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ON

PHYTOCHEMICAL INVESTIGATION OF *Aloe kefaensis* ROOTS AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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PHYTOCHEMICAL INVESTIGATION OF *Aloe kefaensis* ROOTS AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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

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DECLARATION

I, declare that this thesis is my original work except where reference is made and has not been submitted for the award of any degree or diploma in any University. Information taken from the work of others has been properly acknowledged in the text and a list of references is given.

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Tamiru Fayisa

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Abbreviations and Acronyms

ATCC	American Type Culture Collection
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
IC_{50}	50% Inhibitory concentration
LC_{50}	50% Lethal Concentration
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
WHO	World Health Organization
2D NMR	Two Dimensional Nuclear Magnetic Resonance

Abstract

Infectious diseases are a serious and remain health and economic problem due to the emerging of new infectious antimicrobial resistance agents. Therefore, the search for antimicrobial compounds mainly from medicinal plants with traditional practice cannot be over emphasized so as to target these problems. Thus, the aim of this study was to isolate compounds from roots of *Aloe kefaensis* and evaluate their antimicrobial activity. The air-dried and powdered plant material was sequentially extracted with petroleum ether, dichloromethane, acetone, and methanol to yield crude extracts of 3.2 g (0.4 %), 7.5 g (0.93 %), and 10 g (1.25 %), 7 g (0.88 %) respectively. The extracts were evaluated for their *in-vitro* antimicrobial activity against four bacterial (*E.coli*, *S.aureus*, *B.cereus*, *S.typhi*, and one fungal (*C.albicans*) strains using agar disk diffusion method. Superior antimicrobial activity was exhibited against all the strains by dichloromethane extract with the highest activity was observed against *S. typhi* (23.0 ± 0.28 mm). Following this superior activity, serial dilutions of the extract have been prepared with a concentration of 100, 50, 25, 12.5 mg/ml; and showed the maximum inhibition of 18.0 ± 0.28, 15.5 ± 0.71, 11.0 ± 0.28, and 10.0 ± 0.71 mm , respectively against *S. typhi.* The acetone extract and dichloromethane extract were combined, as their TLC profile is similar and subjected to silica gel column chromatography for fractionation and isolation of the compounds. The column was eluted with petroleum ether containing increasing amount of EtOAc and resulted six compounds (AK-1, AK-2, AK-3, AK-4, AK-5, and AK-6) after further purification by using Sephadex LH-20 and small size column chromatography. The structural elucidation of only two compounds were carried on basis of NMR (^{1}H NMR, ^{13}C NMR and DEPT) data and found to be deoxyerythrolaccin (AK-3), and (5Z, 9Z)-26-methyloctacosa-5, 9-dienoic acid (AK-2). Antimicrobial evaluations of the isolated compounds were also carried out, and AK-3 and AK-4 have displayed strong activity against *S. typhi* and *E. coli* (16.5 ± 0.71-19.0 ± 0.28 mm). The compounds were also showed interesting antifungal activity against *C. albicans* with superior activity exhibited by AK-3 with zone of inhibition 17.5 ± 0.71 mm. The antimicrobial activity of the crude extracts and isolated compound support the traditional uses of the plants for the treatments of microbial infections.

Key words: Medicinal plants; A*loe kefaensis*; antimicrobial activity; deoxyerythrolaccin; (5Z, 9Z)-26-methyloctacosa-5, 9-dienoic acid

1. Introduction

1.1. Background of the study

Traditional medicine involves knowledge systems developed over generations within various societies before the era of modern medicine. According to the WHO definition, traditional medicine is sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical, and mental illness [1]. Africa has an immensely rich biodiversity and knowledge in the use of plants to treat various ailments. WHO estimate that 80% of the population in sub-Saharan Africa depends solely on traditional medicine for their primary healthcare needs because of their accessibility, cheapness, and socio-cultural background [2].

Infectious diseases are a serious cause of fatality worldwide, accounting for about 50% of all deaths in tropical countries [3]. To combat these diseases huge resources have been exhausted during the last four decades with the help of various strategies such as high-throughput screening, genomics and vaccine development, but it remains a challenging public health problem because of the emerging of resistant pathogens, climate change, and increasing numbers of immune-compromised patients. In such a period when antimicrobial resistance increases, and limiting the usage of many drugs that are currently in the market the search for novel anti-microbial from the potential sources should take attention [4].

Plants consist of a number of biologically active ingredients such as alkaloids, flavonoids, steroids, glycosides, terrenes, tannins and phenolic compound hence they are used for the treatment of various diseases. These compounds derived from plants are found to be bio-resource of drugs used in medicine. Drug discovery from medicinal plants led to the isolation of drugs such as aspirin (**1**) (anti pain) from *Filipendula ulmar*, pilocarpine (**2**) (parasympathomimetic) from *Pilocarpus jaborandi*, ephedrine (**3**) (bronchodilator) from *Ephedra* species [5]. Quinine (**4**) (antimalarial) from the *Cinchona* tree [6], Neoandrographolide (**5**) (antidysenteric), from *Andrographi spaniculata* [7], Calanolide A (**6**) (anti-HIV) from *Calophyllum lanigerum* [8], reserpine (**7**) (antihypertensive) from *Rauwolfia heterophylla* [9], of which some are still in use.

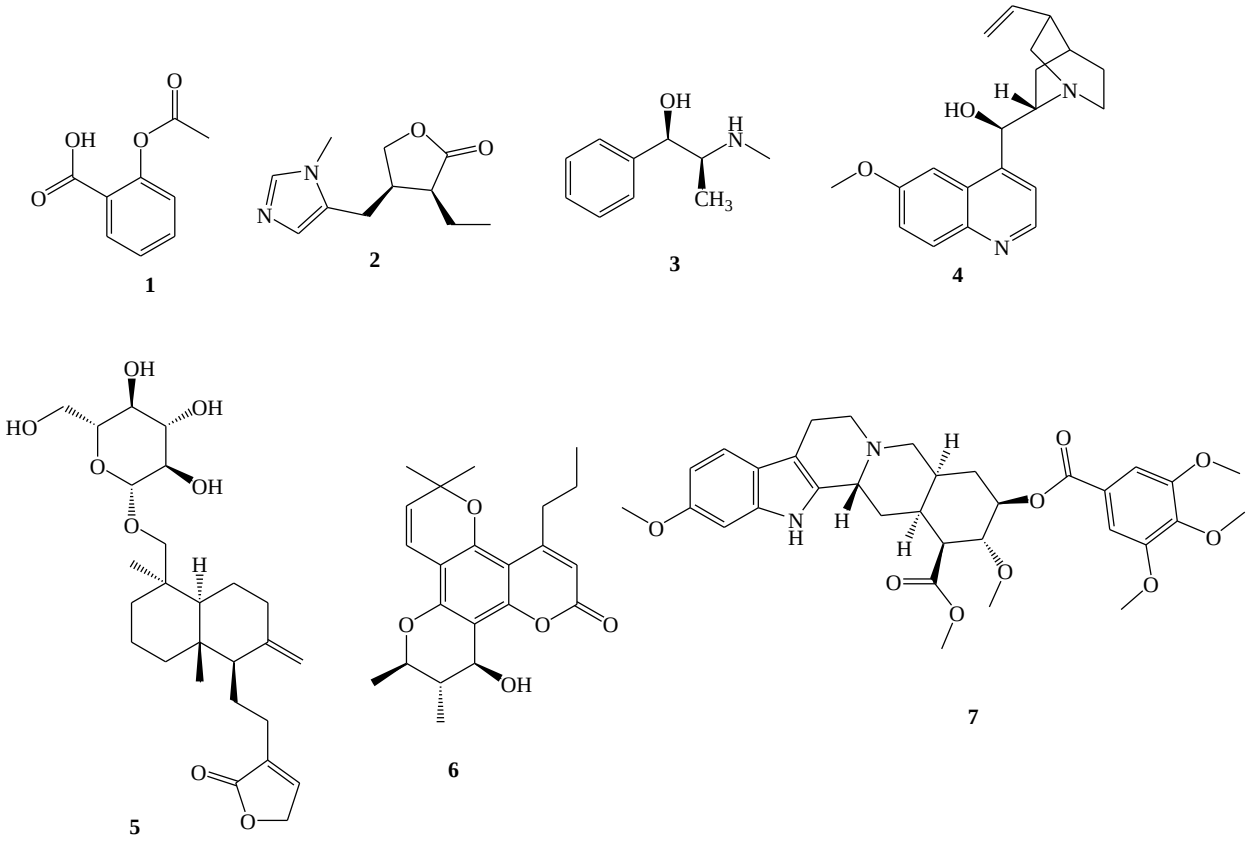


Figure 1: Some Example of Drugs obtained from Medicinal plants

Plants in members of the genus *Aloe* have been utilized since early times to treat a range of ailments including microbial infections, gastrointestinal diseases, and inflammatory conditions [10]. In line to these traditional claims crude extracts from *A.vera*, *A.volkensii,* and *A. secundiflora* showed good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans*, and *Fusarium oxysporum* [11]. *A. kefaensis* are one of the traditionally used medicinal by the community living in Agaro District, Jimma, Ethiopia for treatment of wound healing and fire burn [12]. In addition this plant is also used by residents of Keffa, Bonga District, to treat gastrointestinal disorders. In this research phytochemical profile of *A. kefaensis* was investigated and isolated compounds were evaluated for their antimicrobial activities.

1.2. Statement of the Problem

One of the main public health challenges in this era is the emergence of resistant microbes causing infectious diseases. Despite great resources wasted toward curbing these challenges, increasing of drug-resistant pathogens has been posed fear to public health. Such a serious problem is calling for collective and an organized global effort for the development of new strategies for the treatment of infectious disease. Compounds isolated from plants with the medicinal claim have been used as clinically proved drugs to be supplied as antimicrobial agents [13]. Isolation and characterization of pharmacologically active compounds from medicinal plants has been continued today. *A. kefaensis* is one of the endemic *Aloe* species of Ethiopia, which is traditionally used to treat different ailments such as wound healing, fire burn, and gastrointestinal disorders. However, no studies have been conducted pertaining to phytochemical information and biological activity of this plant. Therefore, the current study aimed at isolating and characterizing antimicrobial compounds from roots of *A. kefaensis.*

1.3. Objectives

1.3.1. General Objectives

To investigate phytochemicals constituents of roots of *Aloe kefaensis* and evaluate its antimicrobial activities

1.3.2. Specific Objectives

- ✓ To extract the roots of *Aloe kefaensis* sequentially with petroleum ether, chloroform, acetone and methanol.
- ✓ To screen the classes of phytochemical of the crude extract following standard chemical test methods.
- ✓ To isolate compounds from the roots extracts of *Aloe kefaensis* using column chromatography.
- ✓ To evaluate antimicrobial activities of the crude extracts and isolated compounds against bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* and a fungi (*Candida albicans*) using agar disc diffusion method.
- ✓ To elucidate the structures of the isolated compounds using NMR spectroscopic techniques (^{1} H, ^{13}C and DEPT-135) and comparison with reported literature data.

1.4. Significance of the Study

Plants play a central role in traditional systems of medicine for the prevention and treatment of disease around the world. The bioactive compounds present in plants have made many contributions to the development of new drugs. Regardless of the traditional medicinal usages of *A. kefaensis* for the treatment of various ailments, the phytochemical information pertaining to this plant and its microbial activity has not been addressed. Therefore, findings of this research will have the following significances:-

- ✓ Provide background information on the phytochemical profile and microbial activity of the plant
- ✓ Provide base line information for the further studies on the same plant genus and use as baseline data for pharmaceutical company.
- ✓ It could create initiation to conserve species of these plants

2. Review of Related Literature

2.1. Botanical Information

2.1.1. Family Asphodelaceae

According to recent classification, the family Asphodelaceae has been recognized to contain two new sub-families; Alooideae (with the genera *Aloe*, *Astroloba*, *Chamaealoe*, *Gasteria*, *Haworthi*a, *Lomathophyllum*, and *Poellnitzia*) and the Asphodeloideae (with the genera *Asphodeline, Asphodelus, Bulbine, Bulbinella, Jodrellia, Kniphofia*, *Smiethis, and Trachyandra*) [14]. These two subfamilies are mostly herbaceous and woody with trunks reaching to several meters high. They are mainly distributed in arid regions of the temperate, subtropical, and tropical zones of the World in which South Africa is considered as the main center of distribution [15].

2.1.2. The Genus *Aloe*

Aloe is the largest genus among the Asphodelaceae family and it comprises of more than 400 species ranging from diminutive shrubs to large trees that are widely distributed in Africa, India, and other arid areas, with the major diversity in South Africa. *Aloe* is represented in East Africa by 83 species, of which 38 grow naturally in Ethiopia, including 15 endemic species. It has played important role in medicinal and economic history since 1500 BC. The gel found in the interior of their leaves has been used to cure human and animals diseases [16]. They are distinguished by having fleshy and cuticularized leaves usually with spiny margins. Its name is taken from the Arabic word “Alloeh”, meaning “shining bitter substance” [17]. There are 20 different groups (group 1 to 20) of the species of these plants according to Reynolds division by their similarities in morphology as depicted in table 1.

Table 1 : Reynold's Grouping of Tropical African *Aloes*

	Description	**Representative species**	**References**
Group 1	grass *Aloe*s small acaulescent plants with fuis form roots	*A. myriacontha*	[14]
Group 2	lepto *Aloes* short-stemmed	*A.nuttii*	[14]
Group 3	bulbous species	*A. bkettneri*	[14]
Group 4	perianth striped	*A. peckii* *A. rugosifolia* *A. pirottae*	[18]
Group 5	compact rosettes or larger with open rosettes.	*A. dorotheae*	[18]
Group 6	sapanariae: plant with perianth pronounced basal inflation	*A. lateritia* *A. dumetorum* *A. graminicola*	[18]
Group 7	hereroenses: short-stemmed	*A. hereroensis*	[14]
Group 8	perianth trigonously indented above the overy	*A. chabaudii* *A. rivae*	[18]
Group 9	verae: plants acaulous solitary or in group	*A. barbadensis* *A. pubescens*	[18]
Group 10	pendet series	*A. veseyi*	[14]
Group 11	laterbracteatae: plants with bracts large, broadly ovate or suborbicular	*A. cryptopoda*	[9]
Group 12	acaulescentor short stemmed	*A. christianii*	[14]
Group 13	perianths clavate	*A. camperi* *A. calidophila* *A. sinana*	[18]
Group 14	orthol*o*phae:flowers second	*A. secundiflora* *A. orthol*o*pha*	[18]
Group 15	racemes bottle brush like, plants with racemes densely flowered	*A. aculeate*	[14]
Group 16	large compact rosettes	*A. percrassa* *A. harlana*	[18]
Group 17	leaves spreading, canaliculated	*A. magalacantha* *A. schelpei*	[18]
Group 18	tall stemed species	*A. volkensii*	[14]
Group 19	shrubs	*A. dawei* *A.arborescens*	[18]
Group 20	trees	*A.eminens*	[14]

2.1.3. *Aloe kefaensis*

A. kefaensis is endemic to Ethiopia; naturally grow in Kaffa region as old boundary demarcation. The species are in a member of the 'saponaria' group and distinguished from the other members of the group by the leaves being much less fleshy, sparser spots on the leaves, and the basal swelling of the perianth being less distinctly globose [19]. The plant is traditionally used for the treatment of ailments such as wound and fire burn in Jimma zone, Agaro district [12].

Figure 2: Photograph of *Aloe kefaensis in* its natural habitat (taken by Tamiru Fayisa, 2020)

2.2. Ethno Medicinal Uses of *Aloe Species*

Plants in genus *Aloe* have been used for a broad range of medicinal purposes by traditional healers from wide variety of cultural groupings in Africa [20]. As well as these plants have been visited by traditional healers to treat various diseases in Ethiopia as depicted in table 2. In rural parts of the country, its mucilaginous fluid applied to cuts and wounds in order to prevent infections and bring about healing [21].

Table 2: Ethnomedicinal Uses of *Aloe Species* in Ethiopia

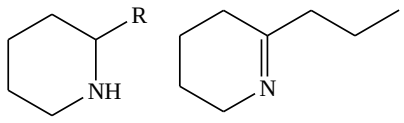
***Aloe* species used as medicine**	**Disease treated**	**Part used for treatment of disease**	**References**
A. macrocarpa	impotency in men	root	`
	malaria	latex	[22,23]
	bloat and fire burn	fresh leaf	
A. trichosantha	malaria, Stomach ache, gonorrhea, Impotency in men	latex	
A. citrina	Swollen foot	latex	[22]
A. monticola	liver disease	root	
	anthrax	root	[22,24]
A. gilbertii	malaria and wounds in humans	leaves gel, roots and exudates	[25]
A. lateritia	eye ailments	exudates	
A. pulcherrima	asthma	sap mixed with other medicinal plants	
	psychiatric disease		[26]
A. Kefaensis	fire burn	latex	[14]

2.3. Secondary Metabolites from *Aloe* Species

Aloes are interesting sources of various classes of secondary metabolites (Table 3). Regarding the different composition of leave portions of *Aloe* species, they are likely to have distinct classes of bioactive compounds; outer green epidermis has been reported to contain alkaloids, anthraquinones, pre-anthraquinones. While the outer pulp region below the epidermis contains latex that predominantly consists of phenolic compounds, including anthraquinones, pre-anthraquinones, anthrones, chromones, coumarins, and flavonoids [27]. Besides leaves and roots are also the site of storage for many interesting secondary metabolites such as anthraquinones, pre-anthraquinones, anthrones, chromones, and alkaloids [28].

Table 3: Secondary Metabolite Reported from *Aloe* Species

Class	Species (plant part)	References
Alkaloids		
Coniine (**8**)	*A.sabaea* (leaves)	[29]
Conhydrine (**9**)	*A. gillilandii* (leaves)	[30]
g-Coniceine (**10**)	*A.krapholiana*(leaves)	[31]
Anthraquinones		
Chrysophanol (**11**)	*A.megalacantha*(root)	[32]
Helminthosporin (**12**)		
Aloeemodin (**13**)		
Aloesaponarin II (**14**)		
Aloesaponarin I(**15**)		
Pre-anthraquinones		
Aloesaponol I(**16**)	*A.megalacantha*(root)	[33]
Aloesaponol III (**17**)	*A.saponaria*	[14]
Aloesaponol IV (**18**)	*(*subterranean parts)	
Aloesaponol-I-6-O-glucopyranoside (**19**)		
Aloesaponol-II-6-O-glucopyranoside (**20**)		
Aloesaponol-III-8-O-glucopyranoside (**21**)		
Anthrones		
Aloin A (**22**)	*A.castanea* (leaves exudate)	[33]
5-Hydroxyaloin A (**23**)	*A.ferox* (leaf exudate)	[34]
Aloinoside A (**24**)		
10-hydroxyaloin B (**25**)	*A.littoralis*	[35]
Deacetyllittoraloin (**26**)	(leaf exudate)	
Naphthoquinones		
6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**27**)	*A.dawei*(root)	[36]
Ancistroquinone C (**28**)		
5,8-dihydroxy-3-methoxy-2-methyl-1,4-naphthoquinone (**29**)		
Malvone A (**30**)		
Droserone (**31**)		
Droserone-5-methyl ether (**32**)		
Hydroxydroserone (**33**)		
Chromones		
8-C-glucosyl-(S)-*O*-aloesol (**34**)	A.*Vera* (leaves)	[37]
8-C-glucosyl-7-*O*-methylaloediol (**35**)		
8-C-glucosyl noreugenin (36)		
Aloesin (**37**)	*A. monticola* (leaf latex)	[38]
7-hydroxy-2,5-dimethyl-chromone (**38**)	*A.ferox (*leaf exudates*)*	[39]
furoaloesone (**39**)		



8) R=$CH_2CH_2CH_3$
9) R=$CH(OH)CH_2CH_3$

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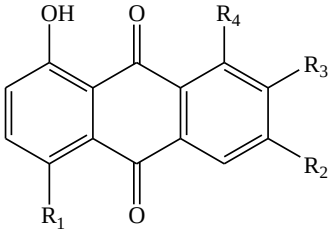
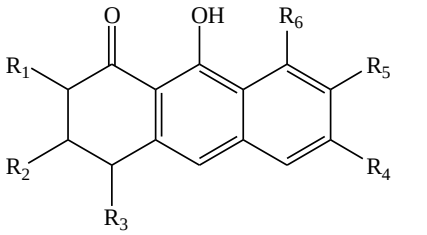
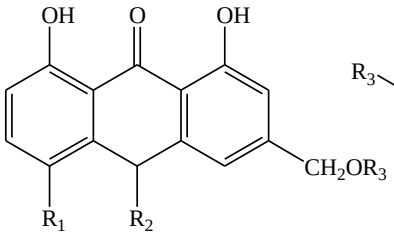


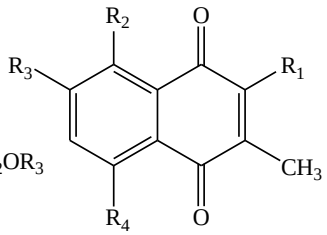
11) R_1=H=R_3=H,R_2=CH_3,R_4=OH
12) R_1=OH,R_2=CH_3,R_3=H,R_4=OH
13) R_1=H=R_3=H,R_2=CH_2OH,R_4=OH
14) R_1=H,R_2=OH,R_3=H,R_4=CH_3
15) R_1=H,R_2=OH,R_3=$COOCH_3$,R_4=CH_3



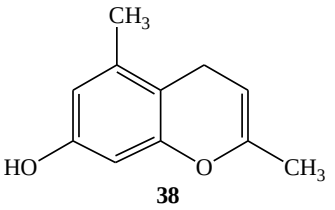
16) R1=R_3=H,R_2=R_4=OH,R_5=$COOCH_3$,R_6=CH_3
17) R_1=R_2=R_5=H,R_3=R_6=OH,R_4=CH_3
18) R_1=OCH_3,R_2=R_5=H,R_3=R_6=OH,R_4=CH_3
19) R_1=R_3=H,R_2=OH,R_5=OGlc,R_6=$COOCH_3$
20) R_1=R_3=R_5=H,R_2=OH,R_4=OGlc, R_6=CH_3
21) R_1=R_2=R_5=H,R_3=OH,R_4=CH3,R_6=OGlc



22) R_1=R_3=H,R_2=Glc
23) R_1=OH,R_2=Glc,R_3=H
24) R_1=H, R_2=R_3=Glc
25) R_1=H=R_3=H,R_2=Glc
26) R_1=H,R_2=Glc,R_3=$COCH(CH_3)$



27) R_1=R_2=OCH_3,R_3=OH,R_4=H
28) R_1=OH,R_2=R_3=OCH_3,R_4=H
29) R_1=OCH_3,R_2=R_4=OH,R_3=H
30) R_1=OCH_3,R_2=R_3=OH,R_4=H
31) R_1=R_2=OH,R_3=R_4=H
32) R_1=OH,R_2=OCH_3,R_3=R_4=H
33) R_1=R_2=R_4=OH,R_3=H



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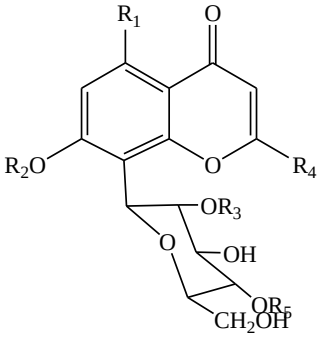

34) R_1=CH_3,R_2=H,R_3=H, R_4=$CH_2CH(OH)CH_3$,R_5=H
35) R_1=CH_3=R_2=CH_3,R_3=H,R_4= $CH(OH)CH(OH)CH_3$, R_5=H
36) R_1=OH, R_2=R_3=R_5=H,R_4= CH_3
37) R_1=CH_3,R_2=R_3=R_5=H,R_4=CH_2COCH_3

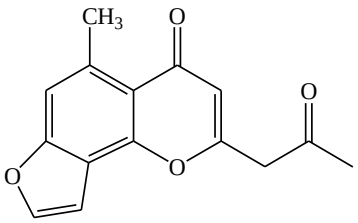

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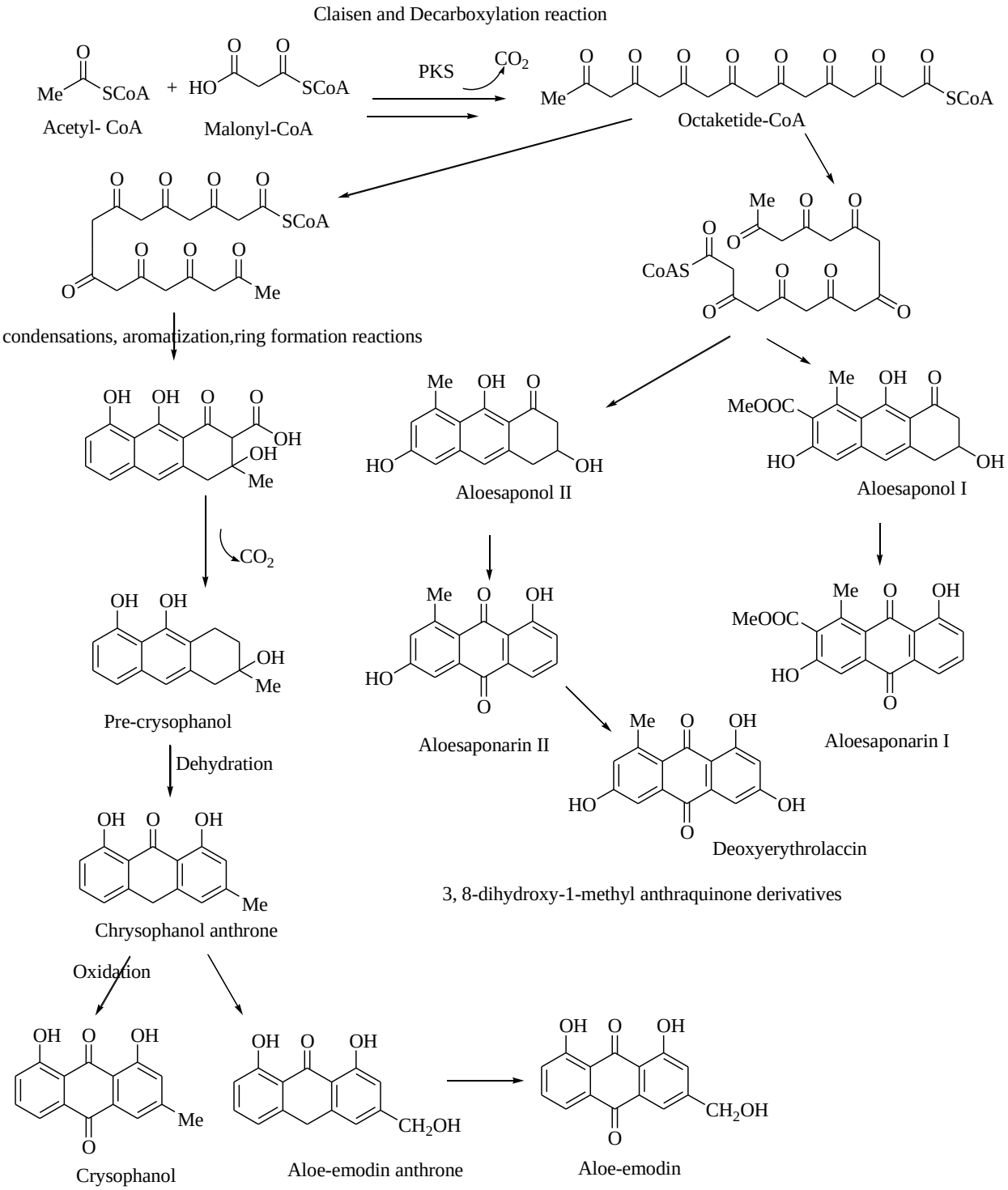
Figure 3: Some Secondary Metabolites Isolated from *Aloe* Plants

2.4. Biosynthesis of Anthraquinones

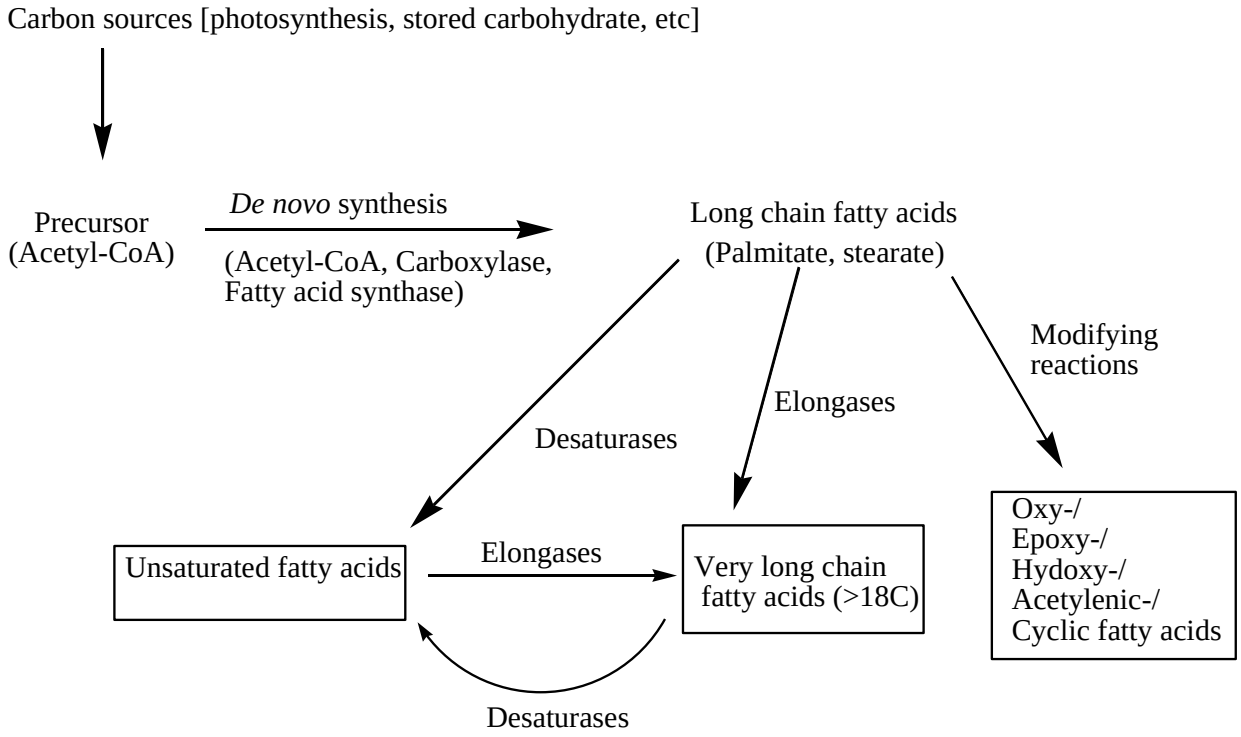
Plants produce their own individual range of chemical components through different biosynthetic pathways to survive, reproduce, and grow and to defend any possible harm in the ecological environment [40]. The quinones of plants in family Asphodelaceae including anthraquinones, and naphthoquinones are derived through the polyketide pathway which governed by the polyketide synthase enzymes. In anthraquinones formation these enzymes catalyze condensation of a number of acetyl units from malonyl-CoA into a growing polyketide chain. In this pathway acetyl-CoA and malonyl-CoA acts as the starter and extender unit respectively, to form a linear octaketide intermediate. This linear octaketide intermediate fold into different ways to form two types of anthraquinone derivatives namely 1, 8- dihydroxy-3-methyl anthraquinone and 3, 8-dihydroxy-1-methyl anthraquinone (scheme 1). In both pathways, after the polyketide chain folded, the ring at the center of the fold is formed first, followed by the next two rings. In general different reactions such as claisen condensations, decarboxylation, aromatization, dehydration, and oxidation reactions are involved in the formation of anthraquinones.



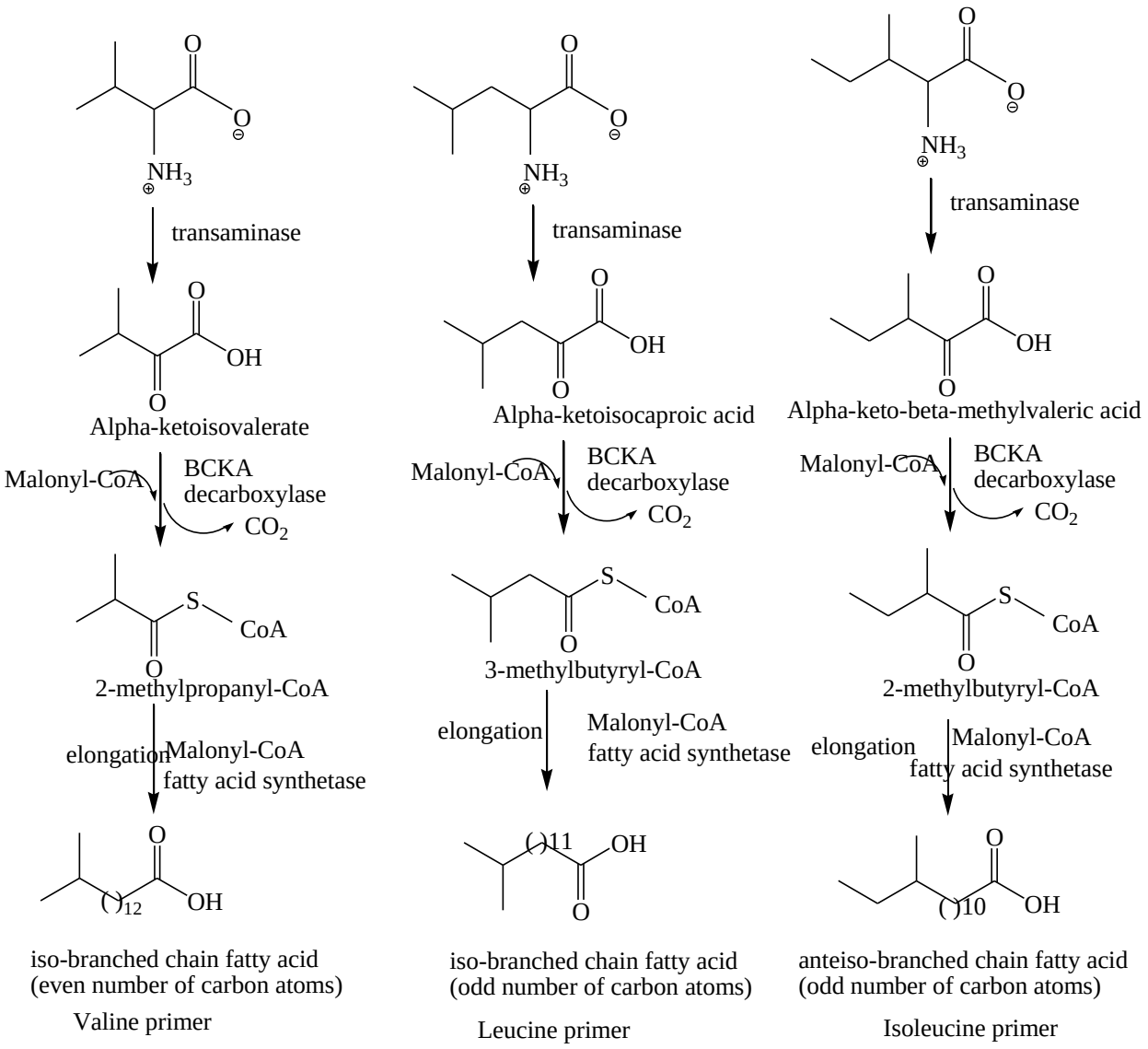
Scheme 1: Biosynthesis of Anthraquinones [41]

2.5. Biosynthesis of Fatty acids

Plants synthesize fatty acids from precursors derived from photosynthesis by using two enzyme systems; acetyl-CoA carboxylase, and fatty acid synthase. The end products of this synthesis are usually palmitate, and stearate with the latter predominating. Once a 16:0 carbon fatty acid has been formed, it can undergo a number of modifications, resulting in desaturation and elongation [42]. Fatty acids synthetases enzymes from various sources are divided into two groups on the basis of their physical structure; (typeI) and (type II). Type I includes fatty acid synthetases from animals yeasts and Fungus. Type II includes fatty acid synthetase from plants and bacteria [43]. Alkyl-branched fatty acids (alkyl FA) are frequently found in all taxa; microorganisms, plants, and animals. Iso- or anteiso-methyl branched FAs (iso- and anteiso FA) are the most common FAs, found in bacteria and plants. In the synthesis of these branched fatty acids, amino acids such as leucine, valine, and isoleucine are used as precursors [44].



Scheme 2A: Biosynthesis of non-branched fatty acids from carbohydrate sources



Scheme 2B: Biosynthesis of branched fatty acids from amino acid sources

2.6. Biological Activities of Genus *Aloe*

As literatures show, the genus *Aloe* has interesting biological activities including antimicrobial, antiplasmodial, antioxidant, anticancer, anti-inflammatory, and larvicidal activities.

2.6.1. Antimicrobial Activities

Number of *Aloe* species such as *A.vera*, *A. volkensii*, *A. secundiflora*, *A. ferox*, *and A. monticola* has been used for the treatment of infections and microbial diseases including stomache, anthrax, gonorrhea, pasterlosis, and tuberculosis [11, 24]. The interruption of the external epidermal barrier by a wound and burn allows microbes to enter and infect the wound there by causing inflammation and hindering wound healing. The antimicrobial activities of the *Aloe* species can be convinced through the use of the plants to combat this problem [45]. In addition to this the antimicrobial properties of these species can also be appreciated from good results of *in vitro* antimicrobial test of the plants extract against different strains of bacteria and fungi as showed in table 4.

Table 4: Antimicrobial Activity of *Aloe* species Extracts against Bacterial and Fungal Strains

Bacterial strains	***Aloe* species (plant part)**	**Zone of inhibition in mm**	**References**
Bacillus cereus	*A. rupestris* (leaf)	22.3 ± 1.52	[46]
Bacillus cereus	*A. maculatavar (*leaf*)*	21.0 ± 2.0	[47]
Salmonella typhiTy2	*A. monticola* (leaf latex)	15.0 ± 0.6	[39]
Escherichia coli	*A. vera* (leaves)	29	[47]
Pseudomonas aeruginosa	*A. vera* (leaves)	20	[47]
Pseudomonas aeruginosa	*A. pulcherrima* (root)	21	[2]
Fungal strains			
Candida albicans	*A. vera* (leaves)	20	[47]
Fusariumoxysporum	*A. volkensii* (leaf pulp)	21 ± 1	[11]
Aspergillusniger	*A. vera* (leaf)	23	[48]

Such genuine *in vitro* antimicrobial activity of the *Aloe* species may attribute to the presence of chromones, anthraquinones, and their derivatives. As their antimicrobial evaluation shows compounds such as chrysophanol (**11**), Aloe-emodin (**13**), Aloesin (**37**), and 7-hydroxyl-2,5-dimethyl-chromone (**34**) are promising antimicrobial agents [38, 49].

2.6.2. Antiplasmodial Activities

Antiplasmodial activity of both crude extract and isolated anthrones (Aloin A (**22**) from the leaf latex of *A. percrassa* was studied *in vivo* using Peter's 4-day suppressive test. After a four day treatment of *Plasmodium berghei* infected mice with the extract at doses of 100, 200 and 400 mg/kg/day; chemosuppression of 45.9 %, 56.8 % and 73.6 % was observed respectively for each doses. Aloin A/B showed chemo-suppression of 36.8, 51.1, and 66.8 % [50]. Compounds isolated from the roots of *A. pulcherrima*; chrysophanol (**11**), aloesaponarin II (**14**) and aloesaponarin I (**8**), were evaluated for their *in vitro* antiplasmodial activity using malaria SYBR Green I-based *in vitro* assay against chloroquine resistant (W2) and chloroquine sensitive (D6) strains of *Plasmodium falciparum*. The isolates; chrysophanol (**11**) (IC_{50} 21.05 ± 0.64), aloesaponarin II (**7**) (IC_{50} 5.00 ± 0.36) and aloesaponarin I (**15**) (IC_{50} 7.80 ± 1.11) showed considerable *in vitro* antiplasmodial activity against chloroquine-sensitive (D6) strain [2]. Moreover these anthraquinones; chrysophanol (**11**) (IC_{50} 36.09 ± 3.32), aloesaponarin II (**14**) (IC_{50} 18.60 ± 7.10) and aloesaponarin I (**15**) (IC_{50} 20.13 ± 5.12) have also showed significant antiplasmodial activity against chloroquine-resistant (W2) [2].

2.6.3. Antioxidant Activities

The antioxidant capacities of crude extract of *A. gilbertii* were evaluated by using reducing power determination method. The methanol, ethanol, and ethyl acetate root extracts of the plant showed good antioxidant activity with 244.5 ± 0.631, 241.5 ± 0.112, and 106 ± 1.05 mg of ascorbic acid per 10 mg dry weight of antioxidant in the reducing power, respectively [51]. The ethanol extracts of the peels of *A. vera* have also been reported to have high antioxidant activity with values of 2.43 mM ET/g MF (DPPH), 34.32 mM ET/g MF (ABTS), and 3.82 mM ET/g MF (FRAP) [52]. Total antioxidant activity was determined as the capturing of the DPPH• and ABTS •+ radicals, while the iron-reducing antioxidant power (FRAP) was analyzed by spectroscopic methods.

2.6.4. Anticancer Activities

Petroleum ether extract of *A. perryi* flowers was evaluated for its anticancer activity against seven human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2 and HeLa) using MTT assay. The percentage inhibition of the extract was reported to be 92.6 %, 93.9 %, 92 %, 90.9 %, 88.9 %, 82 % and 85.7 % for HepG2, HCT-116, MCF-7, A- 549, PC-3, HEp-2 and HeLa cells,

respectively [53]. The *in-vitro* anticancer activity of compounds isolated from *A. turkanensis* was evaluated using MTT assay against the human extra hepatic bile duct carcinoma (TFK-1) and liver (HuH7) cancer cell lines. The anthraquinone aloe-emodin (**13**) and the naphthoquinone 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4- dione (**29**) exhibited high inhibition against TFK-1 cell lines with IC_{50} values of 6.0 and 15.0 µg/mL (in TFK-1 cells) and 31 and 20 µg/mL (in HuH7 cell line), respectively [54].

2.6.5. Anti-inflammatory Activities

The anti-inflammatory activity of aqueous extract of *A. barbadensis* was investigated in rats using formalin- induced hind paw oedema. The results of the anti-inflammatory study revealed that 25, 50 and 100 mg/kg of the extract significantly reduced the formalin-induced oedema at the beginning of 3 hours [55]. The anti-inflammatory activity of aqueous extract *Aloe ferox* leaf was studied using carrageenan and formaldehyde-induced rat paw oedema. The extract exhibited potential anti-inflammatory activity (78.2 and 89.3% for carrageenan and formaldehyde-induced rat paw oedema, respectively) at the dose of 400 mg/kg [56].

2.6.6. Larvicidal Activities

The ethyl acetate soluble extract of *A. turkanensis* was reported to have high larvicidal activity against the common malaria vector, *Anopheles gambie*, where 100 % mortality was achieved at a concentration of 0.2 mg/ml and it had an LC_{50} of 0.11 mg/ml [57]. Crude extract of *A. vera* have been screened for its larvicidal activity against *Musca domestica*. Three instars larvae of housefly were treated with the different concentrations by dipping method for 24 and 48 hrs. The LC_{50} values of *A. vera* extract were found to be 32.67, 36 and 38.67 ppm in 24 hrs; 24, 25.67 and 28.33 ppm in 48 hrs on 1^{st}, 2^{nd} and 3^{rd} instars respectively [58].

2.7. Infectious Diseases, Management Strategies and Current Challenges

Infectious diseases are the second leading cause of death worldwide and the third leading cause of death in developed countries. These diseases are caused by viruses, bacteria, fungus, and parasites. Various strategies such as heightened surveillance; vaccine development, promotion of the rational use of antimicrobials, accelerated basic and applied research in the areas of microbial pathogenesis, vaccine and drug development, sequencing of the genomes of important pathogens have been used

to combat infectious diseases. Despite we have entered the in the era of army with current and future challenges of microbial pathogens through technological advances, the microbial world is extraordinarily diverse and possesses an adaptive capacity [59].

Among the pathogens bacteria are constitute a large domain of prokaryotic microorganisms on earth. They can be classified on the basis of cell structure, differences in cell components, such as DNA, fatty acids, pigments, and antigens. The Gram stain, developed in 1884 by Hans Christian Gram, characterizes bacteria based on the structural characteristics of their cell walls. The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink [60]. Gram-positive bacteria include *Bacillus cereus* and *Staphylococcus aureus* while Gram negatives could represented by *Escherichia coli*, and *Salmonella typhimurium*. *Bacillus cereus* is motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally and it is associated mainly with food borne illnesses, causing severe nausea, vomiting, and diarrhea[61].*Staphylococcus aureus* is the most dangerous of all of the many common staphylococcal bacteria and it is sphere-shaped bacteria mainly causing skin infections but can also cause pneumonia, heart valve infections, and bone infections [62]. *Salmonella typhimurium* and *Escherichia coli* are rod-shaped bacteria causing diarrhea, fever, and abdominal cramps [63]. Besides bacteria fungi can also cause various infection for example *Candida albicans* is an opportunistic fungal pathogen that commonly infects the vagina and results in intense pruritus.

The challenge associated with fighting these diseases is becoming worsened than ever before because of the fast development of resistance to the antimicrobial agents that are currently in market; emergence of resistant strains of a number of microbes, the changing nature of the infections observed in the elderly and other immune-compromised patients [64]. This demands emphasis on potential sources of antimicrobial substances including medicinal plants that are used for treatment of microbial infections like plants in genus *Aloe*.

3. Materials and Methods

3. 1. Chemicals

Solvents that were used in this study include petroleum ether, ethyl acetate, chloroform, acetone and methanol (LobaChemie Pvt Ltd, Mumbai, India) for extraction, gradient column elution, TLC analysis and gel filtration (sephadex LH-2) were analytical gradient. Silica gel 60-120 mm mesh size (Merck, Darmstadt, Germany) were used to pack column chromatography. Hydrochloric acid, ammonia solution, distilled water, chloroform, concentrated sulfuric acid; sodium hydroxide, acetic acid, ferric chloride, potassium iodide and sodium iodide were used during phytochemical screening tests. Mueller Hinton agar and nutrient broth was used as culturemedia and DMSO as a negative control. Gentamicin and chlotrimazole as standard antibiotic and antifungal drug respectively were used during antimicrobial evaluation. Deuterated chloroform and acetone were used for recording NMR spectra of the compounds.

3.2. Apparatus and Instruments

Pistil, mortar, weighing balances, Erlenmeyer flasks, filter papers, conical flasks, Rotary evaporator(Labo Rota 4000, Heidolph Instrument), TLC plates (pre-coated silica gel 60 F254 plates (Merck, Darmstadt, Germany),chromatography chamber, UV lamp, oven, glass columns, sephadex LH-20,beakers, disks, autoclave and NMR (Bruker Avance 400 Spectrometer)

3.3. Collection and Preparation of Plant Materials

The roots of *A. kefaensis* were collected from Jimma zone Oromia regional State, Agaro district, which is 390 Km away from Addis Ababa and 45 km from Jimma town. The selection and collection of the plant has been done by approaching traditional healer living in that district. After being collected it washed roughly with tap water, air dried, grained and powder placed in extraction containers. Identification of the plant has been made by botanist at Biology Department, Jimma University, Ethiopia and the voucher specimen CH8 has been deposited.

3.4. Extraction and Isolation

3.4.1. Extraction

The air-dried and ground roots (800 g) of *A. kefaensis* were sequentially extracted with petroleum ether, chloroform, acetone and methanol (2.5 L) using cold maceration for 48 hrs each. The extracts were concentrated using rotary evaporator.

3.4.2. Isolation

The choice of appropriate solvent to run column was made after carrying out the TLC analyses of the extract in various combinations of solvent systems with different polarity. Based on TLC profile acetone (8 g) and dichloro methane (5.5 g) extracts were combined together and subjected to column chromatography packed with silica gel, which was eluted with petroleum ether containing increasing percentage of EtOAc to afford 69 fractions 200 mL each. The fraction eluted with 13% EtOAc in petroleum gave AK-1 (90 mg). The fraction eluted with 30%-33% EtOAc in petroleum ether gave AK-2 (50 mg) after the combined fractions was purified on Sephadex LH-20 (eluting with CHCl3/MeOH; 1:1). The fractions eluted with 35% EtOAc in petroleum ether gave AK-3 (40 mg) after purified on Sephadex LH20 (eluting with CHCl3/MeOH; 1:1). Fractions eluted with 65-100% of ethyl acetate in petroleum ether were combined and applied on small size column chromatography followed by further purification of the fractions on Sephadex LH-20 (eluting with CHCl3/MeOH; 1:1) yielded three compounds; AK-4 (35 mg), AK-5 (25 mg), and AK-6 (15 mg). The separation was monitored with the help of thin layer chromatographic (TLC) analyses. Detection has been done using ultraviolet light (254 and 365).

3 .5. Qualitative Analysis of Phytochemicals

Preliminary phytochemical analysis was carried out for the extracts using methods described below.

3.5.1. Alkaloids (Wagner's Test): 1 mL of extract treated with Wagner's reagent; formation of brown precipitate indicates presence of alkaloids [65].

3.5.2. Flavonoids (Alkaline Reagent Test): 2 mL of extract was treated with 20 % NaOH solution to form intense yellow color indicating presence of flavonoid [66].

3.5.3. Phenolic (Ferric Chloride Test): 1 mL of extract was treated with 5% ferric chloride solution; formation of blue-black color indicates the presence of phenolic compounds [67].

3.5.4. Tannins (Ferric Chloride Test): 2 mL extract was stirred with distilled water, heated on water bath and filtered. A few drops of 5% ferric chloride were then added. Black coloration has been taken as positive result for the presence of tannins [67].

3.5.5. Quinone (Sulphuric Acid Test): 2 mL of extract treated with concentrated sulphuric acid; formation of red color indicates presence of quinones [68].

3.5.6. Anthraquinones (AmmoniaTest): To 1 mL of extract little 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones [68].

3.5.7. Saponins (Foam Test): To 2 mL of extract, distilled water was added and shaken to form foam indicating the presence of saponins [68]

3.5.8. Terpenoids (Salkowski's Test): 1 mL of crude extract was separately shaken with chloroform followed by the addition of concentrated H_2SO_4 reddish-brown coloration of the interface has been taken to indicate the presence of terpenoids [69].

3.5.9. Anthraquinone glycoside (Borntrager's Test): To 2 mL extract solution, 5% H_2SO_4 was added, heated in a water bath, filtered then the filtrate was then shaken with an equal volume of chloroform and kept standing for 5 minutes. Then a lower layer of chloroform was shaken with dilute ammonia to form rose-pink to red color of the ammoniacal layer indicating the presence of anthraquinone glycosides [69].

3.6. Structural Elucidation

The structure of the isolated compounds was elucidated using NMR (^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra) spectroscopy and comparison with the literature data. Chemical shifts were reported in ppm. All spectroscopic analysis were run at the Department of Chemistry, Addis Ababa University.

3.7. Antimicrobial Assay

3.7.1. Antibacterial Activity Test

The extracts and isolates were screened for *in vitro* antibacterial activities against four bacteria strains (*Escherichia coli* (ATCC25922), *Bacillus cereus*, *Salmonella Typhimurium (ATCC13311) and Staphylococcus aureus* (ATCC25923) by Agar disc diffusion method [38, 70] at Microbiology laboratory, Jimma University. The test solution with concentrations of 200 mg/mL and 10 mg/mL was prepared by dissolving the crude extract and compounds respectively in DMSO. The inoculums having similar turbidity with 0.5 McFarland were then swabbed uniformly over the Müeller-Hinton Agar medium with sterile swab. Then, the filter paper discs (6 mm in diameter prepared from Whatman no. 1 filter paper and sterilized by an autoclave at 121 ^{O}C for 20 min) were impregnated with the test samples and placed on the surface of the medium at appropriate distance from one another to avoid overlap of zones of growth inhibitions. Inhibition zone was measured in mm after 24 hour's incubation at 37 ^{O}C and compared with the standard drugs. 1% DMSO was used as a negative control and gentamycin (10 mg/ml) was used as standard antibacterial drug.

To see a concentration dependent effect, four serial concentrations of the DCM extract (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) were prepared through double serial dilutions of the stock solution and the tests were performed following the same procedure [71]. Further dilution of the extract (6.25mg/ml -0.78125 mg/ml) was also made to study its activity against *Salmonella typhimurium* [72].

3.7.2. Antifungal Activity Test

The antifungal potential of the extract and compounds were also evaluated against *Candida albicans (ATCC14053)* by Agar disc diffusion method [38, 70]. The tests were conducted following similar procedure except the petridishes was incubated at room temperature for 72 hours and clotrimazole (25 mg/ml) has been used as standard drug. Concentration dependent effect for DCM extract has also been conducted by using serial dilution.

4. Results and Discussion

4.1. Percentage Yields of the Crude Extracts

Air-dried roots of *A. kefaensis* (0.8 Kg**)** were extracted sequentially with petroleum ether, dichloromethane, acetone and methanol. The resulting extracts were weighted and the percentage yield was calculated as given below. Relatively higher yield of extract was obtained with acetone (1.25 %, 10 g) as solvent and the lower with Petroleum ether (0.4 %, 3.2 g) as depicted in table 5. Percentage yields of the acetone (1.25 %, 10 g,) and methanol (7 g, 0.88 %) extracts obtained from this plant is greater than the yield obtained from *A. pulcherrima roots* (0.81 Kg) which were sequentially extracted with hexane, chloroform, acetone and methanol to yield 2.0 g (0.25 %), 3.2 g (0.39 %), 4.9 g (0.61 %) and 4 g (0.49 %), respectively as reported by Abdissa *et al*. 2017 [2]. This difference in yields might be due to species variation, and amount of sample used in extraction.

$$\%\ \textbf{yield} = \frac{\textbf{weight of the crude extract (g)}}{\textbf{weight of the sample used for extarction (g)}}\ \textbf{x 100}$$

Table 5: "Percentage Yield of the Crude Extracts from *A. kefaensis* Roots (0.8 Kg)

Type of extract	Mass of crude extract (g)	Percentage yield (%)
Petroleum ether	3.2	0.40
Dichiloromethane	7.5	0.93
Acetone	10	1.25
Methanol	7	0.88

4.2. Qualitative Phytochemical Screening

As phytochemical screening shows various classes of secondary metabolites are present in petroleum ether, dichloromethane, acetone, and methanol extract (Table 6). From screening, it has been observed that quinones were present in all solvents; alkaloids and terpenoids in all extracts except in petroleum ether; Anthraquinone glycoside and tannins in acetone and methanol extract; Whereas, saponins were detected only in methanol extract. Phenolic compounds have been confirmed to be present in petroleum ether extracts that might be pertain to free anthraquinones, which can be obtained with less polar solvents such as hexane and petroleum [73].

Table 6: Qualitative Phytochemical Analysis of *Aloe Kefaensis* Roots Extracts

	Extracts			
phytochemical	**Petroleum ether extract**	**Dichloromethane extract**	**Acetone extract**	**Methanol extract**
Alkaloids	-	+	+	+
Flavonoids	-	-	-	-
Phenolic	+	++	++	++
Tannins	-	-	+	+
Quinones	+	+	+	+
Anthraquinones	+	++	++	++
Terpenoids	-	+	++	+
Saponins	-	-	-	++
Anthraquinone glycosides	-	-	+	++

Key: + = the presence of phytochemical constituents; ++=strong presence; - =the absence of phytochemical constituent

4.3. Structural Elucidation of Isolated Compounds

The chromatographic separation of the combined acetone-dichloromethane extracts of the roots of *A. kefaensis* was resulted in isolation of six compounds (AK-1-AK-6). Their purity has been determined by TLC analysis. However, only two compounds (AK-2 and AK-3) have been characterized structurally. The physical data and the TLC analysis of the compounds have been indicated in table 7 and appendix 2.

Table 7: Physical data of Isolated Compounds

Compounds	**Mass of cpds (mg)**	**Color and state**	**% EA(column)**	**Rf (PE:EA)**
AK-1	90	yellow amorphous	13	Rf=0.2 (9:1)
AK-2	50	Pale yellow amorphous	30-33	Rf=0.44 (9:1)
AK-3	40	yellow amorphous	35	Rf=0.413 (7:3)
AK-4	35	Color less solid	65-100	Rf=0.413 (6:4)
AK-5	25	Color less solid	65-100	Rf=0.538 (7:3)
AK-6	15	red amorphous solid	65-100	Rf=0.457 (6:4)

4.3.1. Characterization of Compound AK-3

Compound AK-3 was obtained as a yellow amorphous substance with an Rf value of 0.413 in PE: EoAc (7:3) solvent system. The ^{1}H NMR spectrum showed the presence highly down field shifted proton signal at δ_H 13.32 indicating the presence of hydroxyl group involved in hydrogen bonding. It also showed four aromatic protons at δ_H 6.64 (d, J = 2.5 Hz), 7.09 (d, J = 2.7 Hz), 7.18 (d, 2.5 Hz) and 7.57 (d, J = 2.7 Hz) ppm, two set of meta-coupling aromatic protons. This could only possible when every two aromatic rings are present. Up-field shifted proton signal at δ_H 2.76 integrated for three proton is also indicating the presence of aromatic attached methyl group *peri* to carbonyl group, as evidenced from its chemical shift. The ^{13}C NMR spectrum of compound AK-3 showed signals for protonated carbons at δ_C 125.5, 113.1, 109.2, 107.9, one aromatic CH3 group at δ_C 24.1, and only two quaternary carbons at δ_C 162.7 and 146.3 due to the small amount of the sample. These spectroscopic data in comparison with the reported literature, compound AK-3 was identified as 1,3, 6-trihydroxy-8-methylanthracene-9, 10- dione (deoxyerythrolaccin) which has previously been reported from the roots of *A. vera* and used as dyes for polyester fabrics [74].

Table 8: ^{1}H (400 MHz) NMR data of Compound AK-3

Position	**Compound AK3**		**Deoxyerythrolaccin [69]**	
	$^{\delta}H$		$^{\delta}H$	
2	6.64	d, J = 2.5 Hz	6.62	d, J = 2.5 Hz
4	7.18	d, J = 2.5 Hz	7.16	d J = 2.3 Hz
5	7.57	d, J = 2.7	7.55	d ,J = 2.8 Hz
7	7.09	d, J = 2.7,0.8	7.05	d, J = 2.5 Hz
8-CH3	2.76		2.75	S
1-OH	13.32(chelated)		13.30	S

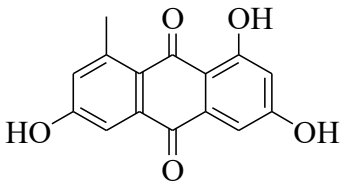


Figure 4. Proposed structure of Compound AK-3

4.3.2. Characterization of Compound AK-2

Compound AK-2 was isolated as a pale yellow amorphous substance with an Rf value of 0.44 in (9:1 petroleum ether: ethyl acetate) solvent system. In the ^{1}H NMR spectrums the peaks at δH 0.90 and 0.91 are due to a methyl group. The peaks between 1.25-2.34 are due to long chain methylene groups. The strong signals at δ_H 2.34, 2.36, 2.38, and 2.51 are due to methylene protons at allylic position. The multiplet signals at δ_H 5.36- 5.41 corresponds to vinyl protons. ^{13}C NMR spectrum of compound AK-2 showed signals for a total of 29 carbon atoms, the signal at δ_C 14.2 and 14.3 are due to the methyl group, peak at δ_C 34.3 is due to the methylene carbon atom α to the carbonyl group, the bunch of signals between 22.7 and 32.1 are due to long chain methylene carbons. Four signals along with depth spectrum in the unsaturated carbon region, at 127.9, 128.0, 130.0, and 130.2 are due to olifinic carbon atoms. Based on these spectral data and by comparing with literature data of analogous compound which has previous reported from Sponge *Geodinella robusta*, AK-2 was proposed to be (5Z, 9Z)-26-methyloctacosa-5, 9-dienoic acid [75]. This is the first report of such compound from plants.

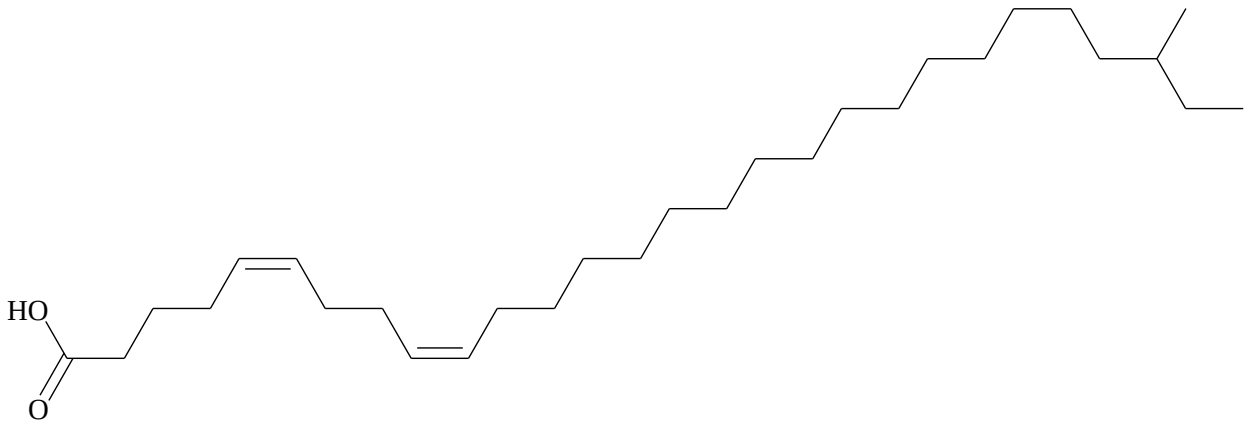


Figure 5. Proposed structure of Compound AK-2

Table 9: ^{1}H NMR and ^{13}C Spectroscopic Data for Compound AK-2

	Compound AK-2		**(5Z, 9Z)-22-methyl-5, 9-tetracosadienoic acid [70]**		
Position	δ_H	δ_C	Position	δ_H	δ_C
1	9.77	-	1	-	178.7
2	2.80	34.3	2	2.76	33.2
3	1.64	25.6	3	1.70	24.6
4	2.36	27.3	4	2.37	26.5
5	5.36-5.41	128.0	5	5.30–5.40	128.5
6	5.36-5.41	130.3	6	5.30–5.40	130.6
7	2.38	27.2	7	2.35	27.3
8	2. 51	27.2	8	2.48	27.3
9	5.36-5.41	128.2	9	5.30-5.40	128.9
10	5.36-5.41	130.1	10	5.30-5.40	130.5
11	2.34	29.0	11	2.32	27.4
12-25	1.27-1.63	29.2-29.7	12-21	1.20–1.40	29.4–30.1
26	1.43	34.0	22	1.51	34.4
27	1.27	32.0	23	1.25	36.7
28	0.90	14.2	24	0.85	11.4
29	0.91	14.3	25	0.84	19.2

4.4. Antimicrobial Activities of Extracts from Roots of *Aloe kefaensis*

The petroleum ether, dichloromethane, acetone, and methanol roots extracts of *A. kefaensis* were screened for their *in-vitro* antimicrobial activity against four bacterial (*E. coli*, *S. aureus*, *B. cereus*, *S. typhi*, and one fungal strain (*C. albicus*) by agar disk diffusion method. The activities of the extracts were assessed by the diameter of the zone of inhibition in millimeters by taking the extracts at concentration of 200 mg/ml. The zones of inhibition for different extracts fall between 9.0 ± 0.71, and 23.0 ± 0.28 mm as compared to gentamicin ranging between 20.0 ± 0.71, and 25.5 ± 0.71 mm as indicated in table 10 and appendix 9. The generate data was taken from two separate experiments in duplicate.

Table 10: *In-vitro* Antimicrobial Activities of the Extracts

Test sample Type		**Strains**				
		S. aureus	*B. cereus*	*E. coli*	*S. typhi*	*C. albicans*
Extracts (200 mg/ml)	PE	9.0 ± 0.71	NI	8.0±0.28	13.0±0.42	10.0±0.56
	DCM	20.5 ±0.71	17.0±0.71	18.5±0.71	23.0±0.28	20.5±0.71
	AC	18.0±0.56	16.0±0.84	15.5±0.71	22.0±0.56	18.5±0.71
	ME	NI	NI	NI	14.0±0.71	9.0±0.42
Serial dilution of DCM	100mg/mL	16.0±0.42	11.0±0.71	17.5±0.71	18.0±0.28	15.0±0.71
	50mg/mL	13.0±0.71	10.0±0.28	16.0±0.28	15.5±0.71	15.0±0.28
	25mg/mL	7.0±0.28	8.0±0.42	11.0±0.56	11.0±0.28	11.0±0.71
	12.5mg/mL	7.0±0.56	7.5±0.71	8.0±0.14	10.0±0.71	9.0±0.28
	G	23.5±0.71	23.0±0.71	20.0±0.71	25.5±0.71	NC
Controls	C	NC	NC	NC	NC	19.5±0.71
	D	NI	NI	NI	NI	NI

Key -: NI: No Inhibition;NC: Not Conducted;PE: Petroleum ether DCM:Dichloromethane; AC:Acetone , ME:Methanol; G: Gentamicin;C: Clotrimazole; D:DMSO

The antimicrobial activity result showed that the dichloromethane and acetone extracts have better against all tested strains. The superior antimicrobial activity was exhibited by dichloromethane extract with zone of inhibition of 17.0 ± 0.71, 18.5 ± 0.71, 20.5 ± 0.71, 20.5 ± 0.71, and 23.0±0.28 mm against *B. cereus, E. coli, S. aureus, C. albicans, and S. typhi*, respectively. Following it good activity, concentration dependent effect of the extract was performed with serially dilution and evaluated against all strains. As can be seen in table 10, different concentrations 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of the extract have shown varying activities against the strains. The serially diluted extract had revealed strong antimicrobial activity against tested organisms at high concentration (100 mg/ml, 50 mg/ml,).The highest dose (100 mg/ml) of the diluted extract revealed the maximum activity (18.0±0.28 mm inhibition zone) against *S. typhi* followed by inhibition effect (17.5±0.71mm) against *E. coli*.). More diluted serial concentrations, 6.25, 3.125, 1.5625, and 0.78125 mg/ml; showed the inhibition zone of 10.5±0.71, 9.0±0.42, 8.0±0.56, and 8.0±0.71, respectively against *S. typhi* (appendix 11). The results of this study has shown that higher concentrations (200 mg/ml potent than diluted concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) on all microbes.

As reported by Biniam *et al*. 2019 [76], the antimicrobial activity of the leaf extracts of *Aloe* species such as *A. camperi, A. elegansi*, and *A. eumassawana* improved against the *E.coli* and *S.*

aureus when the concentration of the extracts was increased. In addition to this, Kedarnath *et al*. 2013 [77] have reported that the chloroform extract of *A. vera* leaf to show highest inhibition zone at higher concentration. In this finding the DCM Extract at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml showed greater anti-bacterial activity against Gram-negative bacteria than Gram-positive bacterial strains. These results are in contrast to other researchers' findings who reported that most plant extracts have more activity against Gram–positive bacteria but it is in agreement with the *in-vitro* effects of *A.Vera* extract on the inhibition of the these bacteria as reported by Tsegaye *et al*. 2014 [78]. Such genuine *in vitro* antimicrobial activity of the extracts may attribute to the anthraquinones whose it's presence strongly detected in all solvents expect petroleum ether as shown in table 6. As the screening for their *in vitro* antimicrobial activity shows chrysophanol (**11**), and Aloe-emodin (**13**) were reported to be promising antimicrobial agents [49].

4.5. Antimicrobial Activities of Isolates from Roots of *Aloe kefaensis*

Owing to the promising antimicrobial effects of the extract, antimicrobial screening of isolates was also carried out for five compounds (AK-1, AK-2, AK-3, AK-4, and AK-5) against all strains as indicated in table 11. These compounds showed antimicrobial activity with varying degrees of potency. AK-3, AK-4, and AK-5 have displayed strong activity against *S.typhi* and *E.coli*. The activity of acetone and dichloromethane extracts could be because of the presence of these chemical components in the extracts. The isolated compounds also showed good antifungal activity against *C. albicans* with superior activity exhibited by AK-3 (deoxyerythrolaccin) as indicated in table 11. There is no report on antimicrobial activity of this compound against human pathogens but it has been tested against plant pathogens including *Xanthomonas campestris*, *Pectobacterium carotovorum* subsp and *Pseudomonas syringae* showing more activity against *Xanthomonas campestris* as reported by Rocio, BA *et al*,2019 [74]. The crude extracts were more active than purified compounds due the synergistic effect, since they have various phytochemicals (table 11 and table 10).

Table 11: *In-vitro* Antimicrobial Activities of the Isolated Compounds

	Compounds					**Controls**		
Strains	AK-1	AK-2	AK-3	AK-4	AK-5	G	C	D
S. aureus	12.0±0.42	14.0±0.28	13.0±0.71	16.0±0.56	13.0±0.42	23.0±0.71	NC	NI
B. cereus	13.0±0.28	14.5±0.71	13.0±0.56	12.5±0.71	13.0±0.42	26.0±0.71	NC	NI
E. coli	10.0±0.56	10.0±0.84	19.0±0.28	16.0±0.42	13.0±0.28	24.0±0.56	NC	NI
S. typhi	13.0±0.28	10.0±0.71	16.5±0.71	17.0±0.71	17.0±0.56	23.0±0.28	NC	NI
C. albican	13.0±0.71	8.0±0.42	17.5±0.71	14.0±0.71	10.0±0.28	NC	16±0.56	NI

Key -: NI: No Inhibition;NC: Not Conducted; G: Gentamicin;C: Clotrimazole; D:DMSO;AK-Compounds code

5. Conclusions and Recommendations

5. 1. Conclusions

In this study the root of *A. kefaensis* (0.8 Kg) were sequentially extracted with petroleum ether, dichloromethane, acetone and methanol. Based on TLC profile acetone and dichloro methane extracts were combined together and subjected to column chromatography packed with silica gel which was eluted with petroleum ether containing increasing percentage of EtOAc to afford 69 fractions 200 mL each. Further purification of fractions by using Sephadex LH-20 and small size column chromatography has resulted total of six compounds (AK-1, AK-2, AK-3, AK-4, AK-5, and AK-6). Among these AK-3, and AK-2 were selected for characterization and their structures determined on the basis of 1D NMR and literature data. The compounds were characterized as deoxyerthrolaccin, and (5Z, 9Z)-26-methyloctacosa-5, 9-dienoic acid, respectively. The extracts showed antimicrobial activity with acetone and dichloromethane extracts have significant antimicrobial activity against both gram negative and gram positive bacteria. The isolated compounds were also showed antimicrobial activity with AK-4, AK-3 and AK-5 showed more active against the test strains. This investigation has shown the potential of the monomeric anthraquinone deoxyerthrolaccin (AK-3) as a lead structure for the development of antimicrobial drugs, provided that further *in vivo* taste should be carried out. The antimicrobial activity of the crude extracts and isolated compound support the traditional uses of the plants for the treatments of microbial infections.

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5.2. Recommendations

Based on the current finding the following recommendations were forwarded:-

- ✓ Antimicrobial activities of isolated compounds and extracts should also be tested against other strains of pathogenic microbes.
- ✓ Phytochemical work with fully bioassay-guided fractionation is required on *A. kefaensis* to get more active ingredients.
- ✓ Structure elucidation of characterized compounds should be supported with EIMS (electron impact mass spectroscopy), and 2 D experiments such as COSY, HSQC, HMBC, and NOESY.
- ✓ Considering that the deoxyerthrolaccin showed good antimicrobial activity against test strains used in current work, it will be interesting to work *invovo* studies of this compound.

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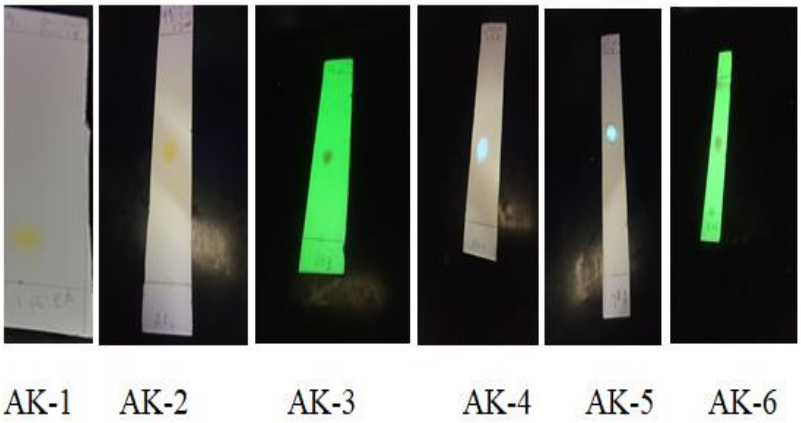
Appendices

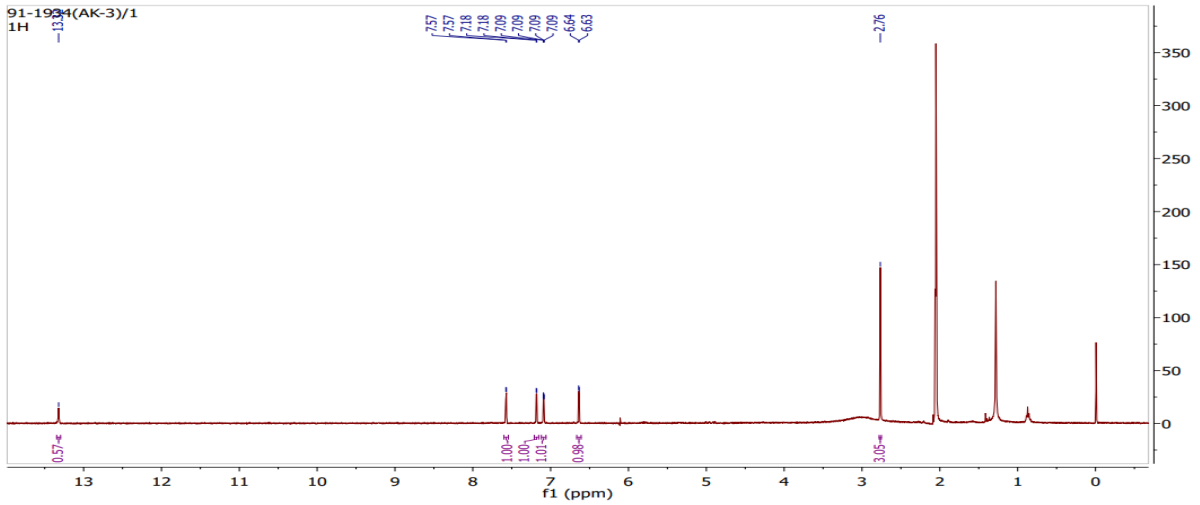
Appendix 1. Phytochemical Analysis of *A.kefaensis* Roots Extracts

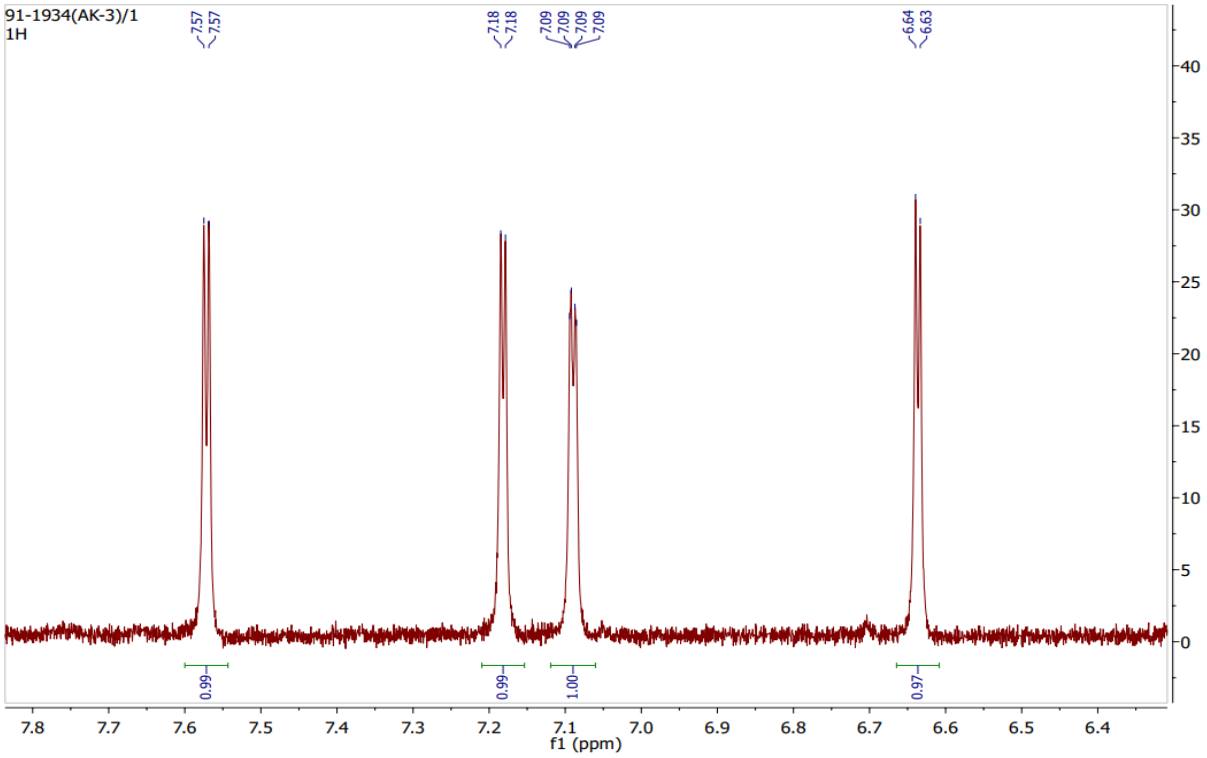
Appendix 2. TLC of isolated compounds



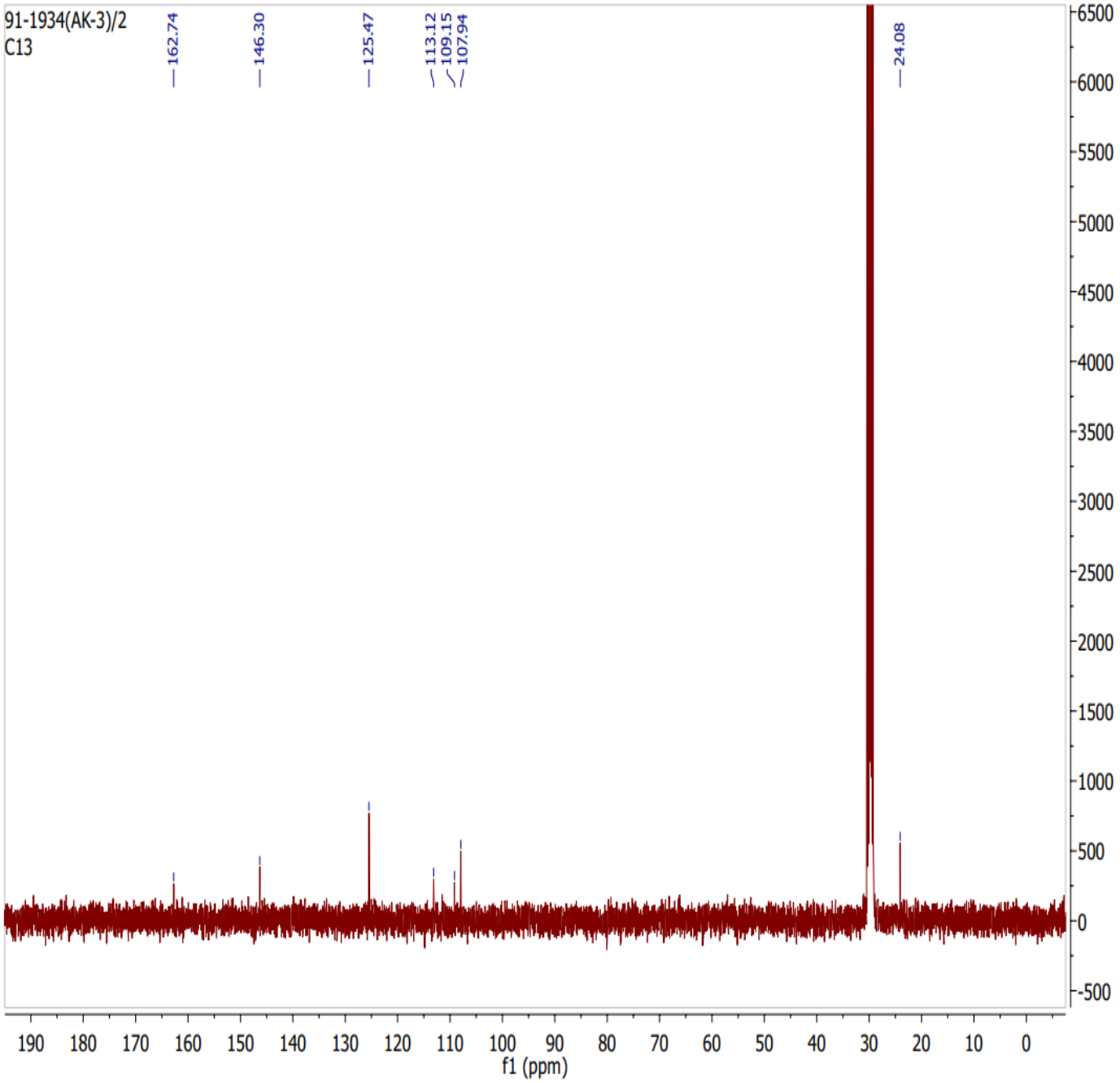
Appendix 3. ^{1}H-NMR Spectrum of compound AK-3 in acetone-d6

1H NMR (400 MHz, Acetone-d6) δ 13.32 (s, 1H), 7.57 (d, J = 2.7 Hz, 1H), 7.18 (d, J = 2.5 Hz, 1H), 7.09 (dd, J = 2.7, 0.8 Hz, 1H), 6.64 (d, J = 2.5 Hz, 1H), 2.76 (s, 3H).

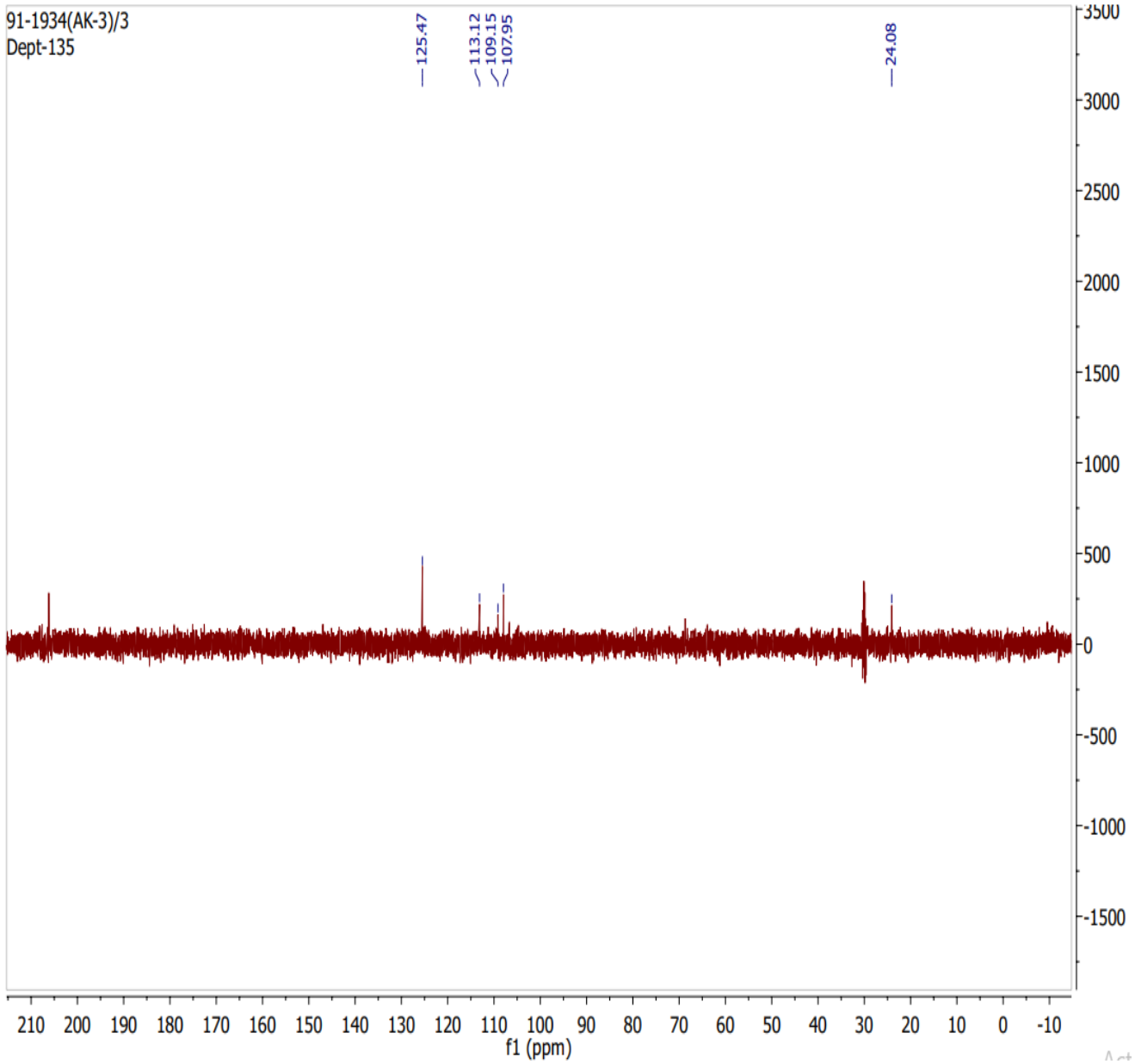




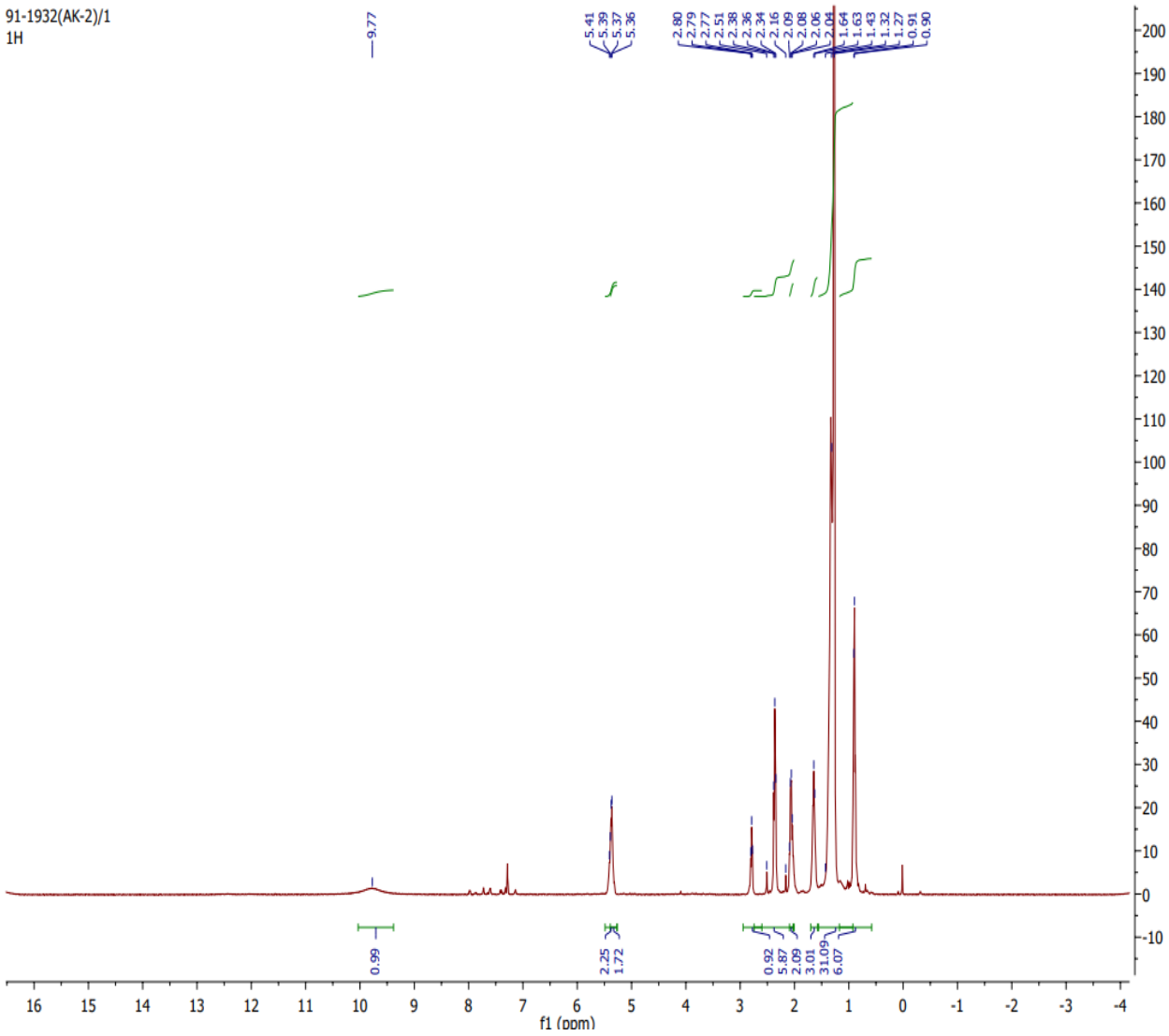
Appendix 4. ^{13}C-NMR Spectrum of compound AK-3 in acetone-d6



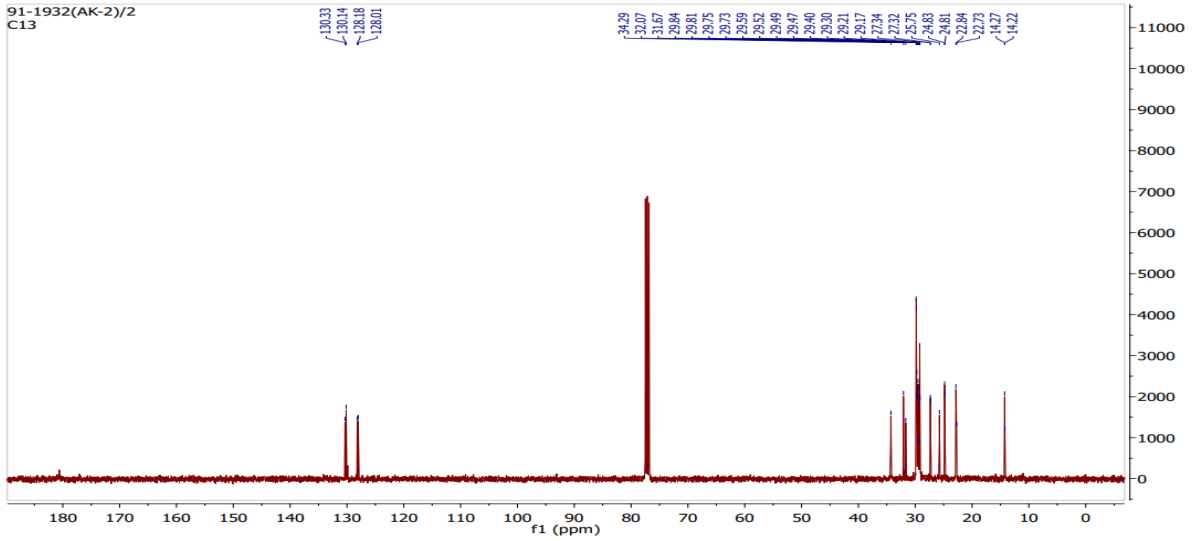
Appendix 5. DEPT-135 Spectrum of compound AK-3

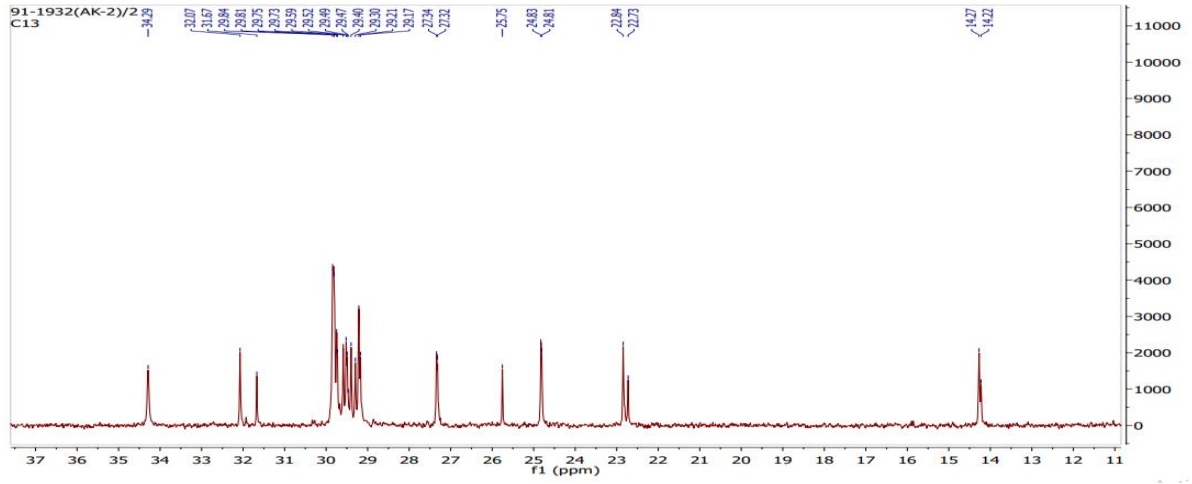


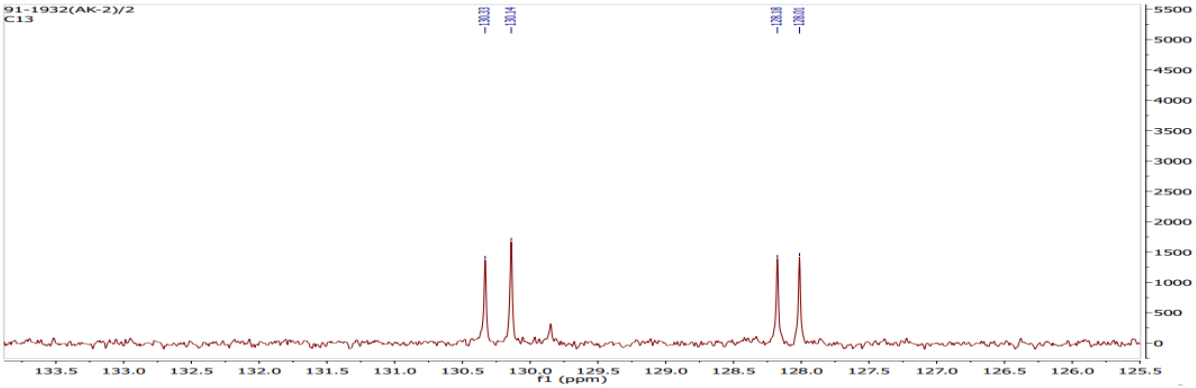
Appendix 6. ^{1}H-NMR Spectrum of compound AK-2 in CDCl3



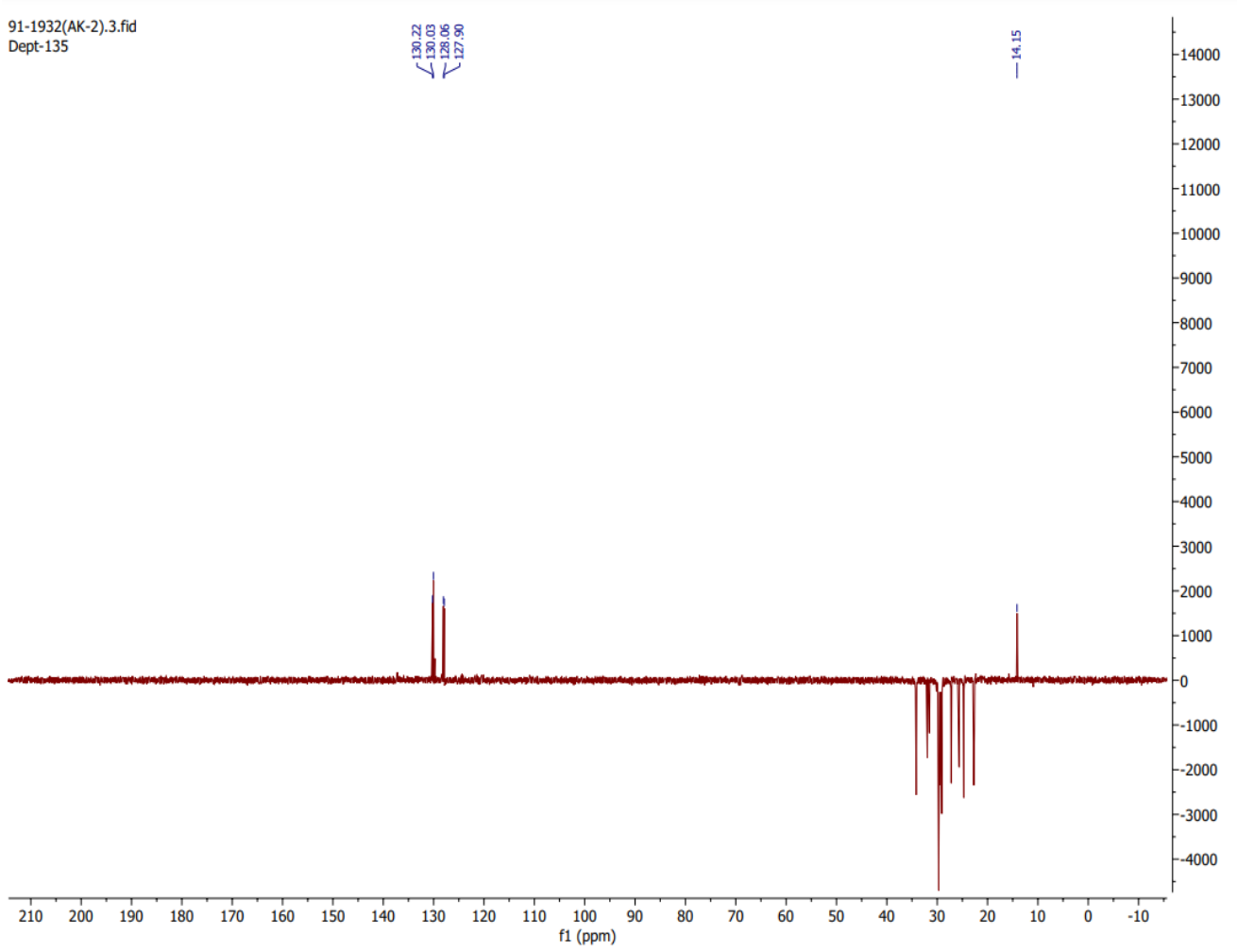
Appendix 7. ^{13}C-NMR Spectrum of compound AK-2 in CDCl3



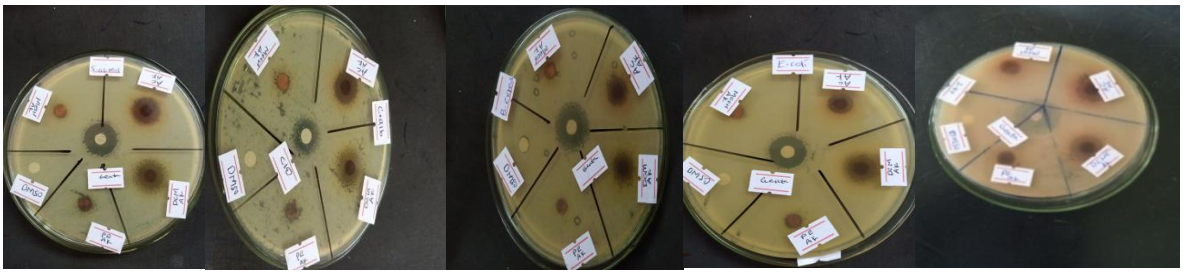




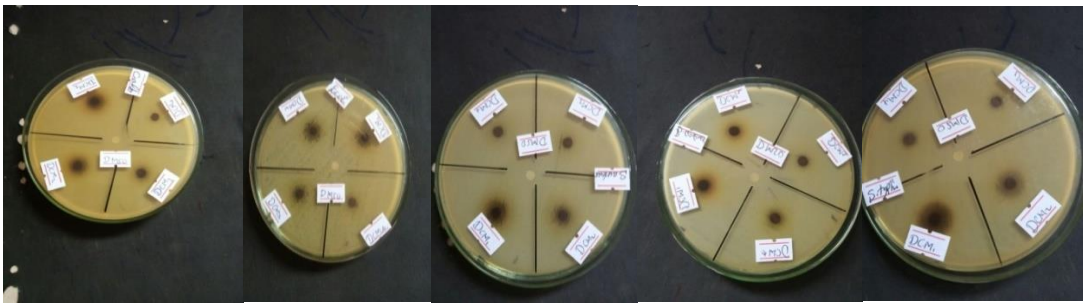
Appendix 8. DEPT-135 Spectrum of compound of AK-2



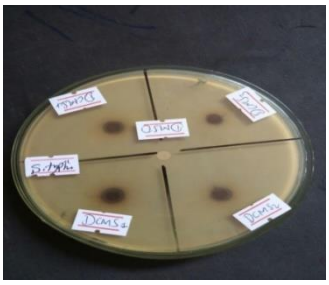
Appendix 9. Antimicrobial activity test of Crude Extract's Zone of Growth Inhibition



Appendix 10. Antimicrobial Activities of Different Concentrations of DCM Extracts



Appendix 11.Anti-Salmonella Activities ofvarious Concentrations of DCM Extracts



Appendix 12. Antimicrobial Activities of the Isolates