metabolites, ligands, hormones, signal transduction molecules ,or neurotransmitter and thus have beneficial medicinal effect on humans due to similarities in their potential target sites (e.g .central nervous system and endocrine system)[7].The development of structural similarity between plant secondary products and the endogenous substances of other organisms could be termed as "evolutionary molecular modeling."[8].These bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols. The bio-active phyto-compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [6].

A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*. Botanical medicines or phyto-medicines refer to the use of seeds, berries, leaves, bark, root or flowers of any plant for medicinal purposes by significant number of people. Knowledge of the chemical constituents of plants is desirable because such information will have value for synthesis of complex chemical substances [9,10]. Phytochemistry or natural product chemistry research is the backbone of herbal industry and directly or indirectly responsible for both failure and success of herbal drugs.. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being [11].

Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs. WHO has suggested that medicinal plants would be the best source to obtain variety of drugs, accordingly about 28,187plant species are currently recorded as being of medicinal use[12]. Since the use of medicinal plant based drugs contain least or no side effects and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs [13]. Since the earliest days of recorded history, man has made use of *Aloe* plants [14, 15, 16] . There are several references to *Aloes* in the Bible [17] but since it was then used as a perfume or incense, identification with the modern species of *Aloe* (family *Liliaceae*), which are not known for their aromatic properties, is doubtful[18].

Aloe debrana is one of these plants which are used as sources of drugs. It is widespread and locally abundant at altitudes between 2,400 and 2,700 m.No threats are known to cause a decline in the population and the population is thought to be stable [19]. According to the

present thesis work it can also grows and abundantly found below 2000m altitude . It is found in Ethiopia, mainly in Shewa, Gojam and Welo and rarely found in other part of the country[20].It is categorized under succulent plants, in *Aloeaceae* (*Aloe*) family. *Aloe debrana* it is an endemic plant of Ethiopia(Africa) which is ever green and needs low water to irrigate. It has red colored flower in which its bloom time is winter/spring. The plant has 2-3 feet height and 3-4 feet width which it is not affected by full sun exposure but can dry in summer.

1.2. Justification of the Study

Plants have been known and used since time immemorial to treat most of the diseases affecting human kind and animals, therefore scientists have found them to be a better choice for bioactive compounds. The introduction of synthetic drugs, however, changed the trend and attracted many to turn to use them on the expense of botanical drugs; a trend which according to researchers is changing at the moment and many people are using medicinal herbs. Of these, the species *Aloe debrana* is known in its traditional use to treat various disease , that is why this study is required to isolate phytochmicals from it. In this thesis work, a comprehensive phytochemical extraction and isolation was carried out on the roots of *Aloe debrana* including spectroscopic analysis, screening and biological activities evaluation against selected strains of microorganisms.

1.3. Statement of the Problem

There is also much biomedical knowledge on causation, prevention, treatment and control of various infectious diseases, nevertheless, it remains a public health problem particularly because of the rapid development of resistance to the available first line drugs. All over the world scientific research is getting momentum to evaluate the pharmacological activities and medicinal properties of different plant species. According to different reports, the secondary metabolites which are found in these plants are used to cure and protect various diseases of human and animals. *Aloe debrana* species is a source of some compounds and exhibits several biological activities. Thus, this species attracts the attention of researchers to isolate and characterize the bioactive compounds. *Aloe debrana's* leaf latex is traditionally used around Bishoftu (Debre zeit) by the farmers to cure the wound of the nape of their oxen made during plough and peoples traditionally use to cleans the eyes that injured accidentally and covered by blood and not removed after medical treatment. As it is believed, the root also have some important compounds which are not extracted yet satisfactorily.Hence this thesis is intended to fill the gap in extracting phytochemicals from the root of *Aloe debrana.*

1.4. Significance of the Study

The antimicrobial activities of most traditional medicinal plants used within various parts of the country are not established on scientific basis. Some herbal remedies may be safe and effective for the treatment of microbial infection and some may not be. Nevertheless, better evidence from randomized clinical tests and cytoxicity assay is required before herbal remedies can be recommended for use. In order to prioritize remedies, there is a need for preliminary survey based on ethnobotanical use of the plants. Considering the fact that the genus *Aloe* is used traditionally to treat various infections disease, this research is believed to provide a scientific basis for the traditional use of the plant on the basis of the identification of the chemical constituents and screening against selected strains of microorganisms. Furthermore, the information obtained from the results of this work will serve as an input for the researchers who are interested to conduct further research on the plant.

1.5. Objectives of the Study

1.5.1. General Objective of the Study

The main objective of this study is extraction , isolation and structural elucidation of some of the major constituents of *Aloe debrana* root and evaluation of their antibacterial activities.

1.5.2. Specific Objectives of the Study

1. To extract roots of *Aloe debrana* using CH_2Cl_2/ MeOH (1:1) and MeOH successively.

2. To conduct phytochemical screening test of CH_2Cl_2/ MeOH (1:1) and MeOH crude extracts of the roots of *Aloe debrana* using standard phytochemical screening protocols.

3. To isolate the secondary metabolites present in the roots of *Aloe debrana* using colum chromatography.

4. To characterize the isolated compounds using a range of spectroscopic techniques such as NMR, IR and UV-Vis and elucidate their structural formula.

5. To evaluate antibacterial activities of CH_2Cl_2/ MeOH (1:1) and MeOH crude extract and isolated compounds from the roots extracts of *Aloe debrana* and compare with the standard drugs.

2. LITERATURE REVIEW

2.1. Botanical Description and Distribution of the Genus *Aloe*

The name *Aloe* was derived from the aerabic "alloeh" meaning "bitter" because of bitter liquid found in the leaves. It is also known as ' lily of the desert ' the plant of immortality and the medicinal plant with qualities to serve as alternate medicine [20]. In the world there are approximately about 600 species of the genus *Aloe* most most of them are confined to Africa [21] .There are about 46 Aloe species found in Ethiopia , of which 24 are endemic, many of these are used in traditional healing [22]. "*Aloes*" is the generic name for the solid residue obtained by boiling and cooling the latex of *Aloe ferox* Miller, its hybrids, and *A. vera* (L.) Burm.f. (also known as *A. barbadensis* Miller) [23].

The sub-family Alooideae (Aloaceae) of the family Asphodelaceae [24]. Asphodelaceae is one of the six smaller families found in the large and heterogeneous family Liliaceae, which consists of two subfamilies, namely, Asphodeloideae and Alooideae [25]. *Aloes* are native to sub-Saharan Africa, the Saudi Arabian Peninsula, and to many islands of the western Indian Ocean, including Madagascar. It has been suggested that the center of diversity for this genus is the highland of southeast Africa [26]. In South Africa about 160 species of *Aloe* are found[27]. Some have been introduced to Asia (*A. chinensis* Bak), Barbados Islands in Central America (*A.barbadensis* Miller) and Europe (*A.arborescens* Miller)[23].

Aloes are adapted to highly disturbed areas and areas with extreme environmental conditions (e.g. arid habitats). They are found flourishing on nutrient deficient, rocky or gravelly soils [28]. Some of the most important adaptations to survive in water stress environments include succulence and a waxy coating on the surface of the leaves [29]. These unique adaptations and others enable them to be dominant and important group in such environments by providing shelter, nectar food and moisture, especially to the avifauna [30]. Members of the genus *Aloe* have been known for their current and potential use in medicine, commerce and horticulture. *Aloe* species have been used in folk medicine, e.g. for treatment of constipation, burns and dermatitis [31]. Gel exudates from leaves of *A. lateritia* has been used in some communities in Ethiopia for treatment of eye ailments [32]. Some other species is playing great role in ecological restoration in Kenya, e.g. *A. secundiflora* that has been used in fencing, hedging and in soil conservation efforts [28].The flora of Ethiopia and Eretria possess 46 species of *Aloe*, out of which 40 are reported to be endemic. Only five species:

A. laterita, *A. macrocarpa*, *A. rivae*, *A. secundifloraand A. vituensis*, are wide spread extending to East and West Africa [33]. *Aloe* species inhabiting in the flora area are highly threatened due to agricultural expansion into marginal lands and habitat destruction due to new development schemes near urban and regional centers [34]. Other species are over collected by succulent enthusiasts and local community for cultivation and their use in traditional medicine. The two species: *A. debrana* and *A. trichosanta* have been collected for their bactericidal property in the suck manufacturing industry [35]. It is obvious that base line data on the biological and ecological attributes of species such as the size and life stage structures, reproductive success; and also threats to its populations and habitats are crucial for conservation decision making. Nevertheless, very few studies have been conducted in these lines on the aloes of Ethiopia, (e.g. reproductive biology of two endemic Aloe species [36]; population structure and dynamics of *Aloe debrana* and *Aloe Pulcherrim* [37].

Aloe debrana is a stemless *Aloe* that suckers from the base with dense rosettes of 20 inch long lanceolate medium green colored leaves that are slightly recurved and have reddish-brown teeth along the leaf margin. In late winter to early spring appear the well branched inflorescences (often with secondary branches) that rise up to 3 feet above the foliage holding dense tightly held buds in 4 inch long capitate clusters that open to display 1 inch long scarlet to rose colored flowers that are yellow at the flared petal tips. Plant in full sun to light shade (flowers best in full sun) in a well-drained soil and irrigate infrequently. Hardiness on this aloe is not well documented. The foliage is a bit plain and not that distinctive but the flowers are stunning. It is widespread and locally abundant in the mountainous areas of central to northern Ethiopia mainly in Shewa, Gojam and Welo regions and rarely in the other area of the country. The specific epithet is in reference to the type locality at Debre Berhan that was formerly spelled Debra-Berhan. In the Amharic language Debre Berhan means the 'place of the light [38].

Figure 1: Picture of *Aloe debrana* taken by Bekele Legesse, November, 2016, Godino

2.2. Ethno pharmacological Uses of the Genus Aloe

Although the use of *Aloe* was recorded by the Egyptians, Assyrians, and Mediterranean peoples dates back from 1500 B.C., the Greek physician Dioscorides was the first to describe the use of *Aloe* to treat mouth infections, sores, and wounds and as a purgative. The genus *Aloe* has been used in India as a cathartic, stomachic, emmenagogue, and anthelmintic and more recently in England and in the USA and Mexico for the treatment of diseases of the immune system [39]. Today Aloe is still a popular folk medicine among peoples of Indian, Chinese, and Mexican origin. Recent surveys have indicated that it is one of the three most used botanicals of middle-aged Mexican women [40].

Aloe debrana is one of the endemic species which grows mostly in Showa, central part of Ethiopia and rarely found in other part of the country [41]. In Ethiopian traditional medicine, the leaf latex of *Aloe debrana* is used as:-

- a laxative in order to clean the digestive system from parasites,
- antidiabetic,
- a wound healing agent, for cleansing the blood,
- antimalarial agent, against nausea and gastric problem [42].

2.3. Biological Activities of the Genus Aloe

The laxative effect of *Aloe* latex is believed to take place through water accumulation in the intestine via active Na^+ transport or by water secretion due to a prostaglandin-dependent mechanism [43]. The genus *Aloe* also encourages wound contraction caused by increased collagen activity [43]. A glycoprotein fraction from *A. vera* was found to accelerate wound healing in a monolayer of human keratinocytes and increase expression of proliferation markers at the immune histochemical level [44]. *A. vera* inhibits inflammation in a dose-response manner and improves wound healing in diabetic mice [45].

Extracts of Aloe gum increases glucose tolerance in both normal and diabetic rats [46], and *A. vera* sap taken for 4-14 weeks has shown a significant hypoglycaemic effect both clinically and experimentally [47]. *A. vera* leaf pulp and gel extracts were ineffective in lowering the blood sugar level of nondibetic rats, but the leaf pulp extract showed hypoglycaemic activity in type I and II diabetic rats [48]. A significant decrease in blood glucose levels after oral administration of the ethanol extract of *A. vera* gel in streptozotocin-induced diabetic rats was ascribed to the antioxidant effect of the extract [49**].**

In vitro, *Aloe-emodin* inhibits the growth of Merkel carcinoma cells [50], liver cancer cell lines, and human promyelocytic leukaemia HL-60 cells [51], has antineuroectodermal tumor activity; and induces apoptosis in lung carcinoma cell lines. Lectin like substances from the leaves of *A. vera have* been shown to promote the growth of normal human cells in culture but inhibit tumor cell growth [52]. However, in contrast, aloe in has been shown to stimulate the proliferation of cultured hepatoma SK-Hep1 cells [53].

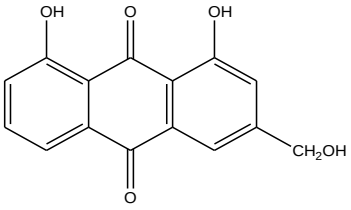
Aloe-emodin has the ability to modulate survival of mouse L929 fibrosarcoma and rat C_6 astrocytoma cells through interference with the activation of inducible nitric oxide synthase and subsequent production of tumoricidal free radical nitric oxide. *Aloe-emodin* rescued interferon-γinterleukin-1-stimulated L929 cells from nitric oxide-dependent killing by reducing their auto toxic nitric oxide release [54].

2.4. Chemical Constituents of the Genus *Aloe*

Chemical investigations on *Aloe* species have revealed the presence of anthraquinones, pre-anthraquinones, naphthoquinones, anthrones, alkaloids, steroids, chromones, pyrones and flavonoides. Some of the compounds isolated from the genus *Aloe* are summarized here below.

2.4.1. Anthraquinones

Anthraquinones consists of a tricyclic aromatic ring with 9, 10-quinone skeleton and several substituent groups at different position. Several free anthraquinones occur in roots and leaves of *Aloe* species. Aloe-emodin **(1)** is a typical leaf constituent and is widespread in the genus. The anthraquinones in leaves may be present as O glycosides as is the case in compounds **(2)** and**(3).** Aloesaponarin I (**4**) and aloesaponarin II (**5**) were isolated first from roots of *A. saponaria* [55]. Anthraquinones of the chrysophanol-type are known to occur both in leaves and roots. Van Wyk investigated 172 root samples of aloe species. It was determined that the compounds isolated from the roots were completely different compared to compounds isolated from the leaves and the anthraquinones and pre-anthraquinones present in the root has chemotaxonomic significance in the genus *Aloe*. These compounds appeared to have been derived through two parallel routes of the polypeptide pathway leading to 1,8-dihydroxy and 1-methyl-8-hydroxy-anthraquinones.The phyotochemistry's of the roots are important to mention due to its chemotaxonomic *Aloe saponarin* [56]. The following are Chemical structures of some of the compounds isolated from the roots of *Aloe ferox* **(6)** and **(7).** In 1956 paper chromatography showed the presence of chrysophanic acid (chrysophanol) in *Aloe ferox* (**8**) [57]. *Aloe vera* is considered to be the most biologically active of the *Aloe* species; astonishingly, more than 75 potentially active components have been identified in the plant, including vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignin, saponins and salicylic acids. Of these potentially active components aloesin **(9)** [58] is commonly known.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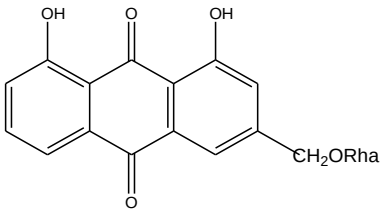
OH
CH2OH
O

(1)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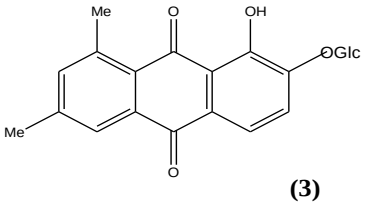
O
OH
CH2ORha
O

(2)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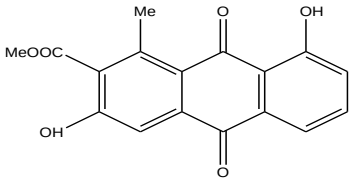
O
OH
OGlc
Me
O

(3)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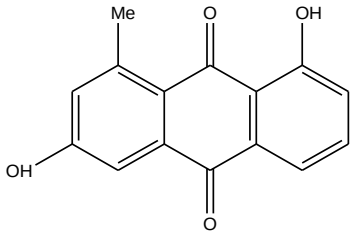
O
OH
MeOOC
OH
O

(4)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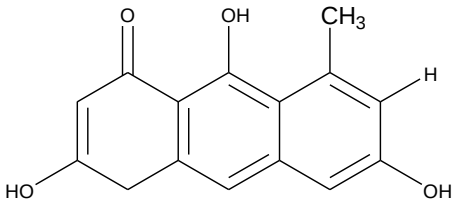
O
OH
OH
O

(5)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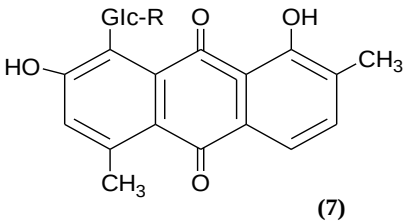
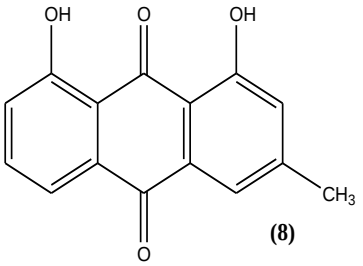
CH3
H
HO
OH

(6)


Glc-R
O
OH
HO
CH3
CH3
O

(7)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CH3
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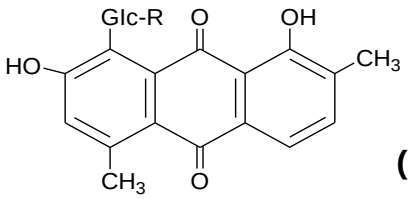
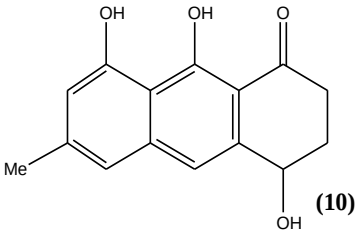
(8)



(9)

2.4.2. Pre-anthraquinones

Biosynthetically pre-anthraquinones are considered to be precursors of anthraquinones. Pre-anthraquinones mainly occur in the roots and subterranean stem of the genus *Aloe*. Some of the isolated pre-anthraquinones are Aloesaponol **III (10) ,** Aloesaponol **IV(11)** , Aloesaponol IV-4-O-glucoside**(12)** and Aloesaponol IV, O-demethyl-4-O-glucosidide**(13)** occur widely in the roots of *Aloe* [59 ,60].



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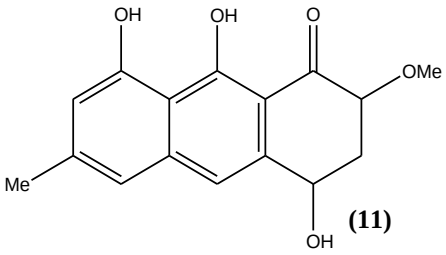

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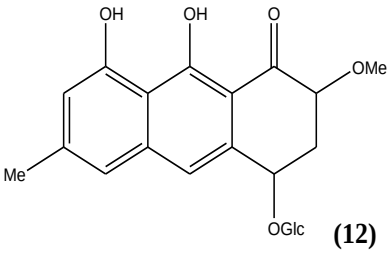

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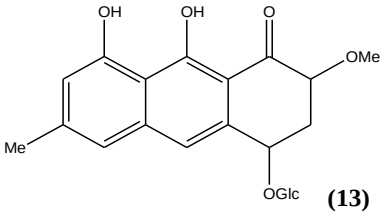
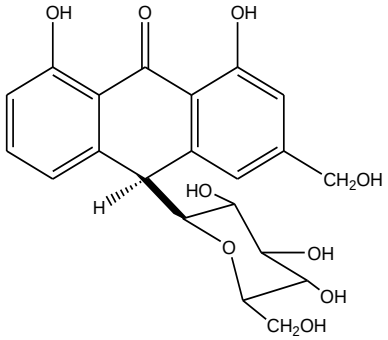


(13)

2.4.3. Anthrones

They are known constituents of the leaves of *Aloe* and possess bitter and purgative properties [61,62]. The taxonomic distribution of aloin A **(14)** and aloin B (**15**) with microdontin A **(16)** and microdontin B **(17)** has been studied and showed anthrones to be the most widely found class of compound in *Aloe* species [63,64].



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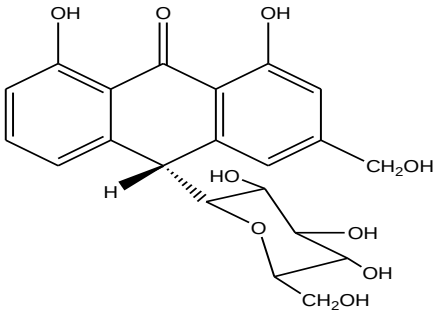

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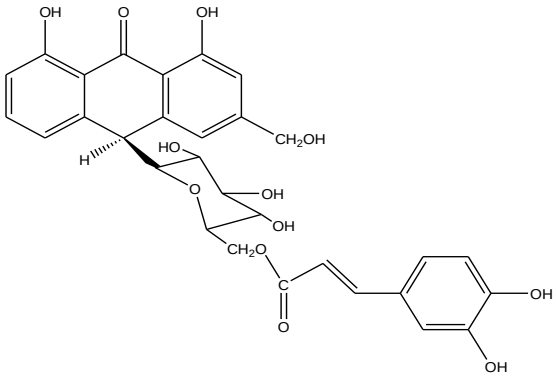

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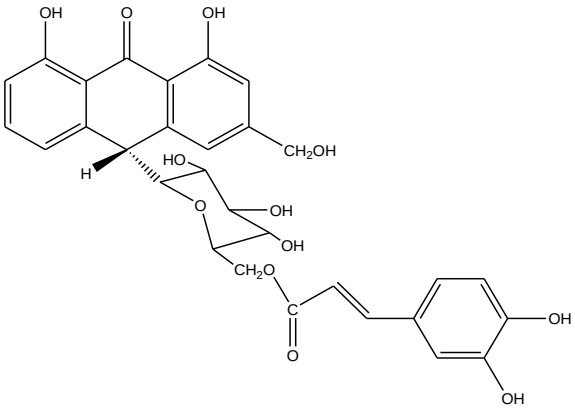
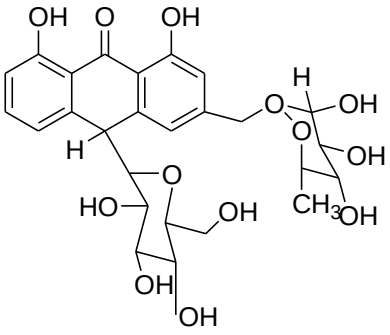
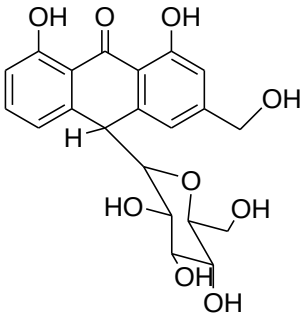


(17)

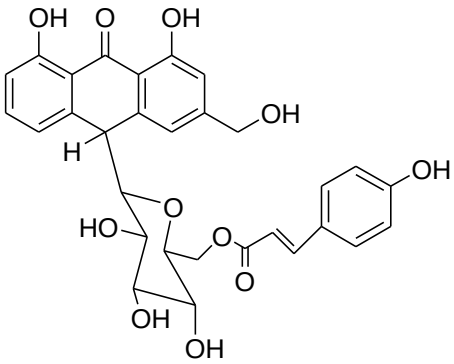
The latex of *Aloe calidophila* dissolved in methanol and directly applied to preparative thin layer chromatography plates over silica gel using a mixture of chloroform and methanol in a ratio of 4:1 as a solvent system to afford Aloinoside **(18)** Aloin **(19)** and microdontin **(20)** [65,66].



(18)

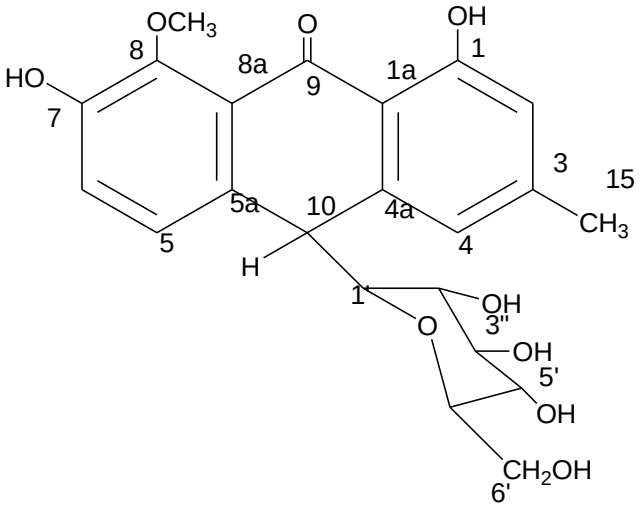


(19)



(20)

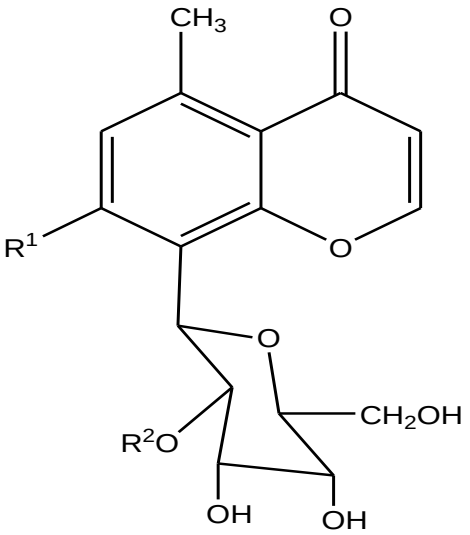
The latex *Aloe citrina* dissolved in methanol and directly applied to preparative thin layer chromatography plates over silica gel using a mixture of chloroform and methanol in a ratio of 4:1 as a solvent system to afford homonataloin A/B **(21)** [67].

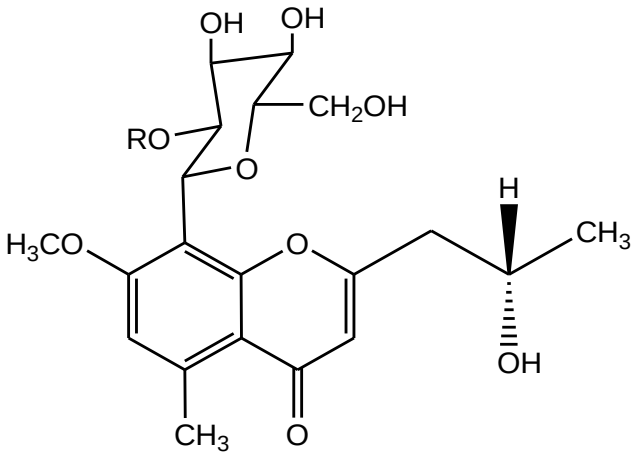


(21)

2.4.4. Chromones

Chromones are derivatives of benzopyron with substitueted keto group on the pyran ring and they are found in *Aloe* leaves. Aloeresin A (**22**), aloeresin B (**23**) [63], 7-*O*-methylaloresin A (**24**) ,7-*O*-methylaloresin (**25**) [63] , isoaloeresin **(26)** [68] are some examples of chromones of the genus of *Aloe*,

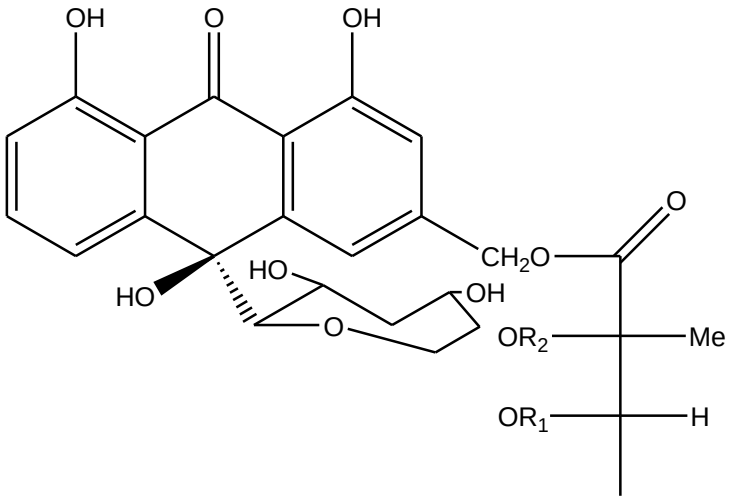




(22) R^1 = OH, R^2 = p-Coumaroyl (**26)** R = H
(23) R^1 = OH, R^2 = H
(24) R^1 = CH_3, R^2 = p-Coumaroyl, **(25)** R^1 = CH_3, R^2 = H

2.4.5. Oxanthrones

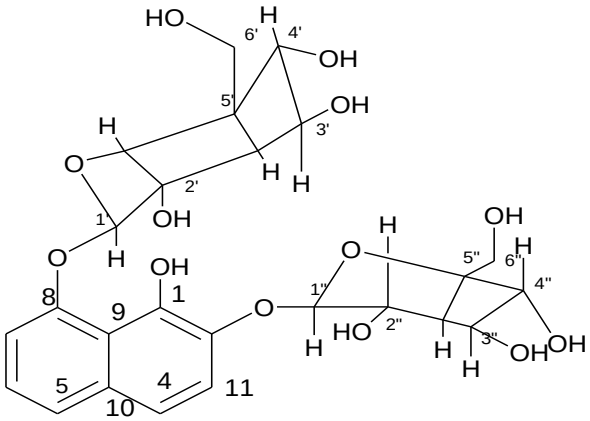
The leaf exudates of *Aloe littoralis*, a plant which grows in South Africa, has led to the isolation of a minor constituent, a structurally novel C, O -diglucosylate oxanthrone, named littoraloside. The isolation and characterization of three related oxanthrones, namely, littoraloin**(27)** , deacetyllittoraloin(**28**) and 10-hydroxyaloin B (**29**) from the leaf exudates of this species were obtained from this plant[69].



(27) R_1=Glc ;R_2=H , **(28)** R_1=H ; R_2=AC , **(29)** R_1=H ; R_2=H

2.4.6. Naphthalene derivatives

The methanol-soluble portion of the leaf exudate which was subjected to preparative thin layer chromatography (PTLC) over silica gel led to the isolation of a naphthalene derivative identified as 2,8-*O*,*O*-di(*β*-D-gluco-pyranosyl)-1,2,8-trihydroxy-3-methylnaphtalene (plicataloside) **(30)** [70].



(30)

3. MATERIALS AND METHODS

3.1. Chemicals and Reagents

CH_2Cl_2, EtOAc, MeOH, Silica gel, *n*-hexane, I_2 Crystal, distilled water, benzene,10% NH_4OH solution, bromine in CCl_4, $HgCl_2$, KI, 10% $(CH_3COO)_2Pb$ solution, $CuSO_4$ solution, NaOH solution, conc.H_2SO_4 solution, and Mayer's reagent.

3.2. Apparatus and Instruments

Analytical TLC was run on a 0.2 mm thick layer of silica gel GF254 (Merck) on aluminium plate. Spots were detected by iodine, vanillin in H_2SO_4 and UV lump. Column chromatography was performed using silica gel 60 (250-400 mesh size) Merck. Samples were applied on column by adsorbing on silica gel. Solvent was removed using rotary evaporator. The UV-Vis spectra was obtained in C_2H_5OH using Hewlett-Packard 8453 spectrophotometer. NMR spectra was recorded using Bruker Avance 400 spectrometer operating at 400 MHz . The IR spectra of compounds was recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) as KBr pellets. Some of the apparatus used are funnels, round bottom flasks, pipettes, vials, refrigerator, Whatmann No1 filter papers, mortar and pestle, drying oven, measuring cylinders, TLC Chamber, capillary tubes, digital balance, test tubes, Erlenmeyer flasks, rotary evaporator, and beakers.

3.3. Plant Material Collection

The roots of *Aloe debrana* were collected in November 2016 from Godino on blink of Wedecha river 20km far North east of Bishoftu (DebreZeit). The plant material was authenticated by botanist Shambel Alemu, the National Herbarium of Ethiopia, Addis Ababa University (AAU) and a specimen was deposited with a voucher number 001. It is locally named as *Hargisa* in Afan Oromo and *Ret* in Amharic.

3.4. Preparation of Plant Material and Extraction Methods

For first preliminary test to identify the appropriate solvent system of maceration, 2gm of grinded root using metal mortar and pestle, sample was macerated in 10 mL of CH_3OH / CH_2Cl_2 (1:1) and 10mL of 100% CH_3OH in a separate Erlenmeyer flask and the filtrate looks deep red for both was filtered off . The TLC of the filtrates was analyzed in mobile phase of EtOAc / n-hexane successively. (1:9) and (2:8) ratios do not show any spot on TLC, (3:7) ratio gave three separate spots ,(4:6) ratio gave four separate spots (5:5) ratio also gave

four separate spots, (6:4) ratio gave five spots each fluoresces yellowish spots using I_2 spray above this no separable spots were observed. This confirms as solvent systems CH_2Cl_2: CH_3OH (1:1) and 100% CH_3OH are recommended for extraction and EtOAc / n-hexane solvent system is recommended as eluent for column chromatography.

The grounded roots (500g) were macerated for 72 hours through shaking and extracted with the solvent CH_2Cl_2/MeOH (1:1) using 1g:5mL sample to solvent ratio at room temperature. The extract was filtered by whatman no. 1 filter papers and concentrated by rotary evaporator at 40^oC . The marc left was further extracted by the same procedure using MeOH. The TLC profile of MeOH crude extracts became similar with CH_2Cl_2 / MeOH (1:1), hence 10g of CH_2Cl_2 / MeOH (1:1) was mixed with 5g of MeOH crude extract for column chromatography.

The TLC test for the concentrated CH_2Cl_2 / MeOH (1:1) crude extract and MeOH crude extract was carried out .

Table 1:- TLC Plate Profile

EtOAc (mL)	n-hexane (mL)	No of spots observed
1	9	no
2	8	no
3	7	3
4	6	4
5	5	4
6	4	5
7	3	no separable spot

3.5. Preliminary Phytochemical Screening Test

Phytochemical screening test were done to identify the type of secondary metabolite present in the crude extract [71,72].

3.5.1. Mayer's test

Mayer's reagent (potassium mercuric iodide solution) is an alkaloidal precipitating reagent used for the detection of alkaloids. It is freshly prepared by dissolving a mixture of mercuric chloride (1.36gm) and potassium iodide (5.00gm) in water (100mL). 3mL of aqueous extract was stirred with 3 mL of 1% HCl on a steam bath. Mayer's reagent were added to the mixture [73, 74] .The formation precipitate confirms the presence of alkaloids.

3.5.2. Molisch's test

Is a sensitive chemical test for the presence of carbohydrates based on the dehydration of carbohydrates by sulphuric acid or hydrochloric acid to produce an aldehyde, which condenses with two molecules of phenols [75].

3.5.3. Lead acetate test

Flavonoids presence were qualitatively tested by dissolving the extract in distilled water and adding 3ml of 10% lead acetate solution [76].The formation white bulky precipitate confirms the presence of flavonoids.

3.5.4. Biruet test

The Biruet test is often used to determine the presence of peptides bonds in proteins .The reagent used is the solution of copper sulphate ($CuSO_4$) and sodium hydroxide solution (NaOH). NaOH is there to raise the P^H of the solution to alkaline levels; the conversion of the blue Biruet reagent to purple shows the presence of peptide bonds,which are the basis for the formation of proteins [77].

3.5.5. Frothing test

2ml aqueous solution of extract was placed in 5ml of distilled water in a test tube and shacked vigorously and then warmed [78].The formation of white stable froth confirms the presence of saponins.

3.5.6. Salkowsky Liberman-Burchardt test

The treatment of 2ml of the extract aqueous solution with few drops of concentrated sulphuric acid and red color formation at lower layer indicates the presence of steroids [78].

3.5.7. Kahelnberg test

The treatment of 2ml of the extract aqueous solution with few drops of concentrated sulphuric acid and formation of yellow color at the lower layer indicates the presence of triterpenoides [78].

3.5.8. Test for anthraquinones

It was tested by the treatment of 0.5g of extract with 10 ml of benzene followed by filtration and addition of 5 ml of 10% ammonia solution to the filtrate [79]. The presence of pink, red or violet color indicate the presence of anthraquinones.

3.6. Isolation and Purification of Compounds

The crude extract (15g) was adsorbed on 15g silica gel and subjected to column chromatography using increasing gradient of ethyl acetate in n-hexane as an eluent. A total of 38 fractions each 50mL were collected. All fractions were monitored by TLC visualized using I_2.

Fraction 1-8 didn't show any spot on TLC. Fraction 9 eluted with the solvent system n-hexane : EtOAc (46 : 4) showed one clear yellow spot. On standing for 24 hours a precipitate formed was a yellow gelatin like substance which gave 8.2mg (R_f = 0.6 , 8% ethyl acetate in n-hexane). The spectroscopic analysis were conducted but the ^{1}H NMR spectrum was not good for this sample and did not characterized. Fraction 10-17 which showed similar spots on TLC were combined and further purified by column chromatography (isocratic mode(25% ethyl acetate in n-hexane) but in purification the yield became very small for spectroscopic analysis and hence it was left out.

Fraction 18 which showed three separable spots was further purified by small column chromatography and eluted with the solvent system n-hexane : EtOAc (35 : 15) (30% ethyl acetate in n-hexane, isocratic mode). From the collected fractions, fraction 7 and 8 showed one clear spot on TLC with similar R_f value. These fractions were combined, and dried to give yellow powdered **compound 1** (12.8mg, coded as **AD-18**, R_f = 0.44 in 30% EtOAc in n-hexane).

Table 2:-solvent systems and fractions collected from CH_2Cl_2 :CH_3OH(1:1) and methanol extracts of the roots of aloe debrana.

Fraction No	Volumeof eluant (mL)	Polarity of eluant	Fraction No	Volumeof eluant (mL)	Polarity of eluant
1	50	100% n-hexane	22	50	n-hexane:EtOAc(25:25)
2	50	n-hexane:EtOAc(49.5:0.5)	23	50	n-hexane:EtOAc(22.5:27.5)
3	50	n-hexane:EtOAc(49:1)	24	50	n-hexane:EtOAc(20:30)
4	50	n-hexane:EtOAc(48.5:1.5)	25	50	n-hexane:EtOAc(17.5:32.5)
5	50	n-hexane:EtOAc(48:2)	26	50	n-hexane:EtOAc(15:35)
6	50	n-hexane:EtOAc(47.5:2.5)	27	50	n-hexane:EtOAc(10:40)
7	50	n-hexane:EtOAc(47:3)	28	50	100% EtOAc
8	50	n-hexane:EtOAc(46.5:3.5)	29	50	EtOAc:CH_3OH(45:5)
9	50	n-hexane:EtOAc(46:4)	30	50	EtOAc:CH_3OH(40:10)
10	50	n-hexane:EtOAc(45.5:4.5)	31	50	EtOAc:CH_3OH(35:15)
11	50	n-hexane:EtOAc(45:5)	32	50	EtOAc:CH_3OH(30:20)
12	50	n-hexane:EtOAc(42.5:7.5)	33	50	EtOAc:CH_3OH(25:25)
13	50	n-hexane:EtOAc(40:10)	34	50	EtOAc:CH_3OH(20:30)
14 -17	200	n-hexane:EtOAc(37.5:12.5)	35	50	EtOAc:CH_3OH(15:35)
18	50	n-hexane:EtOAc(35:15)	36	50	EtOAc:CH_3OH(10:40)
19	50	n-hexane:EtOAc(32.5:17.5)	37	50	EtOAc:CH_3OH(5:45)
20	50	n-hexane:EtOAc(30:20)	38	50	CH_3OH 100%
21	50	n-hexane:EtOAc(27.5:22.5)			

Fraction 19 gave yellow powdered solid on standing at room temperature. The TLC of filtrate (n-hexane soluble)and precipitate (n-hexane insoluble)were analyzed by 40% EtOAc in n-hexane. The filtrate shows multiple spots but the precipitate shows two yellow spots. The precipitate was filtered and further purified by small column chromatography through isocratic mode eluted with the solvent system n-hexane : EtOAc (30:20) affording a compound coded as **AD-19** (16.2mg, **,** R_f = 0.41 in 40% EtOAc in n-hexane). The spectroscopic analysis were conducted and became ready for characterization. Since the NMR spectrums show two compounds and in characterization it became difficult to identify the major compound and separate them, it was left out.

Table 3:- solvent systems and fractions of Fraction 18 Using Isocratic mode

Fraction No	Volume of eluant (mL)	Polarity of Eluant
1	50	n-hexane:EtOAc 30:15
2	50	n-hexane:EtOAc 30:20
3	50	n-hexane:EtOAc 30:20
4	50	n-hexane:EtOAc 30:20
5	50	n-hexane:EtOAc 30:20
6	50	n-hexane:EtOAc 30:20
7	50	n-hexane:EtOAc 30:20
8	50	n-hexane:EtOAc 30:20
9	50	n-hexane:EtOAc 30:20
10	50	n-hexane:EtOAc30:20

Fraction 20 which showed three spots on TLC was further purified by column chromatography with increasing gradient of ethyl acetate in n-hexane. Of the fractions collected, fractions of fraction11-16 eluted with the solvent system n-hexane : EtOAc (25:25:) (50% ethyl acetate in n-hexane) each showed one spot on TLC, by combining them they were made to dry at room temperature for about 48 hours and afforded **compound 2** (20.5mg, coded as **AD-20** , R_f = 0.38, 50 % EtOAc in n-hexane) the spectroscopic analysis were conducted and became ready for characterization.

Table 4:- solvent systems and fractions of Fraction 20 using increasing gradient of ethylacetate in n-hexane.

Fraction No	Volume of eluant (mL)	Polarity of Eluant, n-hexane:EtOAc	Fraction No	Volume of eluant (mL)	Polarity of Eluant, n-hexane:EtOAc
1	50	45:5	10	50	30:20
2	50	45:5	11	50	25:25
3	50	40:10	12	50	25:25
4	50	40:10	13	50	25:25
5	50	35:15	14	50	25:25
6	50	35:15	15	50	25:25
7	50	35:15	16	50	25:25
8	50	35:15	17	50	20:30
9	50	35:15	18	50	100% EtOAc

Each fraction above this, starting from fraction 21 when monitored by TLC even by changing the ratio of eluent exhaustively and visualized using I_2 , spots do not separate rather form tails and became difficult to isolate compounds ,this shows that it needs reverse phase TLC ,hence did not allow for further fractionation.

3.7. Antibacterial Activities Testing

Agar disc diffusion test method is often used as qualitative methods to determine whether a bacterium is resistant, intermediately resistant or susceptible to the crude extracts and pure compound. Disc diffusion method is selected due to its simplicity, capacity to analyze multiple samples simultaneously and its ability to work well with defined inhibitors. The antibacterial activities of the two samples (The CH_2Cl_2:CH_3OH(1:1) crude extract and compound 1 (AD-18)) were tested against two Gram-negative bacterial strains such as: *Escherichia coli* and *Proteus mirabilis* and two Gram-positive bacterial strains such as *Staphylococcus aureus and Bacillus subtilis* using Muller Hinton Agar (MHA) medium. All the microbial were obtained from Oromia Public Health Research Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Standard antibiotic *Gentamicin* was used as positive control prepared by dissolving 10µg powdered *Gentamicin* in 200µL methanol to prepare a concentration of 0.05 mg /ml, and diluent methanol was used as negative control. The CH_2Cl_2:CH_3OH(1:1) crude extracts and compound 1 (AD-18) were prepared for evaluation by dissolving 5mg of each separately in10ml of diluent (methanol) to give a concentration of 0.5mg /ml. Standard antibiotic *Gentamicin* was used as positive control which was prepared by dissolving 10µg powdered *Gentamicin* in 200µL methanol to prepare a concentration of 0.05 mg /ml, and diluent methanol was used as negative control. The CH_2Cl_2:CH_3OH(1:1) crude extracts and compound 1 (AD-18) were prepared for evaluation by dissolving 5mg of each separately in10ml of diluent (methanol) to give a concentration of 0.5mg /ml..

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4. RESULTS AND DISCUSSIONS

4.1. Extraction Yields

The roots were extracted by dissolving 500g grinded and powdered root sample in the solvent CH_2Cl_2/CH_3OH (1:1) and CH_3OH yielded 16.51g (3.3%) and 9.98g (2.0%) successively.

4.2. Preliminary Phytochemical Screening Tests

Table5:- Results of phytochemical screening tests of the crude extract of roots of *Aloe drbrana* using the solvent systsm CH_2Cl_2:MeOH (1:1).

Secondary Metabolite	**Reagent used**	**Observation**
Steroids	Conc. H_2SO_4	+
Alkaloids	$HgCl_2$ + KI aqueous solution(Mayer's reagent)	_
Anthraquinones	benzene + 10% ammonia solution	+
Flavonoids	10%$(CH_3COO)_2Pb$ aqueous solution	_
Saponins	Distilled water	+
Proteins(peptide bond)	$CuSO_4$ + NaOH aqueous solution	_
Triterpenoides	Conc.H_2SO_4	_

Key: Present (+), Absent(-)

Accordingly in the Mayer's test the turbidity of the resulting precipitate was not shown reveled the absence of alkaloids, in Lead Acetate test a bulky white precipitate was not formed indicates the absence of flavonoids . In Biuret test the color remain blue (unchanged) which confirms the absence of peptide bonds, i.e, protein, in Frothing test red color was observed which indicate the presence of saponin. In Salkowsky Liberman-Burchardt test red color was formed at the lower layer, which indicate the presences of steroids. In Kahelnberg test yellow color was not formed at the lower layer, which indicate the absence of triterpenoides. In anthraquinones test red color formation after shaking the filtrate show the presence of anthraquinones .

The presence of steroids, anthraquinones and Saponins in this particular Phytochemical screening test of the crude extract of root of *Aloe debrana* shows the potential uses of the plant root in traditional medicine in accordance to the scientific finding can expressed as follow. SSteroid is used in the treatment of autistic spectrum disorders and other disorders involving mercury toxicity . Anthraquinone compounds are used as laxatives mainly from their glycosidic derivatives and also used in the treatment of fungal skin diseases, it can also

shows antioxidant property, as sliming agents and have been valued for cathartic and presumed detoxifying action. Saponins are plant glycosides, occur in a great many plant species, and have been implicated as pre-formed determinants of resistant to fungal attack by producing detoxifying enzymes.

4.3. Characterization of Compound 1(AD-18)

Compound 1 (AD-18) was isolated as yellow amorphous powder with R_f value of 0.44 (30% ethyl acetate in n-hexane)**.** The UV (EtOH) spectrum revealed absorption maxima (λ_{max}) at 257 and 412 comparable to that of anthraquinone chromophore (Dagne and Steglich, 1984). The IR(KBr) spectrum of AD-18 showed vibration at 3428cm^{-1} (hydroxyl group), 2923cm^{-1} (aliphatic sp3 C-H stretching), 1751cm^{-1} (ester C=O), 1683cm^{-1} and 1631cm^{-1} (ketone C=O), 1586cm^{-1} (aromatic C=C stretching) and 1271cm^{-1} (C-O stretching of ester moiety).The ^{1}H NMR spectrum using $CDCl_3$ solvent displayed the presence of singlet at 12.93ppm attributed to chelated hydroxyl group peri to carbonyl carbon, four aromatic protons, three with an ABX spin pattern at δ_H 7.32 (d, J= 0.8MHz, 1H), 7.65 (t, J=0.7MHz1H), and 7.76 (d, J= 0.4, 1H) and a singlet at δ_H 7.78 (1H, H-4). Another two singlet peaks were observed at δ_H 4.0 (3H, s) and δ_H 2.9 (3H, s). The ^{13}C NMR using $CDCl_3$ solvent shows the presence of two carbonyls at δ_c 189.5 and 182.2ppm, one methyl ester moiety (δ_c 170.4 and 53.2ppm), two sp2 quaternary carbons attached directly to alcohol hydroxyl (δ_c 163.2 and 162.5ppm), six sp^2 quaternary carbons (147.6, 138.7, 132.6,124.4, 121.6, 117.4 ppm) and four aromatic methines (δ_c 135.8, 125.0, , 118.9, 117.4 and 114.9) and one methyl at δ_c 21.7 (also supported by DEPT-135, pointing up in DEPT-135). Based on the spectral data the structure of compound 1 (AD-18) was found to be isomer of aloesaponarin I , anthraquinone in which aloesaponarin I was previously reported compound from the stem of *A. saponaria* [80] .

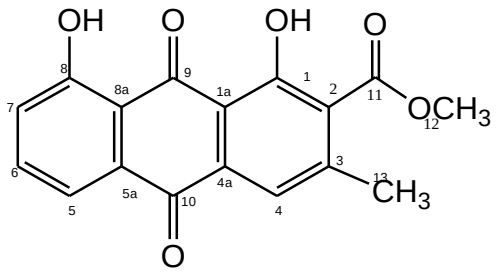

Table 6:-Spectral data of Compound 1 (**AD-18)** and reference [80] (δ in ppm).

Position	^{1}H NMR of AD-18	^{13}CNMR of AD-18	DEPT-135 AD-18	^{1}H NMR of literature [80]	^{13}C NMR of literature [80]
1	OH,12.93(s,1H)	162.5	---	12.93	162.6
2	----	124.4	114.9	---	124.6
3	---	147.6	---	---	148.1
4	7.65(s,1H)	114.9	---	7.80	115.2
1a	---	117.4	---	---	115.3
4a	---	138.7	---	---	138.9
5	7.76(d,J=8,1H)	118.9	118.9	7.62	119.1
5a	---	132.6	---	---	132.8
6	7.78(dd,j=2xs8,1H)	125.0	125.0	7.77	125.2
7	7.32(d,J=8Hz,1H)	135.8	135.8	7.31	136.0
8	OH,12.93(s,1H)	163.2	---	12.93	163.6
8a	---	121.6	---	---	121.2
9	---	189.5	---	---	189.7
10	---	182.2	---	---	182.3
11	---	170.4	---	---	170.7
12	CH_3, 4.08(s, 3H)	53.1	---	4.06	53.3
13	CH_3,1.27(,s,3H)	21.7	---	1.26	22.0

4.3. Characterization of Compound 2 (AD-20)

Compound 2 (AD-20) was isolated as yellow powdered solid with R_f value of 0.38 (40% ethyl acetate in n-hexane). The UV-Vis (EtOH) spectrum revealed absorption maxima (λ_{max}) at 267nm revealed the presence of Π to Π* conjugation due to aromatic ring. The IR (KBr) spectrum revealed vibrations at 3402cm^{-1} (hydroxyl groups), 2926cm^{-1} (methyl C-H bond), 2853cm^{-1} (methylene C-H bond), 1643cm^{-1} (C=O), 1560cm^{-1} (C=C aromatic bond),1298cm^{-1} C-C bond), and 1271cm^{-1} (C-O bond).

The ^{1}H NMR spectrum of AD-20 using $CDCl_3$ solvent displayed the presence of singlet at 9.70 ppm due to hydroxyl group. The presence of three aromatic protons at δ_H 7.28ppm (1H), δ_H 7.16(1H) and δ_H 7.01 with ABX spin pattern coupled with the presence of peaks at δ_H 4.96 (1H), δ_H 3.11 (2H), attributed to oxygenated methine and methylene, suggest that the compound have a chromene skeleton having ABX spin pattern aromatic ring. Moreover, the

peaks at δ_H 2.45 (m,2H) and δ_H 1.27 (t,3H) suggest that there is an ethyl moiety linked to methine at C-2 (δ_H 4.96) and the methylene at δ_H 3.11 is possibly next to carbonyl group. The ^{13}C NMR spectrum usind $CDCl_3$ solvent revealed the presence of one carbonyl at δ_C 203.5, two oxygenated sp2 quaternary carbons at δc 166.2 and 157.9, one oxygenated methine at δ_C 67.7, three aromatic methines at δ_C 118.9, 116.2, and 113.4,two methylenes at δ_C 33.3 and 30.7 , one methyl at δ_C 22.1.

Based on the above spectral data obtained, the structure of compound 2 **(AD-20)** is found to be 2-ethyl-2,3-dihydro-7-hydroxychromen-4-one shown below.

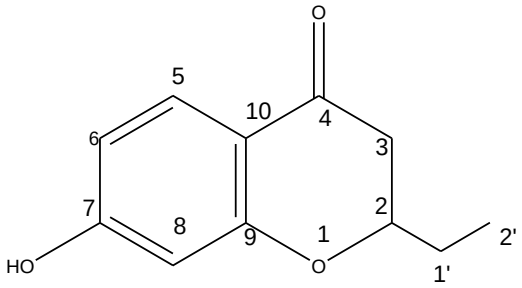

Table7:-.The ^1H NMR, ^{13}C NMR and DEPT-135 spectral data of Compound 2 (**AD-20**)

Position	**^{1}H NMR of** AD-20 **(δ in ppm)**	**^{13}CNMR of** AD-20 **(δ in ppm)**	**DEPT-135** AD-20 **(δ in ppm)**
1	---	---	---
2	4.96(d,1H)	67.7	67.7
3	3.11(m,1H)	33.33	33.3
4	---	203.5	---
5	7.22(s,1H)	118.9	118.9
6	7.16(s,1H)	116.2	116.2
7	-OH ,9.7(s,1H)	166.3	---
8	7.01(s,1H)	113.4	113.38
9	---	157.9	---
10	---	138.8	---
1'	1.48(m,2H)	30.7	30.7
2'	1.27(m,3H)	22.1	22.1

4.4. Antibacterial Activities Testing

Table 8:-Result of Antibacterial Activities Testing

Samples	Zone of inhibition by MIC method with 0.5mg/ml of samples and 0.05 mg/ml of standard drug.			
	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus aureus	Bacillus subtilis	Escherchia coli	Proteus mirabilis
CH_2Cl_2:CH_3OH (1:1) crude extract	11mm	6mm	6mm	6mm
compound 1(AD-18)	7mm	8mm	9mm	6mm
Gentamicin	15mm	15mm	15mm	15mm
Methanol	6mm	6mm	6mm	6mm

Accordingly, 11, 7 , 15 and 6mm were found to be the zone of inhibition breakpoint for crude extract, compound1(AD-18) , standard (Gentamicin) and methanol respectively, against *S. aureus.* whereas 6, 9 , 15 and 6 mm were found to be zone of inhibition breakpoint for crude extract, compound1(AD-18), standard (Gentamicin) and methanol respectively against *E.coli*. Overall evaluation of both compound 1 and crude showed low activity compared to the standard drug. Nevertheless, the crude CH_2Cl_2:CH_3OH (1:1) extract have relatively promising activity against *Staphylococcus aureus.*

5. CONCLUSSIONS AND RECOMMENDATIONS

5.1. Conclusions

Phytochemical screening of the crude extract of root of *Aloe debrana* revealed the presence of steroids, anthraquinones and Saponins. Silica gel column chromatography separation of the DCM:MeOH (1:1) extract yielded isomer of Aloe saponarin I (1,8-dihydroxy-3-methyl-4-anthraquinoic methyl methanoate) and 2-ethyl-2,3-dihydro-7-hydroxychromen-4-one. The structure of the compounds were determined by ^{1}H-NMR, ^{13}C NMR, UV-Vis, and IR spectroscopic analysis. Overall antibacterial evaluation of both compound1 and CH_2Cl_2:CH_3OH (1:1) crude extract showed low activity compared to the standard drug. Neverthless, the crude CH_2Cl_2:CH_3OH (1:1) extract have promising activity against *Staphylococcus aureus* compared to standard drug.

5.2. Recommendations

The scientific findings of the present study proved that the crude CH_2Cl_2:CH_3OH (1:1) extract have some promising activity against *Staphylococcus aureus* which might give a clue about the traditional use of the plant against various infectious diseases. However, considering the TLC profile of the crude extract and Antibacterial activity of the crude extract compared to individual isolated compounds to that of standard drug there are still many unisolated secondary metabolites present in the plant and may need to be screened which initiates further phytochemical analysis of the plant. Starting with limited amount of the plant material (500g) was a bottle neck to isolated other minor constituents of the plant material. Morover, the following recommendation were suggested as a focus areas for future researchers who might be interested to work on this plant further.

- The present study used gravity column using ethyl acetate and n-hexane as an eluent. However, as evidenced by the rich TLC profile of the methanol and CH_2Cl_2:CH_3OH (1:1) extracts, more phytocehmical analysis needs to be carried out on these polar extracts of the plant with the help of reverse phase HPLC (C8 or C18 column) using water/methanol or water/acetonitrile as mobile phase**.**

- The present study started with only 500g of the plant material and could not achieve to isolated and identify some of the minor compounds as supported by the literature reports, Literature reports suggest for the genus as rich source of anthraquinones. Hence, future phytochemical work is recommended starting with higher amount of the plant material and also needs further optimization of extraction solvents for better extraction yield.

- More biological assay on other strains needs to be conducted on various extracts of the plant so as to establish the traditional uses of the plant.

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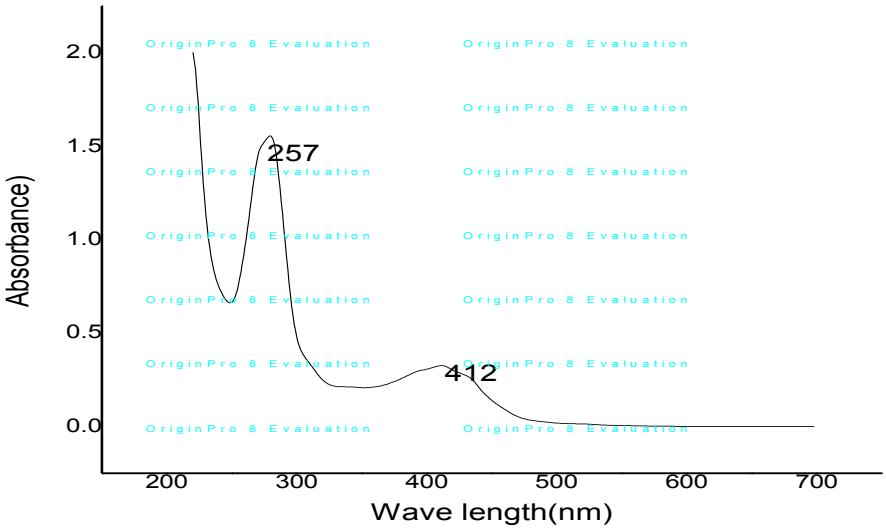
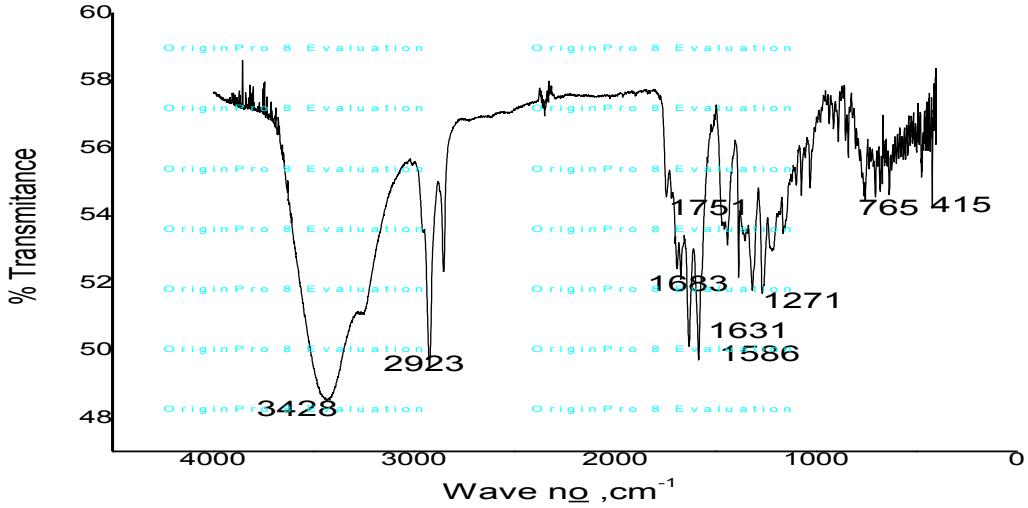
7. APPENDICES

Appendix 1:UV-Vis SPECTRUM OF AD-18

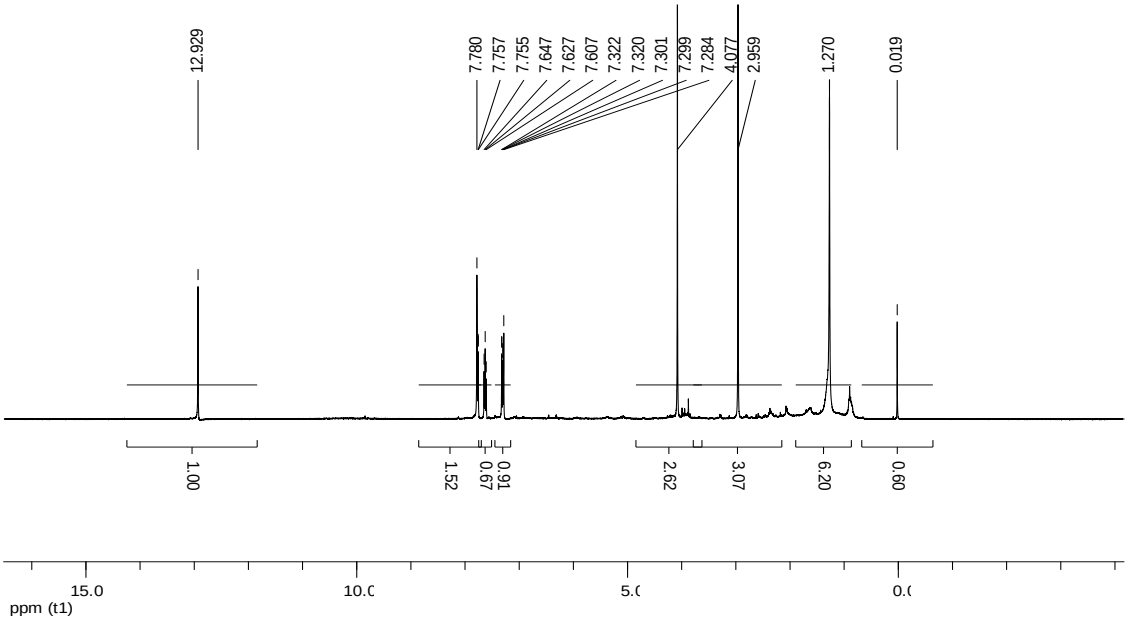


Appendix 2:FT IR SPECTRUM OF AD-18

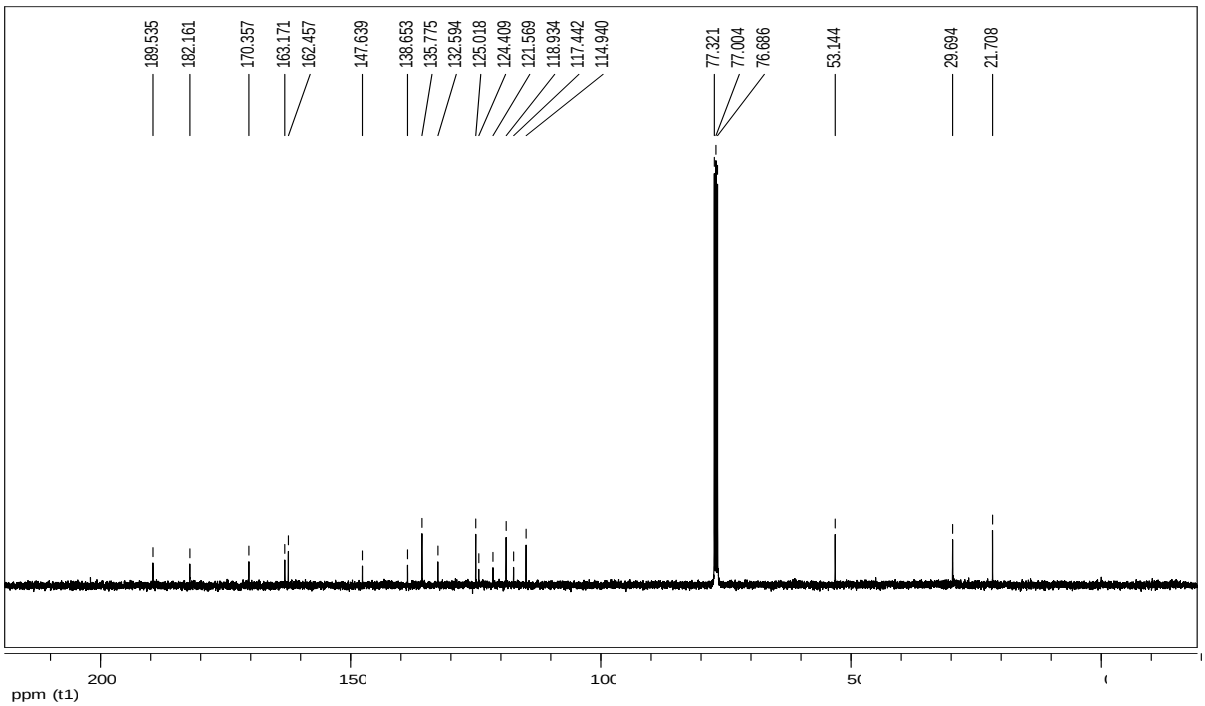


Appendix 3: ^{1}H-NMR SPECTRUM OF AD-18

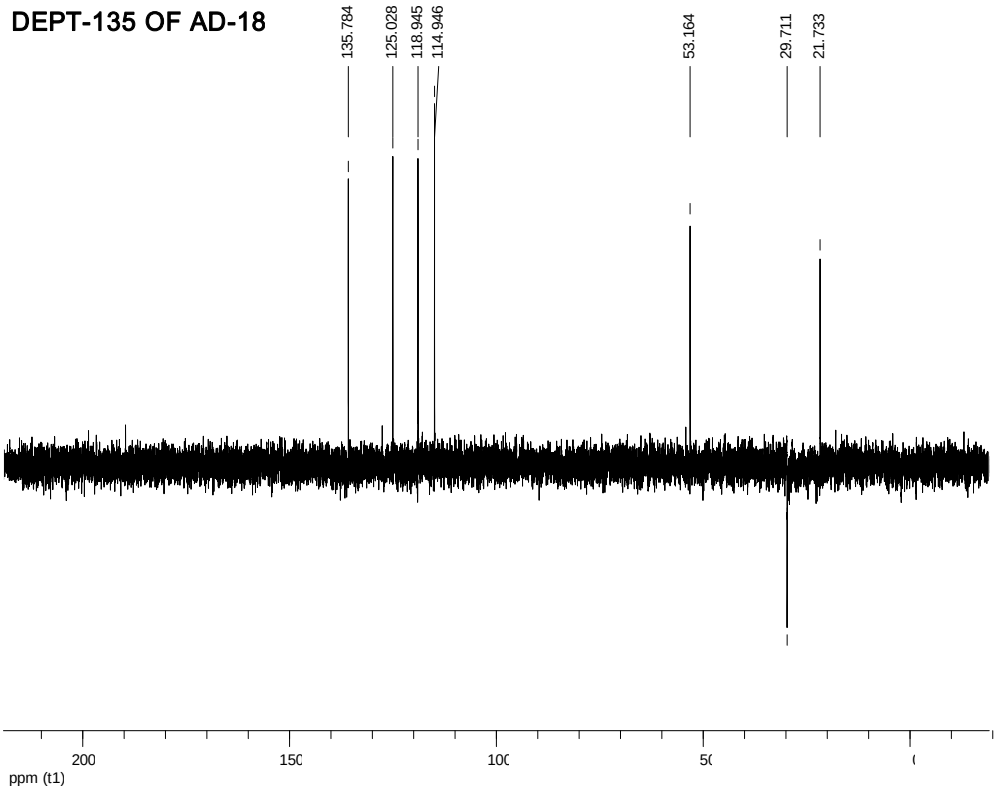
1H-NMR OF AD-18



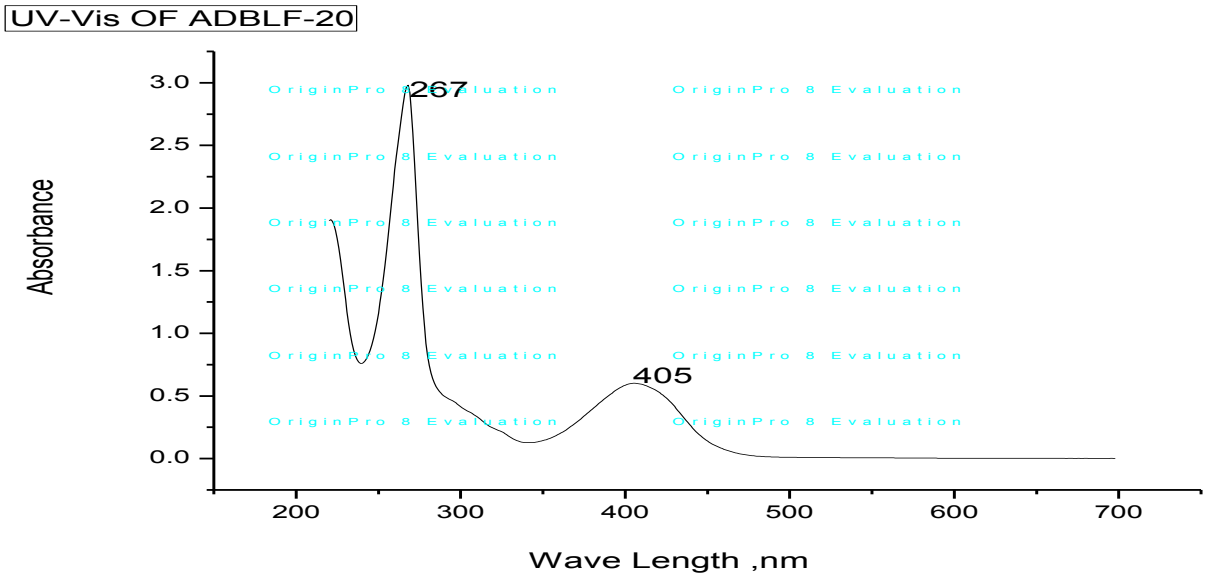
Appendix 4: ^{13}C-NMR SPECTRUM OF AD-18



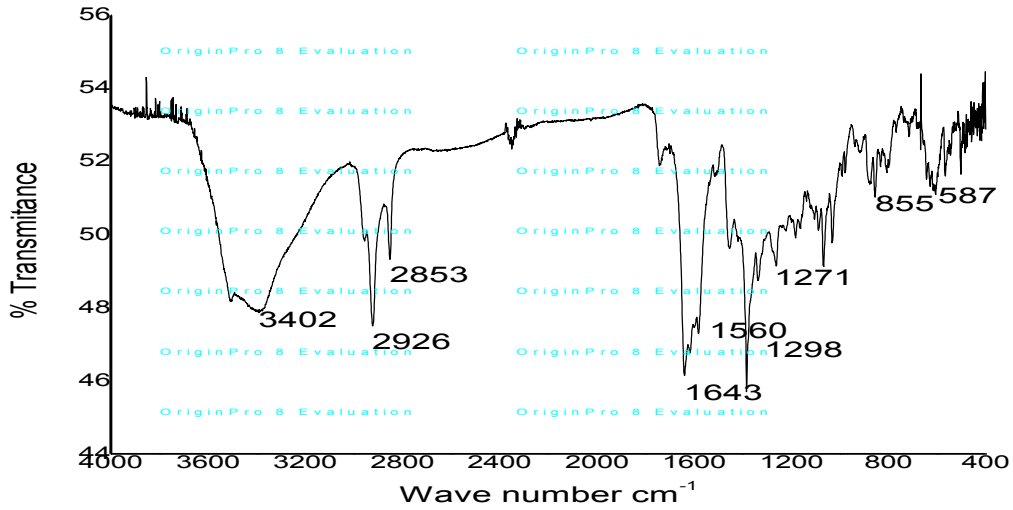
Appendix 5: DEPT-135 SPECTRUM OF-AD-18



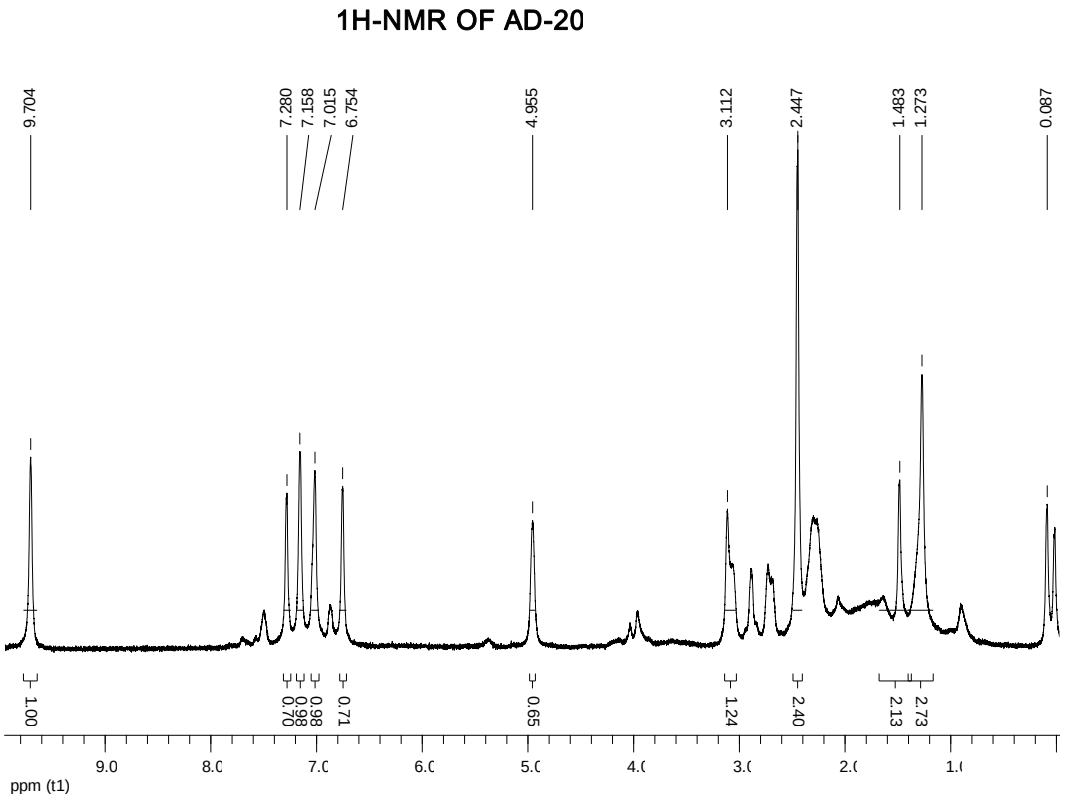
Appendix 6:UV-Vis SPECTRA OF AD-20



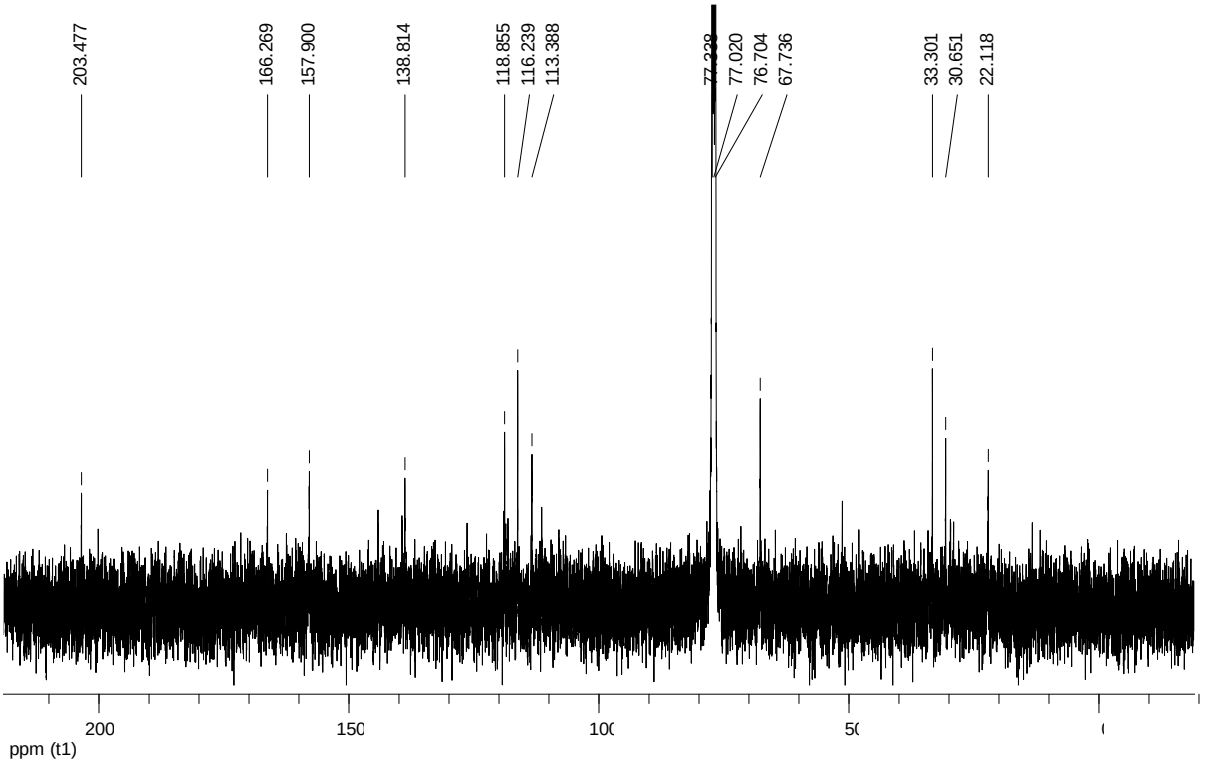
Appendix 1:FT IR SPECTRA OF AD-20



Appendix 8: ^{1}H-NMR SPECTRUM OF AD-20



Appendix 9: ^{13}C-NMR SPECTRUM OF AD-20



Appendix 10:DEPT-135 OF AD-20