Phytochemical Investigation and Antibacterial activity of Stem Bark Extract of *Cordia africana*

BY: Getahun Abraham

A Thesis Submitted to Program of Applied Chemistry

School of Applied Natural Science

Submitted in Partial Fulfillment of the Requirement for the Degree of

Masters of Science in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama Science and Technology University

October, 2018

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Co-Advisor: Yadesa Melaku (PhD)

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Approval Sheet

Submitted by

Name	Signature	Date

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Getahun Abraham. We recommended the paper to be submitted as fulfilling the requirements for the Degree of Master of Science in Chemistry (medicinal Chemistry).

Advisor	Signature	Date
Co- Advisor	Signature	Date

We, the undersigned, members of the board of examiners of the final open defense by Getahun Abraham have read and evaluated his thesis entitled "Phytochemical Investigation and Antibacterial activity of Stem Bark Extract of *Cordia africana*" 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 Applied Chemistry (Medicinal Chemistry).

Examiner-1	Signature	Date
Examiner-1	Signature	Date
Chair person	Signature	Date
Department Head	Signature	Date

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for any degree in any other university and has been submitted in partial fulfillment of the requirements for the degree of Master of Science Adama science and Technology University.

Name: Getahun Abraham

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as a university advisor.

Name: _____

Signature: _____

Date and place of submission: Office of Research and Graduate Programs

Adama

September, 2018

DEDICATION

To my parents, my father and mother and friends and also I dedicate this work as an expression of my deepest respect.

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I owe my deepest respect and humble gratitude to the Almighty God for granting me his sustained loving care, invigorating sprits, strength, patience, and hope during the course of this long and testing work.

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TABLE OF CONTENT

Table of Contents **pages**

ABBREVIATIONS

^{13}C NMR	Carbon-^{13}Nuclear Magnetic Resonance
^{1}H NMR	Proton-Nuclear Magnetic Resonance
CC	Column chromatography
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
HTS	high Throughput Screening
MIC	Minimum Inhibitory Concentrations
NCEs	New Chemical Entities
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

ABSTRACT

Drug discovery involves the identification of new chemical entities of potential therapeutic value, which can be obtained through isolation from natural sources. The use of plants as medicines has a long history in the treatment of various diseases. Cordia africana is one of the main sources of traditional medicines serving to cure different diseases for human being and domestic animals. For example, it helps to cure cough, toothache, stomach ache, fire burn, Michi, diarrhea, jaundice, eye infection, tonsillitis, eye-problem, anthrax of cattle, treat tuberculosis, asthma, skin rash, smell of foot, scabies. In this study, the stem bark of the plant was successively extracted using n-hexane, chloroform and methanol. Methanol extract was found to be more active against bacteria indicating that the antibacterial component(s) of the sample may be residing in the methanol extract. The methanol crude extract was fractionated and purified using column chromatography with increasing polarities of chloroform-methanol mixture through frequent TLC analysis and two compounds were isolated and characterized. The structure of the isolated compounds were characterized by using ^{1}*HNMR* ^{13}*CNMR, and DEPT experiments. The crude extracts and isolated compounds were evaluated for their in vitro antibacterial activity against Staphylococcus aureus, a Gram-positive bacteria and three Gram-negative strains Escherichia coli, Pseudomonas aeroginosa and Kleblesia pnuemonia using agar diffusion method. Crude extract of chlorofrom and n-hexane displayed pronounceable activity against S. aureus and E. coli with inhibition zones of 8mm and 9mm respectively, while the methanol extracts displayed superior activity with 12 and 10 mm zone of inhibition against S. aureus and E. coli respectively. In this study* two compounds isolated, (2) were isolated. *The result is significant compared with ceftriaxone used as positive control and DMSO used as negative control*.

Keywords: Antibacterial activities, *Cordia africana*, Isolation of compounds,

1. INTRODUCTION

1.1. Background of the study

Drug discovery involves the identification of new chemical entities (NCEs) of potential therapeutic value, which can be obtained through isolation from natural sources, through chemical synthesis or a combination of both. The field of natural products drug discovery, despite the success stories of penicillin, paclitaxel, etc., also had aspects that made it less attractive. In the traditional approach, drug targets were exposed to crude extracts, and in case of evidence of pharmacological activity the extract was fractionated and the active compound isolated and identified. This method was slow, labor intensive, inefficient, and provided no guarantee that a lead from the screening process would be chemically workable or even patentable [1]. As natural products usually are molecules with more complex structures it was more difficult to extract, purify or synthesize sufficient quantities of a NCE of interest for discovery and development activities. Enriched or pure material is needed for the initial characterization of the chemical and biological properties as well as the elucidation of structure-activity relationships in drug discovery studies; furthermore, even larger quantities need to be supplied for potential later development activities and ultimately the market [2].

The pharmaceutical industry's interest in natural products diminished with the advent of such promising new technologies like combinatorial chemistry and high throughput screening (HTS) [3]. The prospect of such disciplines, aimed at accelerating drug discovery efforts for NCEs, led some companies to dismiss their natural product programs. The compounds in development today target a variety of indications, mainly cancer and infectious diseases (bacterial, viral, fungal, and parasitic), but also address other therapeutic areas such as cardiovascular diseases, neurological illnesses and depression, metabolic diseases (like diabetes and cholesterol management), and inflammatory diseases (like arthritis) [4, 5]. The cytotoxic properties of many secondary metabolites from marine organisms and bacteria are of particular interest for the development of new anticancer treatments. For infectious diseases, natural products are effective because most of these compounds evolved from microbial warfare and show activity against other microorganisms at low concentrations.

One of the most important sources used for natural products in drug discovery are medicinal plants. Plants produce two types of products, primary and secondary metabolites, as part of their normal metabolism. Medicinal properties of plants are due to the combinations of secondary metabolites such as alkaloids, steroids, tannins, and phenolic compounds that are synthesized and deposited in specific or in all parts of the plant. Composition of secondary metabolites varies between (i) tissues such as the bark,

heartwood, roots, branch basses and wound tissues have higher concentration, (ii) species and (iii) seasons [6]. The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants considered as a rich resources of ingredients which can be used in drug development and synthesis. Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field.

Medicinal plants frequently used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti malaria medications, contain ingredients from plants. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future diseases. Of the 119 plant derived drugs commonly in use,74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine [7].

The use of plants as medicines has a long history in the treatment of various diseases. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Approximately 20% of known plants have been used in pharmaceutical studies, impacting the health care system in positive ways such as treating cancer and harmful diseases [8]. Plants are able to produce a large number of diverse bioactive compounds. Medicinal plant extracts and phytochemical constituents present in the plant tissues with well-known antimicrobial properties play an important role in promoting human health and are nontoxic to the human body [9]. Plant extracts work in synergy with synthetic antibiotics against drug resistant bacteria [10]. They can be defensive substances such as phytoalexins and phytoanticipins, anti-feedants, attractants and pheromones [11].

Different medicinal plants are used in the world today, but we do know that medicinal plants are enormously important in both traditional and modern medicine. Ethnobotany, the study of traditional plant use is a field of growing interest to research scientists and pharmaceutical companies looking to develop new and more effective drugs. In general it has been estimated that over 40% of medicines have their origins in these active natural products [12]. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables [13]. The phytochemical studies have addressed extracting, measuring and identifying bioactive compounds of plants. As such, it is always a challenge for scientific researchers to evaluate safety and efficacy of the complicated plant matrix. Enhancing the knowledge of biological and pharmacological effects of plant constituent and

determination of the structures of the active principles may help in sustaining the use of these products [14].

Figure 1: Photo of C*ordia africana* taken by Getahun Abraham from Adama city on February 07/ 02/2018

1.2. Statement of the problem and Justification of the Study

There are many plants that are traditionally used for the treatment of different various human and domestic animal ailments. *Cordia africana* is one of the medicinal plants found in Ethiopia. Even though *C. africana* is a known medicinal plant in Ethiopia, to the best of my knowledge, there is no report hitherto, on the phytochemical investigations and antibacterial tests conducted on the stem bark of the plant. Different plants in the past have been a source of remedies for many diseases. But still there is need for new drugs to manage emerging and re-emerging diseases and finding new for supplements. Currently, microorganisms have tendency of developing resistance to antibiotics that are in the market. It is believed plants can possibly offer solution to such challenges. Therefore, this project is intended to fill this gap.

1.3. Significance of the study

In the looming era of antibiotic resistance, the human race is doomed to destruction unless new antimicrobial armaments are developed as promptly as possible. One potential source of new drugs for the emerging resistant strains of microbes is medicinal plants. Therefore, new compounds that would be isolated from this work could be used in this fight against resistant strains. In addition, large population in the world (80%) use traditional forms of treatment that is mainly plant based. This research can provide an evidence base for the traditional use of the plants by identifying their chemical constituents of the plant identification of species, extraction, separation, testing antibacterial through biological screening. This study also aims to engender further pharmacological studies on the active ingredients of the plant.

1.4. Objectives of the study

1.4.1. General objective

The general objective of this research is to investigate chemical constituents and their antibacterial activity of the stem bark extract of *Cordia africana*.

1.4.2. Specific objectives

1) To extract stem bark of *C. africana* using, n-hexane, chloroform and methanol.
2) To conduct phytochemical screening of the crude extracts.
3) To isolate compounds from the crude extracts of *Cordia africana.*
4) To characterize the isolated compounds using spectroscopic methods including, ^{1}H NMR, ^{13}C NMR and DEPT.
5) To investigate the antibacterial activity of the crude extracts as well as the isolated compounds.

2. LITERATURE REVIEW

2.1. Genus of *Cordia*

The first reports of the genus *Cordia*, Boraginaceae included botanical and reproductive characteristics. The genus C*ordia* encompasses about 250 species; the majorities are tree- or shrub-sized and native to the Americas, and they occur from Central America down to the central region of Argentina [15, 16]. They are widely distributed in tropical and subtropical regions of the world. Medium sized tree, deciduous, leaves simple, alternate, leathery in texture, variable in shape and size, broadly ovate or cut into margin, tip obtuse, base rounded or cordate. The plant goes in dormancy during peak winter months (December–January) and sheds its leaves during February–March naturally. It also shed off its leaves under unfavorable condition during May– June if not irrigated. Fruit is drupe, round shaped, green at maturity and yellowish brown to light pink at full ripening species of them. Due to the various ethno pharmacological reports attributed to species of the genus *Cordia*, numerous research groups have conducted studies to prove their pharmacological or biological properties through a variety of methods and assays in *vitro* and in *vivo* of pre-clinical nature, which are described in there are published reports describing through a wide variety of methods their diverse activities.

The bioactive and pharmacological properties of *Cordia* genus, its phytochemical constituents and is the most common classes of secondary metabolites identified. *Cordia africana* is one of the medicinal plants within the genus present at various geographical locations with a wide traditional use in Ethiopia. Research groups have carried out phytochemical studies resulting in the identification of different classes of secondary metabolites, as well the isolation of various constituents of different parts (root, stem, leaves, flowers and fruits) of various species of the genus *Cordia*. Work contributes scientifically to recognizing the importance of the genus *Cordia* as a target in the search for new biotechnological investments.

2.2. Medicinal uses of genus *cordia*

The genus *Cordia* is the source of large number of compounds and biological activities plant. Medicinal plants have important contributions in the healthcare system of local communities as the main source of medicine for the majority of the rural population. Plants have not only nutritional value but also, in the eyes of the local people, they have medicinal and ritual or magical values [17]. The ethno medicinal healing systems vary across cultures. In Ethiopia, there is cultural diversity with various patterns of using the flora [18]. One of medicinal plants are *Cordia* genus have important contributions in the healthcare system of local communities as the main source of medicine for the majority of the rural

population. The tropical species of *Cordia* are used widely as antiinflammatory for wound healing [20], antioxidant [21], for skin diseases, antimicrobial, antifungal, antiviral, astringent, antidiarrhea, digestive, antiulcers, anthelementic [20], analgesic, hoemostatic, antirhumatic, arthritic and also for treatment of snake-bite, rabies and malaria, antibiotic-modifying, antinociceptive, antifertility, toxicity, hypolipidemic, immunomodulatory and insecticidal. Some of the previous uses are proved scientifically and used medicinally and the others are common in folk medicine in their native countries.

Cordia africana is one of the main sources of traditional medicines serving to cure different diseases for human being and domestic animals. Several parts of the plant are used in traditional medicine. In Ethiopia, the medicinal uses of *Cordia africana* have been reported by different authors for example, it helps to cure, cough, toothache, stomach ache [22]; Fire burn, michi, diarrheal, jaundice, eye infection, tonsillitis [23] ; Eye-problem, anthrax of cattle [24]; treat tuberculosis, cough and asthma [23]; Skin rash, smell of foot, scabies [26].

2.3. Botanical description of *Cordia africana*

Cordia africana Lam. is a multipurpose tree species that belongs to the boraginaceae and is found widely distributed in tropical Africa. *C. africana* is adapted to sufficiently warm areas with an altitude ranging from 900 to 2400 m and a rainfall range of 700 to 2000mm per year. In Ethiopia, it grows in a wide range of agro-ecological zones, though its habitats are mainly montane forest ecosystems. The species serves as timber, coffee-shade tree, agro-forestry crop, honey bee plant and as a draught season food. Deforestation coupled with the extensive exploitation of the species for timber production has led to its depletion, and the Ethiopian government has banned its cutting from natural forests though the problem remains unabated. Research on genetic variation in *C. africana* has been initiated in order to find out whether the populations of the species in Ethiopia show distinct structures with respect to geographical locations, if forest fragmentation has affected the genetic structure of the species, and whether there are genetic markers that differentiate high quality timber trees from the rest of the population. Leaves have been collected from a total of 25 populations and 800 individuals, which represent different geographical locations, various ecosystems as well as contrasting phenotypes.

Cordia africana Lam. is a tree species native to tropical Africa and tropical Arabia. In Ethiopia, it is widespread in broad leaved Afromontane rain forests, undifferentiated (dry) Afromontane forests and in riverine forests as well as in the western lowlands [27]. Crown umbrella-shaped/rounded, dense, much branched; twigs velvety hairy, becoming glabrous Bole typically curved or crooked [28]. Bark surface smooth in young trees, becoming cracked or longitudinally fissured with age, pale brown to dark brown, inner bark fibrous, whitish, turning grayish to nearly blackish upon exposure [29]. Leaves leathery,

simple, alternate, broadly ovate to egg-shaped, rough to feel, dull dark green, rounded to cordate at base, rounded to acuminate at apex, margins entire to slightly toothed, pinnately veined with 5–7 pairs of lateral veins. Buds oval, stalk less, pleated open into flowers that are bisexual, white, sweet scented, shortly pedicel ate or sub sessile, massed in compact panicles covering the crown, with a white mass of attractive flowers; calyx less than 1cm long, strongly ribbed, back of lobes covered with short, soft, brown hairs; corolla lobes crinkled, white, long-exerted, funnel shaped, about 2.5cm long; cymes many flowered. *Cordia africana* begins flowering when a tree is 3-5 years old [30, 31]. It is monocious species with complete flowers (hermaphrodite) and is known to be pollinated predominantly by bees [29]. It can also grow under drier climatic conditions, by minimizing its water consumption through shading its leaves or by closing its stomata [31, 32]. *Cordia africana* has fast growth performance, is cultural and widely used and is adapted to local conditions [33, 31, and 34].

2.4. The genus *Cordia* pharmacological aspects

2.4.1. Antinociceptive activity

The evaluated the antinociceptive activity of the dichloromethane extract of leaves and stem of *C. curassavica* by using the acetic acid-induced writhing test in rats. Six rats were used in each test group, and the extract in doses of 100, 300 and 1000 mg/kg was administered 1h before of the injection of acetic acid. This biological activity was also evaluated by the hot plate test, but the dichloromethane extract did not show any activity, suggesting that the analgesic effect is limited to the inhibition of the inflammatory process [35].

2.4.2. Toxicity activity

This conducted a study with the objective of obtaining safety data in relation to toxicity of the extract of *C. salicifolia*, considering the extensive use of this species in Brazilian popular medicine. The extract was administered orally or intraperitoneally. There were no deaths or other signs and symptoms of toxicity up to the highest dose tested (2000 mg/kg body weight) in mice when administered orally, and necropsy after 7 days of oral treatment with the extract also did not reveal any pathological change. Lethality was observed only after intraperitoneal administration of 920 mg/kg body weight. In the rats, repeated oral administration (chronic toxicity) of different doses of extract (20, 100, 200 mg/kg) for 90 days did not produce signs characteristic of toxicity, and also, there were not deaths of the animals [36]. The results showed low oral toxicity of the extract of *C. salicifolia* and no evidence that it poses risk after prolonged use. Phototoxicity of the methanolic extract of *C.Verbenacea* was evaluated in an in *vitro* study done by [37], utilizing the

bacteria *E. coli* and *S.aureus*. This species showed significant phototoxic activity against *S. aureus*, but not *E. coli*.

2.4.3. Antinflammatory activity

The antinflammatory activity of the extract of *C. dichotoma* seeds was evaluated in a study carried out [38], where Wistar rats weighing 160–180g of both sexes were exposed to an inflammatory process (footpad edema) with the administration of carrageenan. One hour after the injection of this substance, the ethanolic and aqueous extracts were administered at different concentrations for each test group. At a dose of 500mg/kg, the aqueous and ethanolic extracts showed maximal inhibition of edema (69.52% and 58.09%, respectively) in relation to the control group, demonstrating the efficacy of the extract of *C. dichotoma* as an anti-inflammatory agent, which explains the wide use of this plant as an antiedema agent in popular medicine. Methanolic extract of *C. sinensi* and evaluated their antinflammatory activity also using rats with induced inflammation (footpad edema) by the administration of carrageenan. The petroleum ether extract did not show significant results. These authors utilized male Wistar rats (140–180 g) treated orally with the sesquiterpenes of *C. Verbenacea*: humulene and trans-caryophyllene.

2.4.4. Antioxidant activity

This study the antioxidant activity of the extract of *C. boissieri* using the DPPH free radical assay tested. Moderate antioxidant activity was observed on a chromatographic plate, where this species showed small spots or points with low intensity [39]. Insecticidal activity in evaluated the effect of the aqueous extract of *C. verbenacea* on the development of *Spodoptera frugi perda*, examining the variables duration and mortality for the larval and pupal periods, size, weight and presence of morphological alterations of the pupae and fertility of the adults. As a result, the larval state and pupal period of *S. Frugi perda* were prolonged, and morphological alterations in the pupae were also observed [40]. Antibiotic-modifying activity evaluated the antibiotic-modifying effect of the hexane and methanolic extracts of the leaves of *C. Verbenacea* on *norfloxacin* against *S. aureus.* The extracts at a sub-inhibitory concentration of 32g/ml improved the inhibition zone by 50% when combined with the antibiotic compared to the antibiotic alone. That is, the antibiotic activity of *norfloxacin* was enhanced in the presence of the extracts of *C. Verbenacea*, showing significant synergism [41].

2.4. 5. Antimicrobial activity of *C. africana*

C. africana of crude extracted in the treatment of bacterial and fungal in *vitro* tests showed. Test microorganism the antibacterial activity of plants extract was assessed against three bacterial species gram positive (+ve) bacteria *Staphylococcus aureus* (ATCC 25923) and two gram negative (-ve) bacteria such as *pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), in addition to two fungal species *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763).

The leaves of *C. africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (14-30mm). The largest inhibition against *Apergillus niger* give (30mm). The stem of *C.africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (14-20mm). The largest inhibition against *Candida albicans* gives (20mm). The bark of *C. africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (11-18mm). The largest inhibition against *Bacillus subtilis* gives (18mm). The bark of *C. africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (12-22mm). The largest inhibition against *Staphylococcus aureus* gives (22mm). It is clear from table (1) that the extracts of *C. africana* showed high activity all bacteria and fungi.

Table 1: Determination of the minimum inhibitory concentration (MIC) of methanolic extracts of different parts of *C. africana* against standard organism

Part of plant	Solvent	B.s	E.c	Ps	S.a	A.n	C.a
Leaves	MeOH	6.25	6.25	6.25	6.25	25	12.5
Stems	MeOH	12.5	12.5	6.25	6.25	25	25
Bark	MeOH	12.5	6.25	6.25	6.25	6.25	25
Fruit	MeOH	6.25	12.5	6.25	6.25	25	25
Leaves	Ethyl	12.5	12.5	12.5	12.5	25	25

Standard organisms tested: B.S. = Bacillus subtilis, S.a. = Staphylococcus aureus, E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa, A.n= Aspergillus niger, C.a = Candida albicans MeOH= Methanol, P.E=Petrolium ether, M.D.I.Z= Mean diameter of growth inhibition zone in (mm). Result: >18 mm: Sensitive 14 to 18 mm: Intermediate <14 mm: Resistant. (-): No inhibition zone.

2.5. Phytochemistry of the genus *cordia*

The genus *Cordia* is comprehensively studied for its chemical constituents, and till now, more than 290 compounds from different chemical classes have been identified. These phytochemicals mainly contain quinones, terpenoids, a phenolics, lignans, saponins, steroids, fatty acids, alkaloids, carotenoids, coumarins, porphyrins, essential oil and miscellaneous group of compounds. Quinones, flavonoids and terpenoids are the major constituents isolated from the majority of the *Cordia* species. Many of the isolated compounds were also evaluated for their bio efficacy. Previous studies on different C*ordia* species revealed the presence of the following chemical constituents cadinol (**1**), oleanolic acid (**2**), Oncocalyxone A (**3**), β-Sitosterol- β-Sitosterol- D-glucoside Allontoin (**4**), α -cadinol (**5**), α-muurolol (**6**), epi-α-muurolol (7), were reported from the stem and leave of *C. Trichotoma*. Fig.2: [35, 36, 37], cordianol A (**8)** was reported from the leave of *C. multispicata*. Fig.3: [38], 5-hydroxy-4 7-dimethoxyflavanone (**9**), eriodictiol (**10)** were reported from the leave of *C. globose*. Fig.4: [39], δ-cadinene (**11**) α-bisabolene (**12**), caffeic acid (**13**) gallic acid (**14**), chlorogenic acid (**15**), were reported from the leave of *C. verbenacea*. Fig.5: [40, 41], luteolina (**16**), apigenin (**17**), hentricontanol (**18**), hesperidine (**19**), octasanole (**20**), lupeol (**21**), α-amyrin (**22**), robonine (**23**), rutin Betulin (**24**), dihydrorobenetin (**25**),were reported from the leave of *C. dichotoma*. Fig.6: [42]. Phenol, 2-methoxy4-(2-propenyl) (**26**), 4-ethylcatechol (**27**), Phenol, 2-methoxy-4-(1-propenyl) **(28**), Benzene acetic acid, phenyl methyl ester (**29**), 4-((1E)-3-hydroxy-1-propenyl)-2methoxyphenol (**30**), pentadecanoic acid (**31**), 1,2-Benzenedicarboxilic acid, mono (2-ethylhexyl) ester (**32)**, butonic acid, 3-methyl-ethyl ester (**33**), octadcanoic (**34**) acid were reported from the leave and stem of cordia africana. Fig.7:

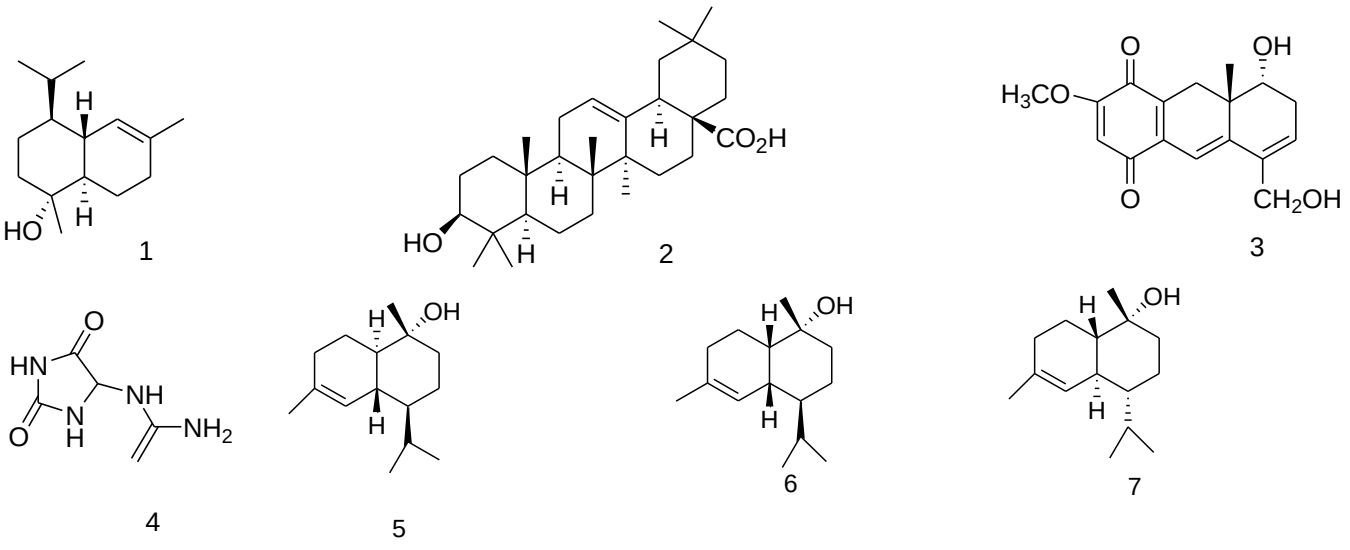

Figure 2: Compound reported from the *C. trichotoma*

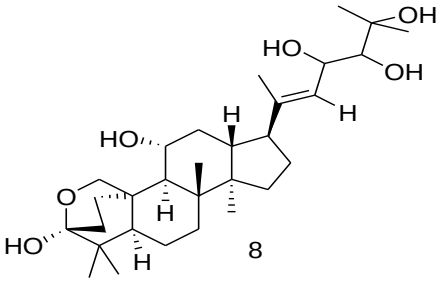

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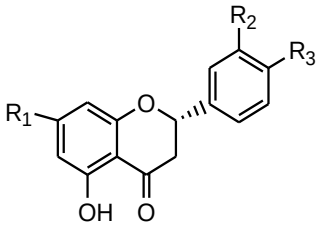
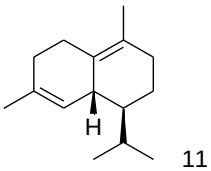
Figure 3: Compound reported from the *C. multispicata*



9 R1=R3=OCH3 : R2=H
10 R1=R2=R3=OH

Figure 4: Compound reported from the *C. globose*



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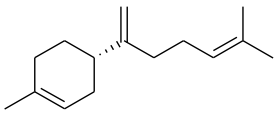

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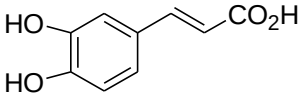

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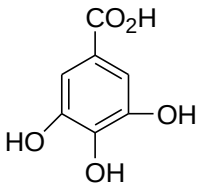

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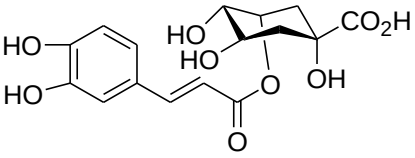

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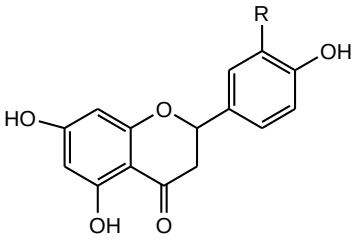
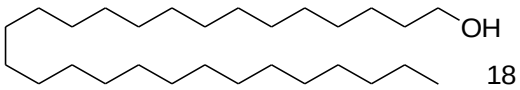
Figure 5: Compound reported from the *C. verbenacea*



16 R=OH

17 R=H



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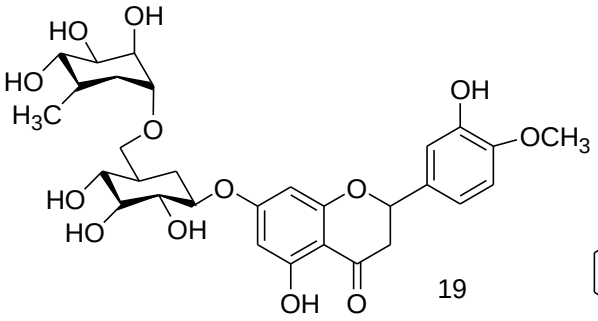

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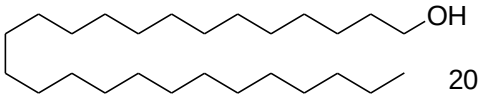

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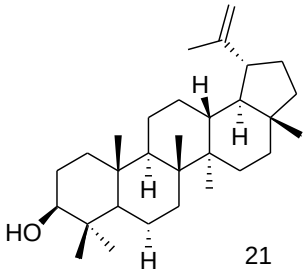

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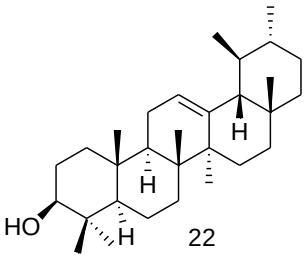

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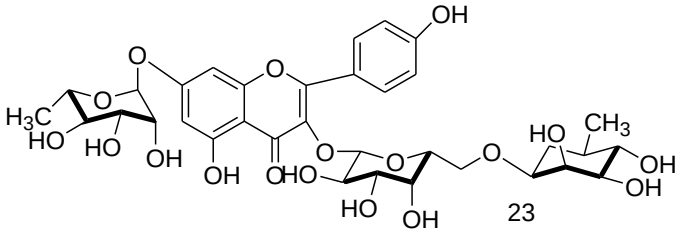

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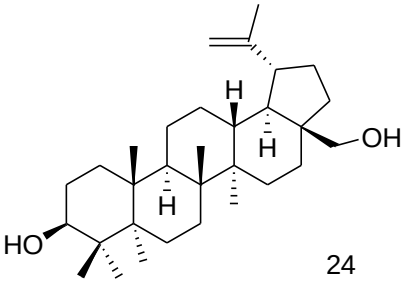

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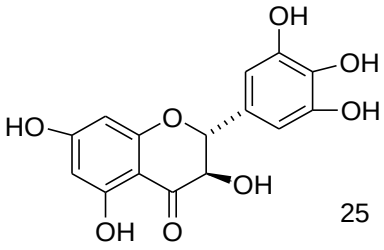

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Figure 6: Compound reported from the *C. dichotoma*

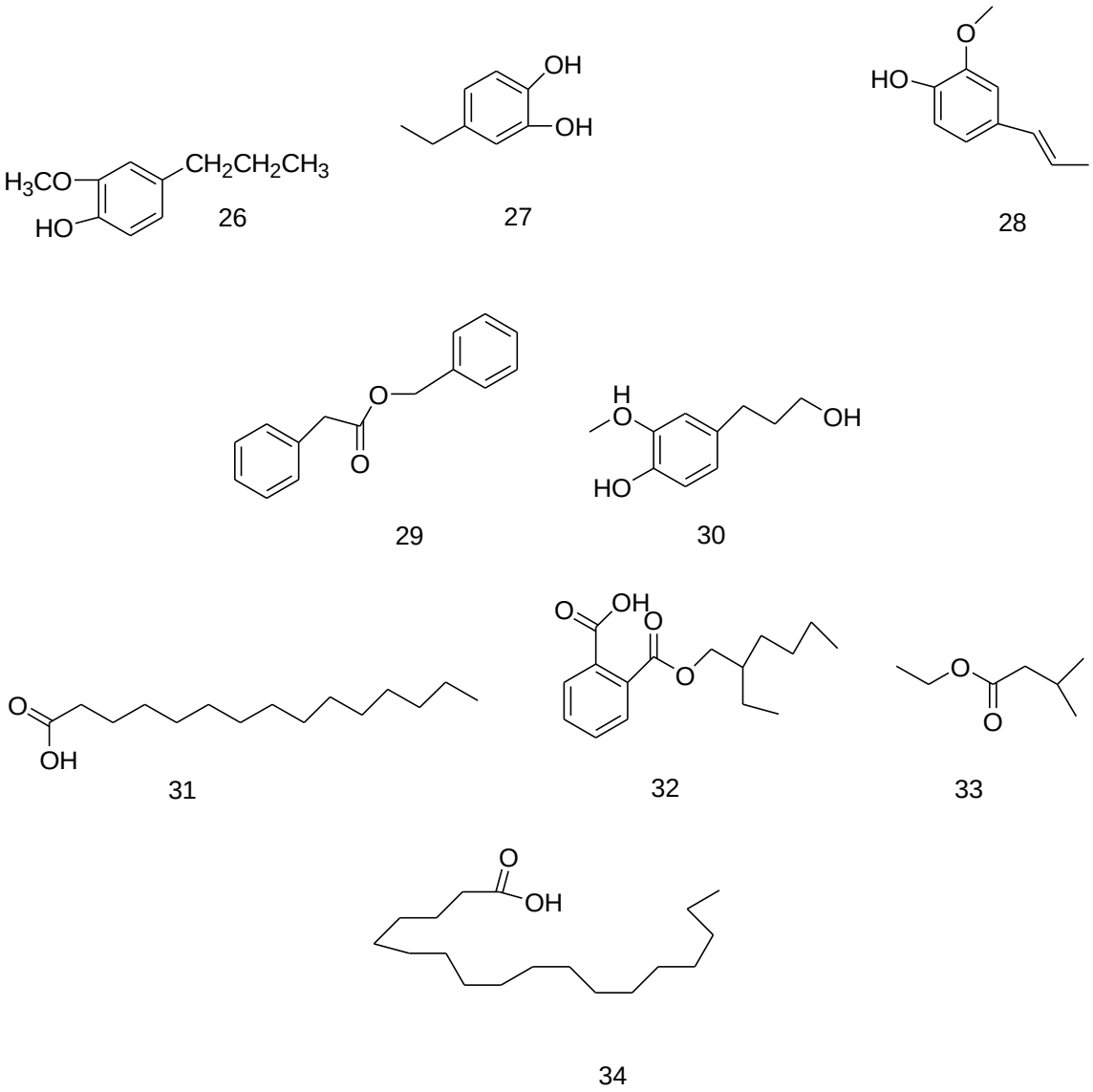


Figure 7: Compounds reported from the *C. africana*

3. MATERIAL AND METHODS

3.1. Chemicals and reagents

The following chemicals were used in this thesis work:- n-hexane, ethyl acetate, methanol, distilled water, silica gel and chloroform, sodium hydroxide, ferric chloride, concentrated sulphuric acid.

3.2. Instruments and apparatus

For this thesis work different apparatus and instruments were used, that included laboratory mill, analytical balance, silica or pre-coated thin layer chromatography (TLC) plates. Column chromatography was performed on normal and deactivated silica gel. Structural assignment was performed based on spectroscopic data analyses and interpretations. The spectra were obtained using Bruker spectrometers for ^{1}H NMR and ^{13}C NMR spectra which were run in DMSO-d_6 solvents depending on solubility of the isolated compounds.

3.3. Collection and preparation of plant materials

The stem bark of *Cordia africana* was collected from Adama city after conducting proper identification with a help from a botanist. Identified and authenticated by a botanist of Shabel and voucher specimen GA001 botanical specimen of the plant is kept in a herbarium for future references. The plant material was dried under shade. The dried plant material was ground into fine powder using mortar and pestle in preparation for extraction.

3.4. Extraction procedures

The ground dried powder of stem barks of C*ordia africana* (500g) was macerated for three consecutive days each at room temperature using n-hexane, chloroform and methanol 2.5L each. The extracts were filtered using Whatman filter paper and evaporated under vacuum to obtain the respective crude extracts. The filtrate was dried by evaporating the solvents using Rota vapor. The dried extracts were weighed and placed in refrigerator until analysis. Removal of the solvents gave 2g of n-hexane extract, 1.5g of chloroform extract and 12.2g of methanol extracted. Column chromatography on silica gel was conducted for separation of the chemical constituents of the crude extracts depending on the TLC profile and biological activity.

3.5. Isolation of compounds

Isolations were carried out by repeated column chromatography of the crude extracts over silica gel column eluted with organic solvents with increasing polarity. But prior to column chromatography, the crude extracts were extensively analyzed by thin layer chromatography (TLC) and their respective

compositions and the nature and type of solvent mixtures that were used in isolation were established. The visualizations of the TLCs were aided by either observing the TLC under UV lamp or spraying with appropriate spay reagents. The TLCs were repeatedly improved by changing the solvent systems until a system that gives the best separation. The crude extracts were repeatedly chromatographed on columns packed with silica-gel, until pure compounds were obtained. Fractions were collected. The compositions of the fractions were continuously inspected by using TLC. Finally, an isolated compound was identified by using spectroscopic methods.

3.6. Phytochemical investigation of the stem bark of *cordia africana*

Methanol crude extract (6.0g) was subjected to silica gel column chromatography (CC) using different proportions of chloroform-methanol mixtures as eluent. A total of 41 fractions were collected (table 3). Those fractions that showed similar profile on thin layer chromatography (TLC) were combined. Fractions 1-4 that were eluted by 9:1 $CHCl_3$-MeOH solvent systems upon analysis by TLC showed no fluorescence when viewed under UV light but presented a single spot when sprayed with vanillin. After removal of the solvents, visible in UV light and showed three spot then by selected solvent ratio system further fractionated two single spot showed. Fractions 5-14 showed similar profile on thin layer chromatography displaying three spots that showed fluorescence of red in long wavelength (365nm) under UV light; they were eluted by 8.5:1.5 ratio of $CHCl_3$: MeOH solvent system. Fractions 15-28 were eluted by 4:1 solvent systems and all showed similar profile on thin layer chromatography, which was a mixture of several compounds that fluoresce when viewed under UV light. Fractions 29-31 were eluted by 7.5:2.5 solvent systems. They showed similar profile on thin layer chromatography and were combined. This mixture was further rechromatographed on a smaller column using mixtures of $CHCl_3$-MeOH solvent system as eluent. It yielded two fractions each of which contained a single spot on TLC with different retention factors. Fraction 32 from the main column showed a single spot on its thin layer chromatography. Fraction 33 showed similar spot like fraction 32 but another additional spot was present. Fraction 34 which was eluted by 13:7 solvent systems showed a single spot. Fractions 35-38 eluted by 6:4 solvent systems, which fluoresced when viewed under UV light, were mixtures. Fraction 39 was eluted by 1:1 ratio solvent systems; fraction 40 by 4:6 solvents ratio and fraction 41 by100% ethanol was all mixture.

Table 2: Fractionation of the MeOH extract

Solvent system	Ratio	Fractions
$CHCl_3$ –MeOH	9:1	1-4
"	17:3	5-14
"	4: 1	15-28
"	3: 1	29-31
"	7: 3	32-33
"	13:7	34
"	3: 2	35-38
"	1:1	39
"	2:3	40
"	100%	41

From the above table two compounds were isolated which as label fraction 32 and further fractionated of 29-31 fractions (1') were as characterized.

3.7. Qualitative analysis of the chemical constituents of crude extracts

Phytochemicals are chemicals derived from plant and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. To tests for phytochemical constituents of the medicinal plant of *Cordia africana* was carried out for the all extract of C. *africana* [42].

Test for tannins: To a 3mg of extracts, a few drops of 10% ferric chloride solution were added. Appearance of a green or blue color indicates the presence of tannins.

Test for steroids: 3mg of extracts was mixed with 1mL of chloroform and 2-3 drops of conc. H_2SO_4 was added to it. Appearance of a pink or red color indicates the presence of steroids.

Test for terpenoids (Salkowiski test): 3mg extracts was mixed with 2mL of chloroform and 3mL of conc. H_2SO_4 solution. A reddish brown colour at the interphase indicates the presence of terpenoids.

Test for alkaloids (Wagner's test): 3mg of the extract was added with Wagner's reagent (1.27 g of iodine and 2g of KI along with 100mL of distilled water) and observed for the formation of reddish brown coloured precipitate.

Test for flavanoids: 3mg of extracts was treated with conc. H_2SO_4 solution. Formation of a yellowish-orange color indicates the presence of flavanoids.

Test for saponins (Foam test): 3mg of extracts was diluted with 5mL of distilled water, shake vigorously and was observed for a stable persistent froth.

Test for phenolic compounds (Ferric chloride test): 3mg of extracts was treated with diluted $FeCl_3$ solution. Appearances of a violet color indicate the presence of phenol like compounds.

3.8. Spectroscopic data of the isolated compounds

Structural elucidation was done on the basis the spectroscopic techniques. Isolated pure compounds were characterized by using ^{1}H NMR, ^{13}C NMR and DEPT spectra which were recorded on a 'Bruker-400' instrument with tetramethylsilane (TMS) as the internal standard and DMSO-d_6 used as a deutriated solvent.

3.9. Bacterial test cultures

Microorganisms that were used for antibacterial tests were *Staphylococcus aureus*, Gram-positive bacteria and three Gram-negative *bacteria viz. Pseudomonas aeroginosa, Klebsiela Pnuemonia and Escherichia coli*. All the procedures were done according to clinical laboratory standard procedures and quality control [43, 44]. Briefly, a fresh culture was obtained by growing the test strains overnight at $37^{o}C$ for bacteria. Nutrient agar (Merck) and blood agar were used to maintain the clinical isolates of the bacteria.

3.10. Anti-bacterial screening tests

The plate agar diffusion method was used to test the extracts and isolated compounds against the bacteria. Antibacterial sensitivity and resistance was confirmed by use of standard discs. Different concentrations of stem bark extracts of *Cordia africana* were prepared. The concentrations were incorporated into sterile blank paper discs and were dried at $37^{o}C$. The paper disc was weighed carefully for confirming exact amount of the extract being incorporated. Clinical isolates of the microorganism which was isolated from stool specimens in the clinic was identified according to routine cultural properties and biochemical tests. After 24h of incubation bacterial suspension (inoculums) was diluted

with sterile solution to the concentration of bacterial suspensions are adjusted to 10^8cells/mL. Measuring the diameter of the zone of inhibition was used for comparison of inhibition zones.

3.11. Agar dilution method antibacterial assays

According to the guidelines of clinical and laboratory standard institute evaluation of the antibacterial activity of plant extracts was conducted according to the agar dilution method. The plant extracts was dissolved in 2.5% dimethylsulfoxide (DMSO), which is maximum volume of DMSO that can be use to dissolve solid extracts. The solvent DMSO (2.5%) that cannot inhibit the growth of microorganism was used as the negative control for all the experiments. The antibacterial assay was performed by an agar diffusion method for solvent extracts. For agar diffusion method a well was prepared in the plates the extracts were introduced into the well. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain controls was maintained where pure solvents was used alternative of the extracts. The result was obtained by measuring the zone diameter.

4. RESULTS AND DISCUSSION

4.1. Extract yields

Dry powdered stem barks of *Cordia africana* (500g) was sequentially soaked over a period of nine days in the three solvents in order of increasing polarity starting from n-hexane, then chloroform and finally methanol. The amount of crude extracts and percentage yields obtained were 2g, 1.5g and 12.2g respectively and percentage yields 0.4%, 0.3% and 2.44% respectively. The methanol extracts represented the highest yield from among the three extracts.

Table 3: Masses of sequential extraction of *C. africana* and percentage yields

Stem bark	Extraction solvent	Mass (g)	% Yield
"	n- hexane	2	0.4%
"	Chloroform	1.5	0.3%
"	Methanol	12.2	2.44%

4.2. Phytochemical screening of the stem bark of *Cordia africana*

Each of the crude extract was subjected to phytochemical screening analysis. The phytochemical screening tests indicated the presence of terpenoids, alkaloids, flavanoids and saponins but absence of tannins, steroids and phenols in the n- hexane extract. The chloroform extract contained alkaloids, terpenoids and flavanoids but lacked tannins, steroids, saponins and phenols. In methanol extract, the tests indicated presence of tannins, terpenoids and saponins and absence of steroids, alkaloids, flavanoids and phenols. The results of the phytochemical screening tests are summarized in Table 4:

Table 4: Summary of phytochemical screening of *Cordia africana* extracted of secondary metabolites

Sample	Alkaloids	Flavanoids	Phenolic	Saponins	Steroids	Tannins	Terpenoids
n-hexane	+	+	_	+	_	_	+
Chloroform	+	+	_	_	_	_	+
Methanol	_	_	_	_	_	+	+

Note: '+' indicates presence '-' indicates absence

4.3. Structure elucidations

4.3.1. Characterization of compound 35

Compound 35 was isolated as a yellow amorphous solid from a fraction 32 of methanol crude extract over silica gel CC of *C. africana* stem bark extract using a solvent ratio of 7:3 MeOH /$CHCl_3$. It was active under UV-light (254nm) and turned purple on spraying with vanillin. Analysis of ^{1}H NMR (Table 5, Appendix 1) ^{13}C-NMR (Table 5, Appendix 2) and DEPT (Table 6, Appendix 3) spectra of the compound led to the proposed structure of the compound as in Figure 8. The ^{1}H NMR spectrums of compound 35 showed proton resonances between δH 4.2 and 3.1. The signals appearing at δH 3.4 integrated for multiple protons and are due to a methoxy group. The complete assignment of the ^{1}H NMR signals to this compound 35 is summarized in Table 5.

Table 5:^{1}H NMR data (DMSO) compound 35

Position	δ H (ppm)	Multiplicity	J (Hz)	Integral
1	4.2	s	-	H
2	3.8	s	-	H
3	3.4	t	10	H
4	3.1	s	-	2H

The ^{13}C NMR spectrum (Appendix 2) showed a total seven of signals and showed in the regions resonances between δ 174.5 and 53.8. The DEPT experiment (Appendix 3) revealed that the compound has four carbons at δ 67.5, 63.0, 55.5 and 53.8 were as oxygenated carbons. Quaternary carbon atoms appeared at δ 157.5, 158.2, and 174.5. 174.5 indicated the presence of carbonyl group in the compound and these quaternary carbon atoms appeared. The DEPT-135 spectra of the compound showed four signals out of which two are negative signals indicating the presence of two methylene carbons that are oxygenated carbons in the compound. Based on the ^{13}C NMR and DEPT-135 spectral data, compound 35 was accounted to have seven carbons comprising of methoxy, carbonyl and four quaternary carbons.

Table 6: ^{13}C NMR data and DEPT-135 NMR (DMSO) of compound35

Position	δ C (ppm)	DEPT 135 δ
1	67.5	CH_2
2	63.0	CH
3	55.6	CH_2
4	53.8	CH_3
5	157.6	-
6	158.7	-
7	174.5	-

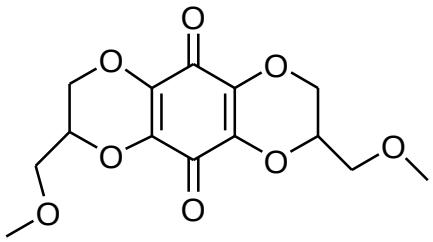


Figure 8: Proposed structure of compound **35**

4.3.2. Characterization of Compound 36

The ^{1}H NMR spectrum of compound 36 showed eleven proton resonances between δ_H 4.38 and 3.1 indicating that all of the carbon atoms to which the protons attached are oxygenated the proton resonances at δ_H 4.38, 4.35, 3.9, 3.87, 3.78, 3.56, 3.47, 3.45, 3.4, 3.15 and 3.1. ^{1}H NMR spectrum recorded in DMSO-d_6 (Table 7 Appendix 4) showed additional proton resonances at δ_H 7.5, 7.2, 7.0, 6.4, 6.2, 5.4, 5.18, 5.17, integrated for one proton each. These signals disappeared and therefore revealed the presence of eight hydroxyl groups.

The ^{13}C NMR spectrum showed a total of 12 carbon resonances between δ 104.49 and 60.9 and also the signal at δ 104.49 indicated the presence of a quaternary carbon showed. The ^{13}C NMR spectrum of the compound exhibited signals at δ 92.2, 83.0, 77.5, 74.75, 73.3, 72.34, 71.1, 70.3, 62.6, 62.0 and 60.9. The DEPT-135 spectrum of the showed three signals were negative signals indicating the presence of three methylene carbons that are oxygenated carbons in the compound at δ 62.6, 62.0 and 60.9 carbons (Table 8). It was evident from the spectroscopic data generated for compound 36 that it is a polyhydroxylated hydrocarbon. From the ^{1}H NMR, ^{13}C NMR and DEPT-135 NMR spectra, the molecular formula could

be deduced for compound 36. This, coupled with the spectroscopic data, clearly suggested the presence of two rings in compound in this proposed compounds 36.

Table 7: ^{1}H NMR data (DMSO) for compound 36

Position	δ H (ppm)	Multiplicity	J (Hz)
1	7.5	d	2
2	7.2	s	-
3	7.0	d	-
4	6.4	d	1.6
5	6.2	d	2
6	5.4	d	4
7	5.18	s	-
8	5.17	s	-
9	4.38	s	-
10	4.35	s	-
11	3.9	s	-
12	3.87	s	-
13	3.78	s	-
14	3.56	s	-
15	3.47	s	-
16	3.4	s	-
17	3.13	s	-
18	3.1	s	-

Table 8: ^{13}C NMR data and DEPT-135 NMR (DMSO) of compound 36

Position	δ C (ppm)	DEPT 135 δ
1	104.5	-
2	92.2	CH
3	83.0	CH
4	77.5	CH
5	74.5	CH
6	73.3	CH
7	72.3	CH
8	71.1	CH
9	70.3	CH
10	62.6	CH_2
11	62.0	CH_2
12	60.9	CH_2

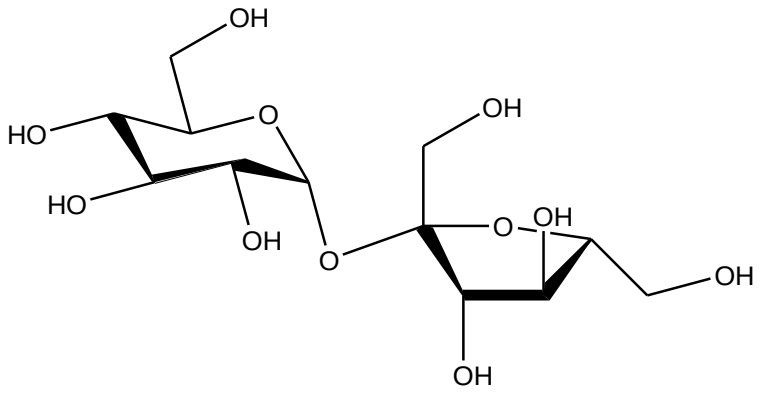


Figure 9: Proposed structure of compound **36**

4.4. Antibacterial activity

All the crude extracts were tested for their *in vitro* antibacterial activity against one Gram-positive bacteria strain, *viz. Staphylococcus aureus* and three Gram-negative strains, *viz. Escherichia coli, P. aeroginosa and K. pneumonia* using agar diffusion method. The zones of inhibition of bacterial growth were measured after 48h and the measurements were done (in mm) from the end of the growth of one side of the disc to the beginning of growth of the other side including the diameter of the disc. Ceftriaxone**,** one of the commonly used third generation cephalosporin antibiotics, was used as positive control, while DMSO was used as a negative control**.** Results were recorded and tabulated as shown in table 9. The n-hexane extract showed activity against *E. coli* with zone of inhibition of 9mm; chloroform extracts against *Staphylococcus aureus* with zone of inhibition 8mm. Methanol extract showed activity against *Staphylococcus aureus* and *E. coli* with zone of inhibition of 12mm and 10mm respectively as shown in table 9.

Table 9: The inhibition zones (in mm) of the crude extracts

No	Extract	*S.aureus* (Zone size in mm)	*E. coli*	*P.aeroginosa* (Zone size in mm)	*K. puemonia* (Zone size in mm)
1	n-hexane	6	9	6	6
2	Chlorofrom	8	6	6	6
3	Methanol	12	10	6	6
4	Ceftriaxone	21	32	20	15
5	DMSO	0	0	0	0

Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Klebsiella pneumoniae,

Table 10: The inhibition zones (in mm) of the isolated compounds

Isolated compounds	S.aureus	E.Coli	P.aeroginosa	K. puemonia
Compound **35**	6	6	6	6
Compound **36**	6	6	6	6
Ceftriaxone	21	32	20	15
DMSO	0	0	0	0

Ceftriaxone, sold under the trade name rocephin, is an antibiotic useful for the treatment of a number of bacterial infections. Ceftriaxone and other third-generation cephalosporin antibiotics are used to treat organisms that tend to be resistant to many other antibiotics [45]. It is within the β-lactam family of antibiotics. Ceftriaxone selectively and irreversibly inhibits bacterial cell wall synthesis by binding to transpeptidases, also called transamidases, which are penicillin-binding proteins (PBPs) that catalyze the cross-linking of the peptidoglycan polymers forming the bacterial cell wall [46]. The peptidoglycan cell wall is made up of pentapeptide units attached to a polysaccharide backbone with alternating units of N-acetylglucosamine and N-acetylmuramic acid [47]. PBPs act on a terminal D-alanyl-D-alanine moiety on a pentapeptide unit and catalyze the formation of a peptide bond between the penultimate D-alanine and a glycine unit on an adjacent peptidoglycan strand, releasing the terminal D-alanine unit in the

process. The structure of ceftriaxone mimics the D-alanyl-D-alanine moiety, and the PBP attacks the beta-lactam ring in ceftriaxone as if it were its normal D-alanyl-D-alanine substrate. The peptidoglycan cross-linking activity of PBPs is a construction and repair mechanism that normally helps to maintain bacterial cell wall integrity, so the inhibition of PBPs leads to damage and destruction of the cell wall and eventually to cell lysis.

The *syn*-configuration of the methoxyoxime moiety confers resistance to beta-lactamase enzymes produced by many Gram-negative bacteria. The stability of this configuration results in increased activity of ceftriaxone against otherwise resistant Gram-negative bacteria. In place of the easily hydrolyzed acetyl group of cefotaxime, ceftriaxone has a metabolically stable thiotriazinedionemoiety [48].

None of the isolated compounds demonstrated activity against any of the test bacterial strains, as can clearly be seen; the isolated compounds do not posses any structural similarity either with the control compound, ceftriaxone, or any other known antibacterial class of compounds.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

Phytochemicals may be important tools for the effective control of infectious diseases caused by bacteria. This study reported the effects of this stem bark part of plant in antibacterial activity. This study focused on the antibacterial effects of plant and phytochemical investigations of stem bark part of *Cordia africana* plant. *Cordia africana* crude extract n-hexane extract had a low activity against E. coli with inhibition zones of 9mm, chlorofrom extract low activity against *S. aureus* with inhibition zones of 8mm and methanol extract of *Cordia africana* stem bark had activity against *E.coli* and *S.aureus* with inhibition zones of 10 and 12mm, respectively. As part of this thesis, phytochemical analysis was conducted on the MeOH extract of the stem bark of *Cordia africana* two compounds isolated, (2) were isolated and characterized had no biological activity.

5.2. Recommendations

This study has demonstrated that there was need for further investigation and bioassay guided isolation of pure compounds from stem bark of *Cordia africana*, finally in addition to the following recommendations.

i. The crude extracts of plant need to be subjected further to other disease causing microbes (bacteria and fungi).

ii. Further studies should be carried out to clarify the biochemical status of this plant by isolation, purification, and characterization the active principles responsible for the claimed properties of *Cordia africana.*

iii. Further pure compounds need be isolated from plant and subjected to bioassay tests.

6. REFERENCES

1. Rouhi. A.M, (**2003**). Rediscovering Natural Products. *Chem. Eng. News*. pp. 104-7.

2. Koehn. F.E, Carter. G.T, (**2005**). The evolving role of natural products in drug discovery. *Nature reviews Drug discovery*. 4(3), pp. 206- 20.

3. Ortholand. J.Y, Ganesan. A, (**2004**). Natural products and combinatorial chemistry: back to the future. *Current opinion in chemical biology*. 8(3), pp. 271-80.

4. Newman. DJ, Cragg. GM, (**2012**). Natural products as sources of new drugs over the 30 years. *Journal of natural products*. 75(3), pp. 311- 35.

5. Butler. MS, (**2005**). Natural products to drugs: natural product derived compounds in clinical trials. *Natural product reports*. 22(2), pp. 162- 95.

6. Gottlieb. O.R, (**1990**). Phytochemicals: differentiation and function. Phytochemistry, 29(6), PP. 1715- 172.

7. Karborne. J.B, Grayer. R.J, Harborne. J.B, (**1994**). In the Flavonoids. *Chapman and Hall, London*, pp. 589- 618.

8. Naczk. M, Shahidi. F, (**2006**). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J. Pharm. Biomed. Anal*., 41, pp. 1523.

9. Sheeba. E, (**2010**). Antibacterial activity of solanum surattense burm. *F. Kathmandu University Journal of Science, Engineering and Technology*, 6(1), pp. 1- 4.

10. Ncube. N, Afolayan. S, Okoh. A, (**2008**). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, 7(12), pp. 1797-1806.

11. Gupesh. P, Pal. S, Pavani. A, Gadge. M, (**2010**). Quantitave estimation of the active constituents of Parkiabiglandulosa by using HPTLC and FTIR. *International Journal of Pharma and Bio Sciences*, (1), pp. 315-322.

12. James. J, Dubery. I, (**2009**). Pentacyclic Triterpenoids from the Medicinal Herb, *Centellaasiatica (L.)* Urban. *J. Ethnopharmacol, Molecules*, 14, pp. 3922 - 3941.

13. Suffredini. I.B, Sader. H.S, Gonçalves. A.G, Reis. A.O, Gales. A.C,Varella. A.D, Younes. R.N, (**2004**). Screening of antibacterial extracts from plants native to the brazilian amazon rain forest and atlantic forest. *Braz. J. Med.Biol. Res*, 37, pp. 379.

14. Lequesne. P.W, Cooper-Driver. G.A, Villani. M, Do, M.N, (**1986**). In new Trends in natural product chemistry. *proceeding of the 2nd international symposium Pakistan U.S, Binational workshop, Shamin printing press; Karachi*, pp. 271.

15. Barroso, F.d. Oliveira, (**2009**). Pharmacognostic diagnosis of fruits of Cordia sellowiana Cham. and Cordia myxa L. (Boraginaceae Jusssieu). *Rev. Bras. Farmacogn*.19, pp. 458–470.

16. Corrêa, M.P, (**1984**). Dicionário de plantas úteis do Brasil e das exóticas cultivadas, Dicionário de plantas úteis do Brasil e das exóticas cultivadas. Imprensa Nacional Brasília.

17. Abbink. J, (**1995**). Medicinal and Ritual Plants of the Ethiopian Southwest. *An Account of Recent Research. Indigenous Knowledge and Development Monitor.* 3(2), pp. 6- 8.

18. Abebe. T, (**2005**). Diversity in homegarden agroforestry systems of Southern Ethiopia, PhD thesis, Wageningen University, Wageningen.

19. Thirupathi. K, Kumar. S.S, Raju. V.S, Ravikumar. B, Krishna. D.R, Mohan. G.K, (**2008**). A Review of Medicinal Plants of the Genus Cordia: Their Chemistry and Pharmacological Uses. *Journal ofNatural Remedies*. 8, pp. 1- 10.

20. Kuppast, Nayak, (**2006**). Wound healing activity of *Cordia dichotoma* Forst. fruits. *Journal of natural product and research*. 5(2), pp. 99- 102.

21. David. N, Cohen. M.X, Newen. A, wernick. B.H, Shah. N.J, Fink. G.R, (**2007**). The extrastriate cortex distinguishes between the consequences of one's own and others' behavior. *Neuroimage.* 36, pp. 1004– 1014.

22. Regassa. R, (**2013**). Diversity and conservation status of some economically valued indigenous medicinal plants in Hawassa College of Teacher Education Campus, Southern Ethiopia. *International Journal of Advanced Research.* 1(3), pp. 308- 328.

23. Teklay. A, Abera. B, Giday. M, (**2013**). An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *Journal of Ethnobiology and Ethnomedicine*. 9(65), pp. 2.

24. Chekole. G, (**2011**). An Ethnobotanical Study of Plants Used in Traditional Medicine and as Wild Foods in and around Tara Gedam and Amba Remnant Forests in Libo Kemkem Wereda, South Gonder Zone, Amhara Region, Ethiopia. *M.Sc. Thesis. Addis Ababa university, Ethiopia*.

25. Amsalu. N, (**2010**). Ethnobotanical Study of Medicinal Plants in Farta Wereda, South Gonder Zone of Amhara Region, Ethiopia. *M.Sc. Thesis. Addis Ababa university, Ethiopia.* pp. 127.

26. Etana. B, (**2010**). Ethnobotanical Study of Tradational Medicinal Plants of Goma Wereda Jima Zone of Oromia Region, Ethiopia. *M.Sc. Thesis. Addis Ababa university, Ethiopia*.

27. Friis. I, (**1992**). Forests and Forest Trees of Northeast Tropical Africa. Kew Bulletin. 15, pp. 396.

28. Warfa. AM, (**1988**). Cordia (Boraginaceae) in NE Tropical Africa and Tropical Arabia. Ph.D. *Thesis, Uppsala University*. PP. 396.

29. Breitenbach. FV, (**1963**). The Indigenous trees of Ethiopia. Second revised and enlarged edition, Ethiopian Forestry Association, Addis Ababa.

30. ICRAF (International Centre for Research in Agroforestry). Agroforestry data base: Cordia Africana Lam. ICRAF, **1998**.

31. Negash. L, (**1995**). Indigenous Trees of Ethiopia. Biology, Uses and Propagation Techniques. Printed by the SLU Reprocentralen, Umea, Sweden. pp. 105-113

32. J. Gindaba. J, Rozanov. A, Negash .L, (**2004**). Response of seedlings of two Eucalyptus and tree deciduous tree species from Ethiopia to severe water stress. *Forest Ecology and Management*, 201, pp. 119- 129.

33. EFAP (Ethiopian Forestry Action Program), (**1994**). Ministry of Natural Resources Development and Environmental Protection, Addis Ababa, Ethiopia.

34. Hailu. T, Negash. L, Olsson. M, (**2000**). Millettia ferruginea from Southern Ethiopia: Impacts on soil fertility and growth of maize. *Agrofoestry Systems*, pp. 48:9- 24.

35. Menezes. J.E, Lemos. T.L, Silveira. E.R, Braz-Filho. R, Pessoa. O.D, (**2001**). Trichotomol, a new cadinenediol from Cordia trichotoma. *J. Braz. Chem. Soc*.12, pp. 787– 790.

36. Lago. J.H, Soares. M.G, Batista-Pereira. L.G, Silva. M.F, CorreaA.G, Fernandes. J.B, Vieira. P.C, Roque. N.F, (**2006**). Volatile oil from Guarea macrophylla ssp. tuberculata: seasonal variation and electroantennographic detection by Hypsipyla grandella. Phytochemistry .67, pp. 589–594.

37. Sylvestre. M, Pichette. A, Longtin. A, Nagau. F, Legault. J, (**2006**). Essential oil analysis and anticancer activity of leaf essential oil of Cordia trichotoma from Guadeloupe. *J. Ethnopharmacol*. 103, pp. 99–102.

38. Kuroyanagi. M, Seki. T. Hayashi. T, Nagashima. Y, Kawahara. N, Sekita. S, Satake. M, (**2001**). Anti-androgenic triterpenoids from the Brazilian medicinal plant, Cvordia multispicata. *Chem. Pharm. Bull.* 49, pp. 954– 957.

39. Silva. S.A.S.D, Agra. M.D.F, Tavares. J.F, Cunha. E.V, Barbosa-Filho. J.M, (**2010**). Flavanones from aerial parts of Cordia globosa (Jacq.) Kunth, Boraginaceae. *Rev. Bras. Farmacogn.* 20, pp. 682–685.

40. De Souza. M.F, Manganotti. S.A, Souza. P.N.S, Meira. M.R, de Matos. C.D.C, Martins. E.R, (**2011**). Influência do horário de coleta, orientac¸ ão geográfica e dossel na produc¸ ão de óleo essencial de Cordia verbenacea DC. *Rev. Biotemas*. 24, pp. 9– 14.

41. Matias. E.F.F, Santos. K.K.A, Falcão-Silva. V.S, Siqueira-Júnior. J.P, Costa. J.G, Coutinho. H.D, (**2013**). Modulation of the norfloxacin resistance in Staphylococcus aureus by Cordia verbenaceae DC. *Indian J. Med. Res*. 137, pp. 178– 182.

42. Jones. P, Kinghorn. D,(**2006**). Extraction of plant secondary metabolites. Methods in Biotechnology. Natural Products Isolation. *2nd ed. Totowa, New Jersey: Human Pres*. pp. 269–273.

43. Chhabra. S.C. and Uiso. F.C, (**1992**). Antibacterial activity of some Tanzania plants, used in traditional medicine. Fitoterapia. 62, pp. 499- 504.

44. McChesney. J, Clark. A, and Silveira. E, (**1991**). Antibacterial dipterpenes of Croton sonderianus. *Journal of Natural Products.* 54, pp. 1625- 1633.

45. Katzung, Bertram, Masters, Susan, Trevor, Anthony (**2012**). Basic and Clinical Pharmacology. Mc Graw-Hill. pp. 797–801.

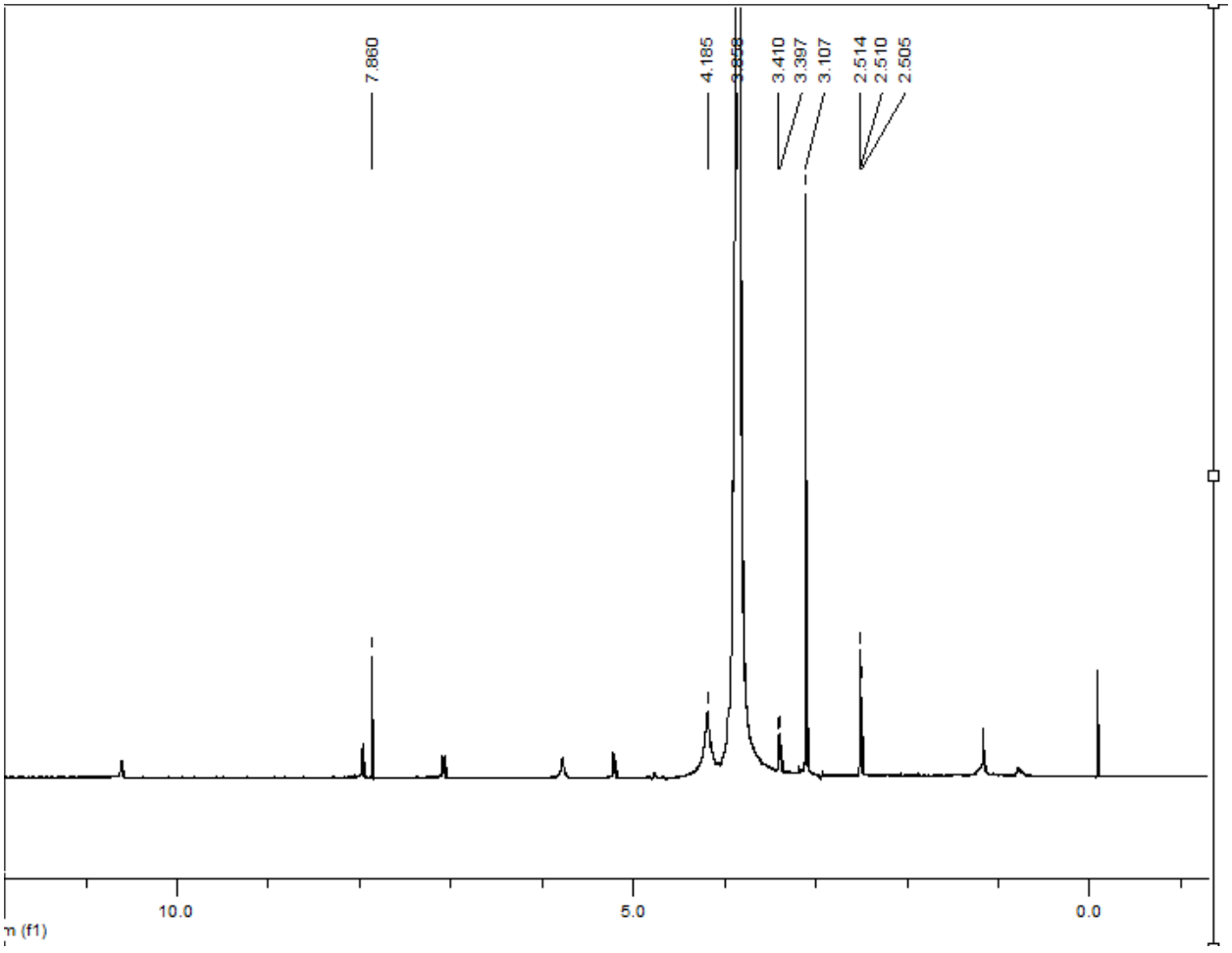
46. David. A, Lemke, Philadelphia. PA, Thomas. L, Lippincott Williams & Wilkins. Pp, Williams (**2013**). Foye's Principles of Medicinal Chemistry (Seventh ed.). pp. 1099–1100.

47. van Heijenoort, J.(**2001**). "Formation of the glycan chains in the synthesis of bacterial peptidoglycan". Glycobiology. 11.

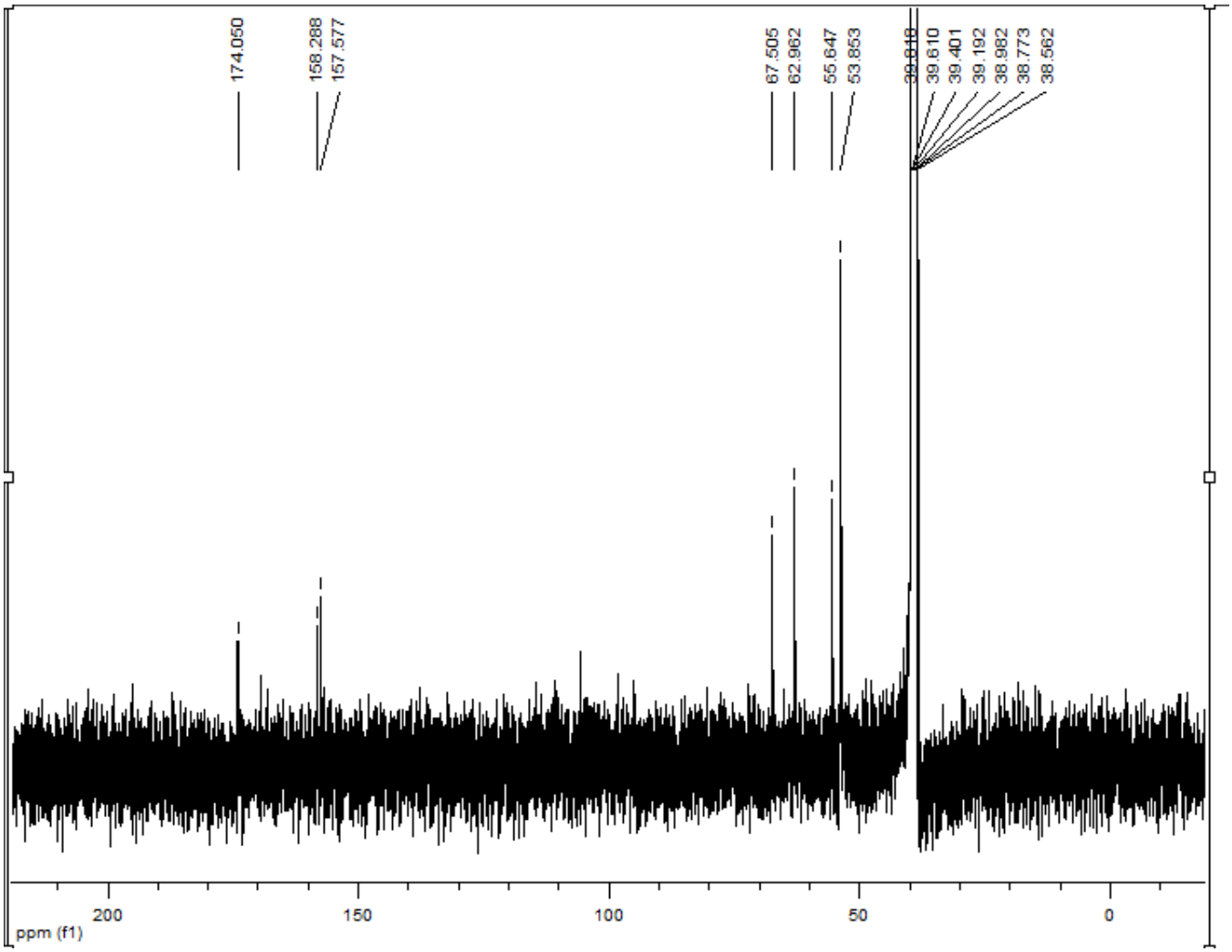
48. Scheffers, Dirk-Jan; Pinho, Mariana G. (**2005**). "Bacterial Cell Wall Synthesis: New Insights from Localization Studies". *Microbiology and Molecular Biology Reviews*. 69. pp. 585–607.

7. APPENDIXES

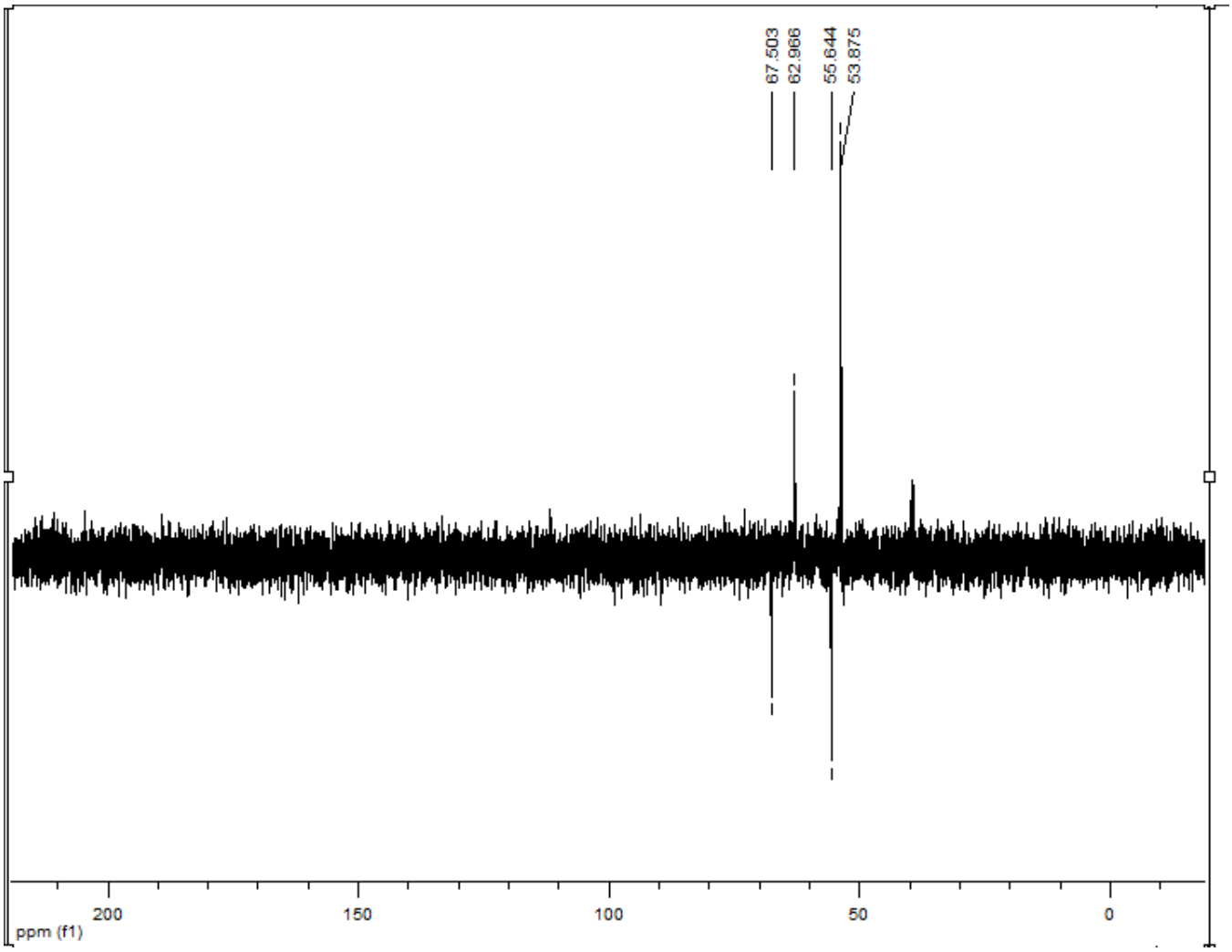
Appendix 1:^{1}H-NMR spectrum of compound 35



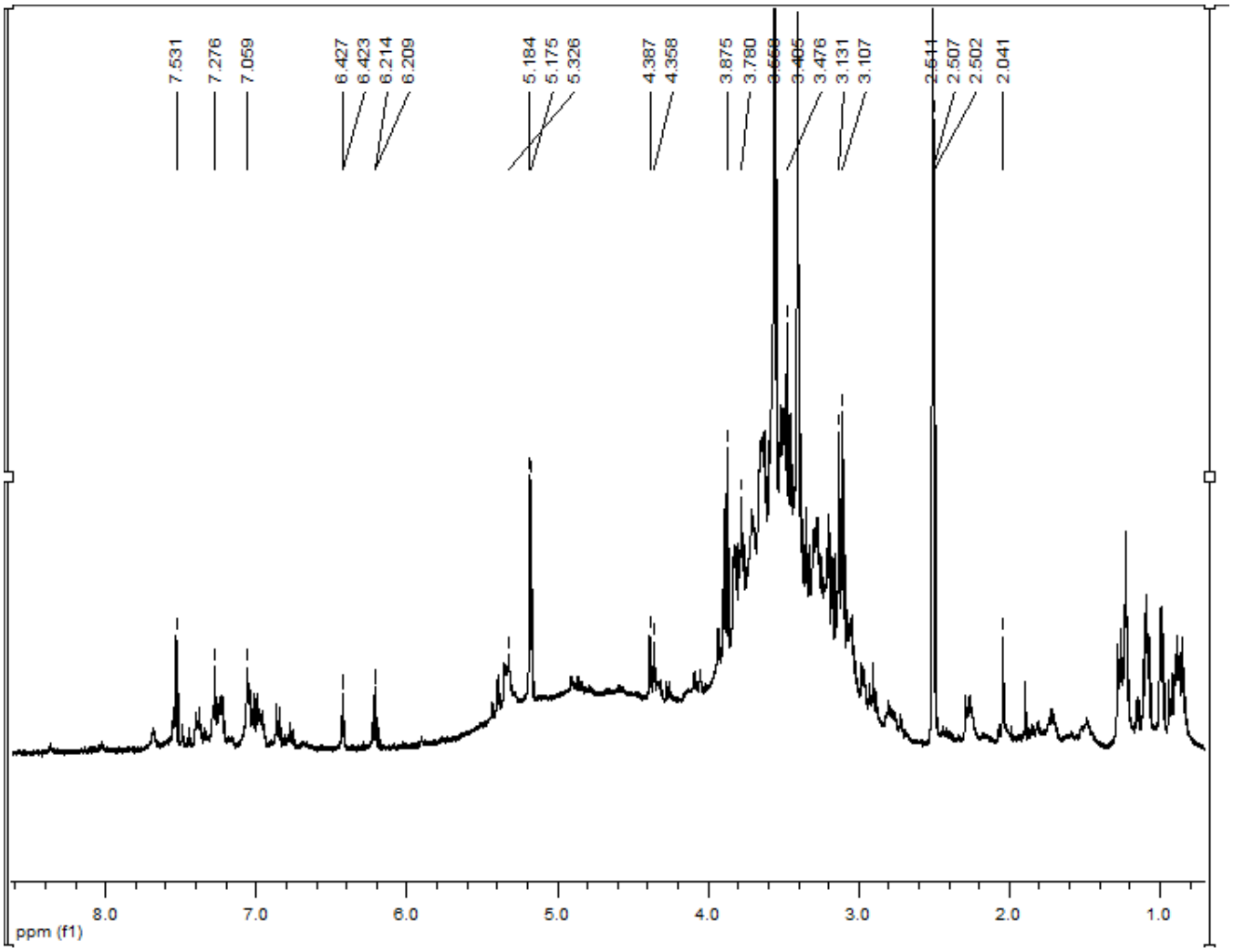
Appendix 2:^{13}C-NMR spectrum of compound 35



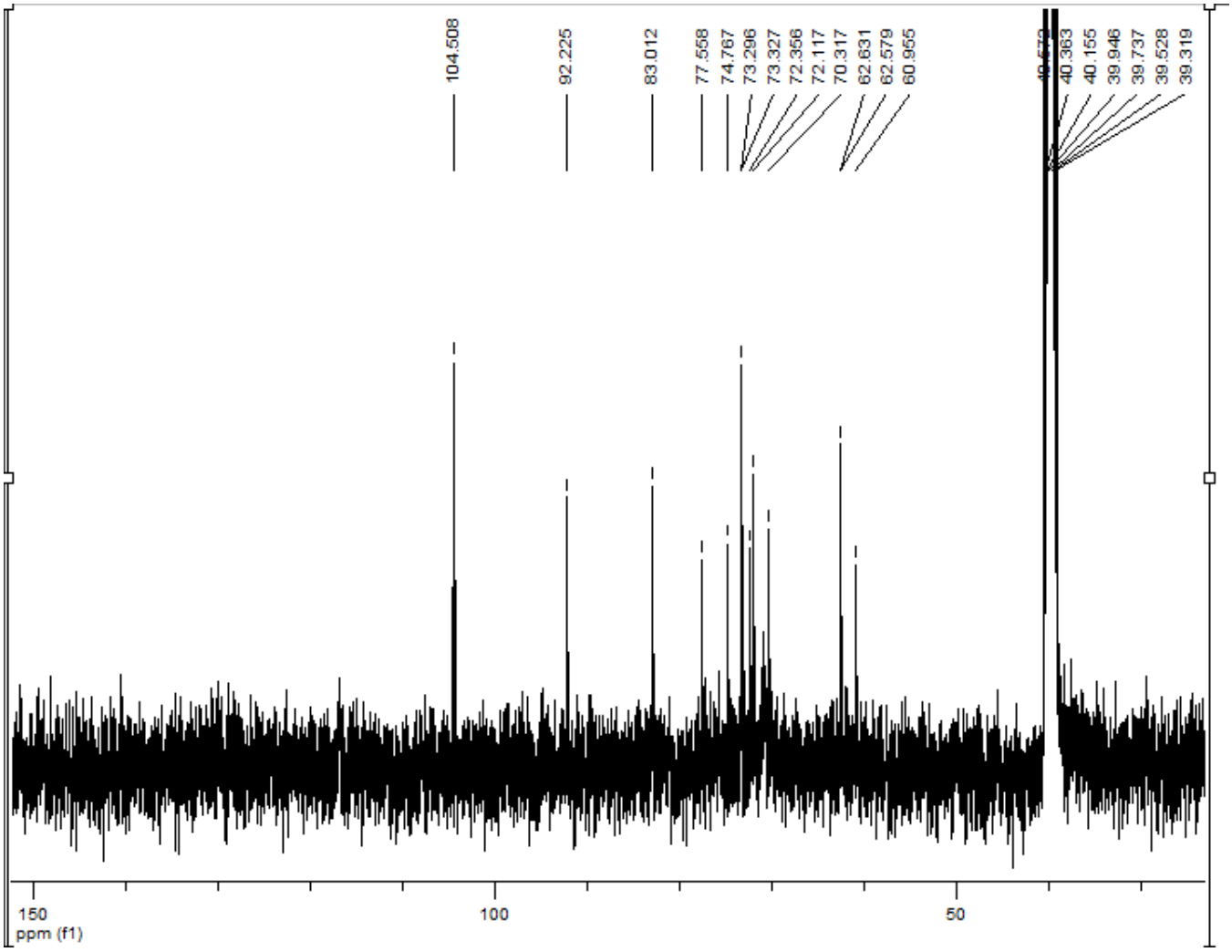
Appendix 3: DEPT-135 NMR spectrum of compound 35



Appendix 4: ^{1}H-NMR spectrum of compound 36



Appendix 5: ^{13}C-NMR spectrum of compound 36



Appendix 6: DEPT-135 NMR spectrum of compound 36