PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY OF THE STEM BARK OF *SECURIDACA LONGEPEDUNCLATA*

By Markos Addisu Defa

A THESIS SUBMITED TO

THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

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

OFFICE OF THE GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

September 24, 2018

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APPROVAL OF BOARD OF EXAMINERS

We the undersigned members of the board of examiners of the final open defence by **Markos Addisu** have read and evaluated his thesis entitled **''*phytochemical investigation and anti-bacterial activity of the stem bark of Securidaca longepedunclata''*.** This is, therefore, to certify that the thesis has been accepted in partial fulfilment of the requirement of the degree of Master's of Chemistry**.**

Approved by**:**

Yadessa Melaku (Ph.D)	_____	_____
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Chairperson	signature	Date
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Examiner (internal)	signature	Date
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Examiner (external)	signature	Date

DECLARATION

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

Name Markos Addisu

Signature _____

This MSc Thesis has been submitted for examination with my approval as thesis advisor.

Name Yadessa Melaku (Dr.)

Signature_____

Date of submission _____

ADVISOR'S APPROVAL SHEET

To: Chemistry department

Subject: Thesis Submission

This is to certify that the thesis entitled ‘‘***phytochemical investigation and anti-bacterial activity of the stem bark of Securidaca longepedunclata’’*** submitted in partial fulfilment of the requirements for the degree of Master‘s in Chemistry, the Graduate program of the department of Chemistry, and has been carried out by **Markos Addisu** ID.NO**.GSS/0218/05** under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

_____ _____ _____

Name of the main adviser Signature Date

ACKNOWLEDGEMENT

First of all I like to give thanks to my almighty God for the wonderful things He has done for me not only in this study work but also in every aspect of my life. I can do nothing and I am nothing without Him. Next I would like to bring my deepest thanks to my advisor Dr. Yadessa Melaku who devoted his time and stands on my side in giving advice, encouraging and support honestly. My gratitude also goes to Dr. Hailemichael Tesso, for the advice and encouragement he offered to me. My in-depth appreciation goes to the School of Natural Science and Adama Science and Technology University Post-Graduate Programme for financial support. I thank my colleagues and staff of the Post-Graduate Organic Chemistry laboratory for all their encouragement and support. I would also like to express my deepest gratitude to my sweet wife Bezunesh Tadese for her patience while I devote much time away from her for the study and for her constant support and pray. Finally I'd like to say 'God bless you' to my senior graduates Mr. Tokuma Getahun and Mr. Fekadu Asefa for their high contribution in providing important materials, and encouraging me from their experience.

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LIST OF ABBREVIATIONS

CC	Column chromatography
^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}HNMR	Proton nuclear magnetic resonance
HSDP	Health sector development program
IR	Infra red
MS	Mass spectroscopy
Rf	Retention factor
TLC	Thin layer chromatography
UV-Vis	Ultra violet visible
ZI	Zone of inhibition

ABSTRACT

Securidaca longepedunclata is traditionally used in Africa for treating different diseases such as hernias, coughs, fever, ascariasis, constipation, headaches, stomach ache, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections etc. With the aim to explore the chemical constituents and antibacterial activity of S. longepedunclata, the stem bark was successively extracted with n-hexane, EtOAc and MeOH, to furnish 0.52%, 1% and 10.2% yield respectively. Phytochemical screening of the extracts show that the stem bark of the plant contains secondary metabolites such as alkaloids, phenols, flavonoids, terpenoids, cardiac glycosayides, and saponins. The EtOAC extract after silica gel column chromatograp hy yielded three compounds namely 1, 7-dihydroxy-4-methoxyxanthone, 9H-Xanthene-3,5-diol and Oleic acid. The structural elucidations of these compounds were done by the use of spectroscopic methods including FTIR, ^{1}H NMR, ^{13}C NMR and DEPT 135 NMR. To the best of my knowledge compound 21 and compound 22 were isolated for the first time from this species. Furthermore the n-hexane, EtOAc and MeOH extracts and the isolated compounds were evaluated for anti bacterial activity by using Hinton agar well diffusion method against four bacteria strains namely, Escherchia coli, Salmonella typhi, Bacillus subtillus and Staphylococcus aureus. The n-hexane, EtOAC and MeOH extracts show ZI of 14, 15 and 12mm against S. aureus and 12, 11, and 11mm against E.coli. The two compounds: 1, 7-dihydroxy-4-methoxyxanthone and 9H-xanthene-3, 5-diol show ZI of 15 & 16mm against S.aureus and 9 & 11mm against E. coli. The results obtained substantiate the traditional use of this plant against diarrhoea, pneumonia and other inflammatory diseases caused by bacteria.

Key words: *antibacterial activity, NMR, Securidaca longepedunclata, 1, 7-dihydroxy-4-methoxyxanthone, 9-H xanthene-3, 5-diol*.

1. INTRODUCTION

1.1 Background of the study

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Many of these isolations were based on the uses of the agents in traditional medicines [1]. Medicinal plants have been used as a means of curing or preventing disease which is now called phytotherapy [2]. Medicinal plants serve as a source of medicines in a variety of communities for the treatment of numerous pathogenic infections [3]. An enormous amount of information about medicinal properties of various plants exists in various ethnic cultures and can be used to provide sources of new medicines [4]. Ethno-botany and ethno-medical studies are today recognized as the most viable methods of identifying new medicinal plants for bioactive constituents [3]. Some African medicinal plants have been ethno-botanically and scientifically implicated in the treatment of a variety of human infections [5]. The use of medicinal plants as a curing and preventing agents against different diseases arises from their chemical compositions. They contain compounds that are active against microbes [6]. These bioactive compounds have a broad range of activities and are potentially antibacterial, antifungal and antitumor agents [7]. The medicinal values of the plants are due to the chemical substances that produce a definite physiological action on human body and are called phytochemicals [8]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids [9]. They are chemicals extracted from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances [10]. The use of medicinal plants for treatment of all kind of diseases is due to their less harmful effects as compared to drugs synthesized in the laboratory [11, 12]. The leaves of *Securidaca longipedunculata* are used for treating wounds, sores, cough, venereal diseases, snake bite and as a purgative to treat tuberculosis, bilharzias, skin diseases and convulsion in children [13]. Although protected under provincial and national legislation, *S. longipedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa [14].

The uses of this plant include a great variety. In particular there are many different medicinal uses for this tree around Africa. It can be used to treat sicknesses as small as headaches or as severe as arthritis. The roots of the tree can be used for treatments to human ailments such as coughs, chest complaints, toothaches, gout, fevers, constipation, diabetes and microbial infections. It also possesses anti-inflammatory properties that help to reduce arthritic pains [15].

The antibacterial studies so far carried out on the root extract of *S. longepedunclata* imply that many of the compounds obtained from root bark of the plant show positive anti bacterial activities. For example as a report in [16] six bacteria strains namely *Staphylococccus aurous, Streptococcus pyogenes, Pseudomonas aurginosa, Pseudomonas flouresens, Klebsiella pneumonia* and *Salmonella typhi* show positive sensitivity to the hexane, chloroform, ethyl acetate and methanol extracts of the root bark of this plant. As explained above even though some phytochemical investigations were done on the leaves and roots of this plant, it is also important to carry out phytochemical investigation on its stem bark. So this study aims at the investigation of phytochemical and anti bacterial studies of the stem bark of this plant.

The stem bark of *S. longepedunclata* is a thick bark with a golden colour. When it is chopped and dried the golden colour is fade. It has special irritating odour which causes sneezing when inhaled. *S. longepedunclata* stem bark has a salty taste. It forms foam when introduced in to water. The foam of *S. longepedunclata* feels slippery to touch thus it is traditionally used for washing clothes in some parts of Ethiopia.

The stem bark of *S. longepedunclata* is used for treating toothache, headaches, rheumatic pains and venereal diseases in some parts of Africa [17, 18]. In agreement with this report in Ethiopia, in West Wollega zone of Oromia region people use the chopped stem bark of this plant to treat toothache, headaches and rheumatic pains. The stem bark is chopped and the paste is put in the hole of the sick tooth to cure. During the treatment of rheumatic pains with stem bark of *S. longepedunclata*, the powder of the bark is rubbed against the pain feeling part of the body. Despite the traditional uses of this plant against the wide range of diseases, there is a limited scientific report on the chemical constituents and antibacterial activities of the stem bark of *S. longepedunclata*. On the basis of this gap we have performed this study on the stem bark of *S. longepedunclata* and we have isolated three compounds, and then characterized the obtained compounds. Finally we have tested the anti bacterial activities of

the extracts and the compounds by applying them on four different bacteria strains namely *Escherchia coli, salmonella typhi, Staphylococcus aureus* and *Bacillus subtillus.*

1.2 Statement of the problem

Despite the discovery of many drugs and medicines, people that live in sub-Sahara region are still suffering from different infectious diseases [19]. Studies reveal that many new diseases are discovered in tropical and sub-tropical regions in different times [20]. The discoveries of new medicines and drugs are crucially important either pharmaceutically from industries or traditionally from medicinal plants with the same magnitude along with the newly discovered diseases. These diseases are the causes for one half of all deaths in developing countries due to poverty, unavailability of medicines and resistance of microbial to the drugs that administered for the diseases. The high cost of synthetic drugs is another problem for people with less income for their daily life in addition to insufficient health facilities [19]. In order to tackle this problem the search for antimicrobial compounds from medicinal plants is essential.

In Ethiopia there are many medicinal plants used for different diseases. *S. longepedunclata* which belongs to *Securidaca* genus is traditionally used to treat sicknesses such as cough, chest complaints, toothaches, fevers, diabetes and other microbial infections in Africa and in other parts of the world. Hence this research is aimed at finding out antibacterial compound from the stem bark extract of *S. longepedunclata.*

1.3 Significance of the study

Mankind started to use medicinal plants starting from ancient times until now days in treating illness and preventing diseases. Even though there are many medicinal plants that people used to treat different diseases, some plants could be harmful for human health. This study has focused on the extracting chemical compounds from the crude extract of the stem bark of *S. longepedunclata* and the elucidation of the compounds obtained from the plant. Also the study has targeted at the application of the compounds on different bacteria strains to test the activity of these compounds on them. Understanding the structures of the compounds obtained from *S. longepedunclata* has much significance for industrial and pharmaceutical applications. The phytochemical investigation of the stem bark of *S. longepedunclata* may help us to arrive at new natural products. It also provided information

about the structure of the compounds obtained from *S. longepedunclata*. Moreover compounds isolated from this species may elicit good antibacterial activities. Finally this research has laid base for other researchers to initiate them to conduct further investigations on *S. longepedunclata*.

1.4 Objectives of the study

1.4.1General objective

The main objective of this research is to conduct phytochemical investigation and antibacterial study of the stem bark of *S. longepedunclata*.

1.4.2 Specific objectives

- To successively extract the stem bark of *S. longepedunclata* by using n-hexane, ethyl acetate and methanol.
- To conduct phytochemical screening test on the crude extract of stem bark of *S.longepedunclata* using standard protocols.
- To isolate compounds from the crude extract of stem bark of *S. longpedunclata* by using column chromatography.
- To characterize the isolated compounds by employing some physical properties and different spectroscopic methods including FTIR and NMR (^{1}H-NMR, ^{13}C-NMR and DEPT-135).
- To test the antibacterial activities of the crude extracts and the isolated compounds from the stem bark of *S. longepedunclata* against four bacteria strains namely *Escherchia coli, salmonella typhi, Staphylococcus aureus* and *Bacillus subtillus.*

2. LITERATURE REVIEW

2.1. The family Polyglaceae

The Polyglaceae or the milkwort family is made up of flowering plants in the order Fabales. They have a near-cosmopolitan range, with about 21 genera and 900 known species of herbs, shrubs and trees. Over half of the species are in one genus, Polygala, the milkworts [21].

Under the Cronquist classification system, Polygalaceae were treated in a separate order of their own, Polygalales. Currently, according to the Angiosperm Phylogeny Group, the family belongs to Fabales [22].

The centre of diversity of polyglaceae is tropical and sub-tropical areas [22].

Polyglaceae is a remarkable family for the superficial resemblance of the flowers to the well known papilionaceous flower, but the floral structure is quite different from the fabaceae one. The polyglaceae comprises the genus *polygala, Salmoniya, Securidaca* and *Xantophylum.* The economic importance of the first three genus is medicinal while that of Xantophylum is a fine wood [23]. *Polygala* is the largest genus in the polyglaceae family which has about 325 species that range from woody trees up to 15m tall to smaller herbs about 15cm high [24].

2.2. The genus *Securidaca*

The generic name *Securidaca* is derived from Latin securis, as the shape of the wing on the nut recalls a hatchet. The specific name *longepedunculata* hints at the long peduncle on which the flowers are borne [25].

The genus *Securidaca* comprises about 80 species, characterised by papillionaceous purplish flowers and mostly scandent shrubs and lianas, which produce compounds known as securixanthones with antimicrobial and antioxidant properties [26].

The Polygalaceous genus *Securidaca* occurs throughout the tropics. Most of the species are climbers, while a few are shrubs or small trees. The majority of the 80 recognized species occur in America, with only two recorded from Africa and eight described from Indo-Malaysia. *Securidaca* was established in 1754 by Linnaeus and was based on material from America. The genus was characterized by its keel with or without a small fringed crest, its eight stamens with filaments fused toward the base, and its 2-locular, winged fruit. The genus

Securidaca can be separated from other Polygalaceae on the combination of one locular samaroid fruit, eight stamens and three petals [27].

2.3. Botanical information of the *S. longepedunclata*

S. longepedunclata belongs to the family Polygalaceae. It is a species of tree in the *Securidac*a genus. Its leaves are oblancelate and obtuse at apex. The flowers are purple or blue in colouration and the seeds are winged [27]. It is a fairly small to medium-sized tree, measuring between 6 and 12 meters tall. It has pale grey, smooth bark with leaves that grow in clusters. Its small branches are covered in very fine hair. The fruits are round and are attached to a wing that becomes up to 40 mm long. They are sweetly scented and grow in small bunches on a peduncle [28].

2.4. Distribution of *S.longepedunclata*

S. longepedunclata is one of the traditional medicinal plants distributed in many parts of the world. The tree is most commonly found in the tropical and sub tropical areas of Africa [29, 30]. The documented species distribution of *S. longepedunclata* includes Angola, Benin, Botswana, Burundi, Cameroon, Chad, Cote-d'ivoire, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Mali, Malawi, Niger, Nigeria, Rwanda, Senegal, South Africa, Sudan, Uganda, Zambia and Zimbabwe [31]. It is found in various types of woodlands and in arid savannas, and its habitat varies according to climate and altitude. It may be found on sandy, acidic or rocky soils. It is native to the North West and Limpopo provinces of South Africa, and is locally present northwards in the African subtropics and tropics. In tropical Savannas, it is especially found in Miombo and Caesalpinoid wood land. The plant is a savannah shrub with twisted bole or slender erect branches and grows up to 30ft high [26].

2.5. Medicinal values of *S. longepedunclata*

The uses of *S. longepedunclata* plant include a great variety. In particular there are many different medicinal uses for this tree around Africa. It can be used to treat sicknesses as small as headaches or as severe as arthritis [23].The leaves and roots are used to treat wounds, coughs, venereal diseases, diarrhoea, snake bites, bilharzias and other ailments [15].

The roots of the tree can be used for treatments to human ailments such as coughs, chest complaints, toothaches, gout, fevers, constipation, diabetes and microbial infections. It

also possesses anti-inflammatory properties that help to reduce arthritic pains [8]. In Limpopo, the Vhavenda people use the roots to prevent mental disorders and they believe that this remedy will also protect children from illnesses during breastfeeding. People in Zimbabwe use the roots to treat people who seem to be possessed by evil spirits and it is often used on snake bites [9]. In northern Nigeria, the Nupe and the Hausa tribes utilize *S. longepedunculata* ethno-medicinally as a remedy for numerous human and animal ailments. This plant is also used for the treatment of every conceivable ailment such as head-ache, rheumatism, tuberculosis, cancer, venereal diseases, diabetes as well as abortifacient and probably that is why the Hausas refer to it as "uwar magunguna" meaning ''the mother of all medicines'' [24]. They are used for eye complaints (conjunctivitis), malaria, venereal diseases, urethral discharges, stomach problems, dysent ery, rheumatism, fibrositis, toothache, headache, sleeping sickness, cough, chest complain ts, snakebite, and wound dressing. An infusion of the roots is used as a mouthwash in cases of toothache and is applied to cuts on the legs to treat inflammatory conditions [27].

The extract of *S. longepedunculata* was effective in neutralizing lethal effects of Cobra (*Naja nigricollis*) venom in rats. The water extracts of *S. longepedunculata* has potent snake venom neutralizing capacity against venom of cobra (*N.* n*igricollis*). This could be explained by the fact that the combined root bark and leaves extract might possess a toxic potential or activity that acted against the snake venom, which yielded highest number of survival at 300 mg/mL and 33.33% mortality at 200 mg/mL on rats. This is in agreement with the study conducted by Ahmed *et al*. (2010) [32] to determine anti-snake venom activity of different extracts of *S. longepedunculata* against Russel viper venom. Therefore, the root bark and leaves extract of *S. longepedunculata* can be said to possess an anti snake venom activity thereby neutralizing the toxic effects of *N. nigricollis* venom. The potent snake venom neutralizing capacity of this extract could potentially be used for therapeutic purpose in case of snake bite envenomation [33].

Different parts of the *S. longepedunclata* have different traditional medicinal values.

2.5.1. Roots

The smoke resulting from burning the root of *S. longipedunculata*, combined with that of *Zanthoxylum zanthoxyloides,* is inhaled to treat malaria and fever [34]. A root decoction may also be drunk to treat fever, malaria, hernias, gonorrhoea, palpitations, headaches,

oedema, rheumatism, sexual impotence, toothache, fungal infections and malaria [35].

Roots may also be ground into powder form, dissolved in water and taken orally for constipation, pneumonia, back ache, blood purification, sexually transmitted infections and as an aphrodisiac [36]. Dried roots are soaked in water, along with *Citrus aurantifolia* and the resulting juice is taken orally for three days to treat constipation while the dried root is boiled in distilled water and used to treat pneumonia [37].

2.5.2. Leaves

Fresh leaves are made into paste with little or no water along with the bark of *Gardenia erubescens* and applied externally twice a day for sixty-three days to treat skin cancer [34]. Moreover, fresh leaves are made into paste with little or no water along with leaves of *Jussiaea suffruticosa* and shea butter and the resulting mixture is applied externally, twice a day to treat a variety of skin infections. Dry leaves are also ground into powder and put into the fire and the resulting smoke is inhaled to treat headaches while the boiled leaves are taken orally for contraceptive purposes [38]. The leaves are either chewed fresh or both orally and nasally administered to treat epilepsy, headaches, stomach ache, infertility, snakebite, toothache and to expel the placenta [39].

2.5.3. Stem bark

A spoonful of powdered stem bark is mixed with *Mondia whitei* (stem bark), *Uvaria afzelii* (root bark), *Allium ascalonicum* (bulb) and *Parkia biglobosa* (seeds) and then taken with hot porridge to treat a variety of viral infections [40]. The dried bark is ground into a powder and taken orally with cow's milk or porridge for fourteen days to treat dysentery [38]. A decoction from the stem bark may be taken orally to treat stomach ache, headaches, inflammation, chest complaints, abortion, jaundice, ritual suicide, constipation, snake bites, infertility problems, epilepsy and venereal diseases [41]. The powdered stem bark is also mixed with hot water and taken orally to treat syphilis and gonorrhoea [42].

Different parts of *S. longepedunclata* are used as traditional medicine to cure and prevent different diseases in different parts of Africa [43] as indicated in Table 1.

Table 1: Ethnomedicinal uses of *S. longepedunclata* in different parts of Africa

Country	Plant part	Ethnomedicinal uses
Zimbabwe	Roots	Venereal diseases, syphilis, pains, fever, epilepsy, pneumonia, tuberculosis.
Nigeria	Leaves	Dislocated jaw, headaches, skin cancer, skin infections, contraceptive purposes
South Africa	Leaves	Flu, blood purifier, aphrodisiac, psychoactive purposes
Kenya	Whole plant	Malaria, tick prevention in animals
Burkina Faso	Roots	Malaria
	Stem bark	Skin disease
Uganda	Roots	Fever, malaria, ascariasis
Nigeria	Roots	Abortion, constipation, coughs, fever, pneumonia, sexual boost, toothache, tuberculosis, rheumatism
	Stem bark	Treat infections related to nervous Dysentery, malaria, typhoid and frequent stomach ache
Botswana	Roots	Coughs and as an aphrodisiac
Ethiopia	Stem bark	Toothache, arthritic pains and pneumonia

2.6. Chemical constituents of *S. longepedunclata*

Different literatures have reported the existence of different chemical constituents so called secondary metabolites in the leaves and root bark of *S. longepedunclata*. For example the phytochemical investigation done on the aqueous root bark extract of this plant revealed the existence of alkaloides, tannins, flavonoids, saponins and cardiac glycosides [44]. The phytochemical investigations so far done on the root bark and leaves of *S. longepedunclata* reveal that the plant possesses different compounds. Ethyl acetate and methanol extracts of root bark of *S. longepedunclata* were reported to have alkaloids, flavonoids, tannins and saponins [16} where as the methanol extract of the leaves of *S. longepedunclata* contains a member of glycosides named Quercetine-3-O-D-xyloside [17].

Table 2: Phytochemical constituents of the aqueous root bark of *S. longepedunculata* [17]

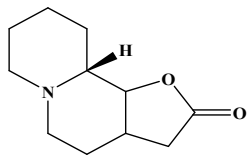
Constituents	Inference
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	+
Cardiac glycosides	+

(+) present

2.7. Phytochemistry of *S. longepedunclata*

The phytochemical investigations carried out on the roots and leaves of *S. longepedunclata* suggest that different compounds were isolated from the plant's roots and leaves. Most classes of compounds have been isolated from the roots of *S. longepedunclata* using a variety of solvents such as methanol, ethyl acetate and dichloromethane [45]. The volatile oil of the roots contains large amounts of methyl salicylate [46]. The report agrees with the report that revealed the major component (over 90%) of the volatile material from the root bark is methyl-2hydroxybenzoate (methyl salicylate) [47]. Furthermore, securinine **(1)**, presenegenin **(2**), 2-hydroxybenzoate esters such as methyl-2-hydroxy-6-methoxybenzoate **(3)** and its benzyl analogue were also reported. Some of the compounds that isolated from the roots and leaves of *S. longepedunclata* are Methyl salycylate **(4)**, benzyl-3-hydroxy-2-methoxybenzoate (R1=H, R2=OH) or Benzyl-2-hydroxy methoxybenzoate (R1=OH, R2=H) **(5),** 4-Methoxy-benzo [1,3] dioxo-5-yl phenyl methanone **(6)** and 2-Methoxy-3,4-methylene dioxy benzophenone **(7).** The very interesting compounds with active anti bacterial activities, xanthones such as 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone**(8),** 5-O-phenyl-1-hydroxy-2,3,6,7,8-pentamethoxyxanthone **(9),** 1,6-dihydroxyxanthone **(10),** 2-hydroxy-1,5 dimethoxy xanthone **(11**) and other members of xanthones were isolated from the leave extracts of *S. longepedunclata* [43]. In addition to the isolated and reported compounds above other compounds with good anti microbial activities were also isolated from different parts of *S. longepedunclata*. For example as reported in [13] a liquid compound named benzyl-2-hydroxy-5-methoxy benzoate **(12)** was isolated from the root bark petroleum ether extract of this plant. Other compounds reported from the root bark of this plant are benzyl-6-methoxybbenzoate **(13),** Cinnamic acid **(14),** Caffeic acid **(15),** Sinapic acid **(16),** P-coumaric

acid **(17)** and β-sitosterol **(18)** [43].



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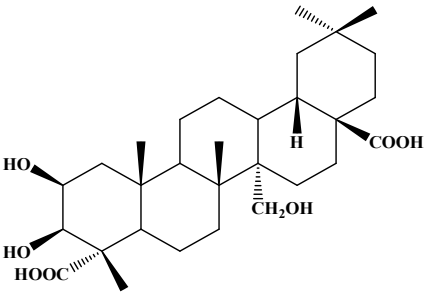

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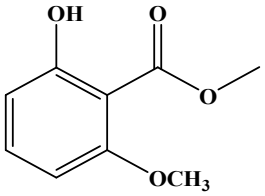

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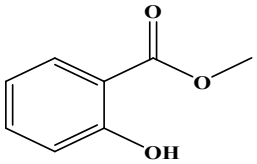

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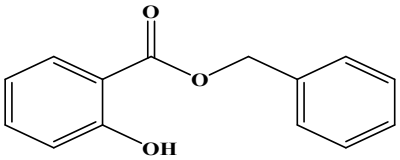

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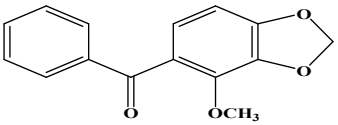

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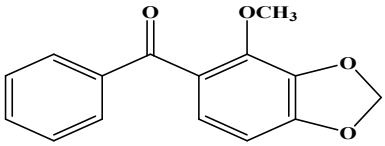

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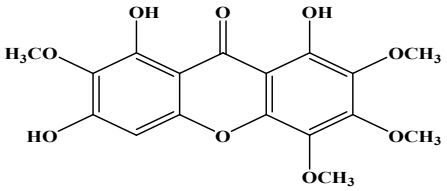

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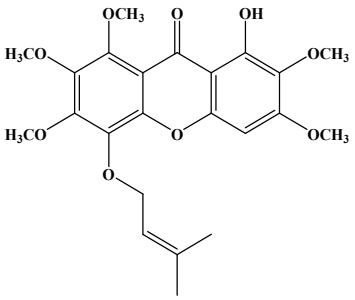

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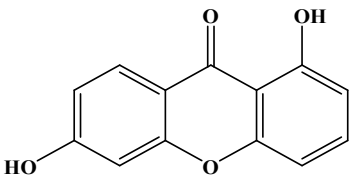

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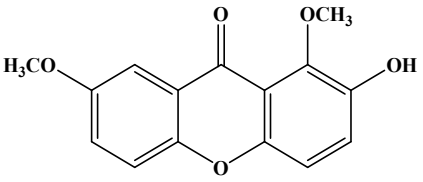

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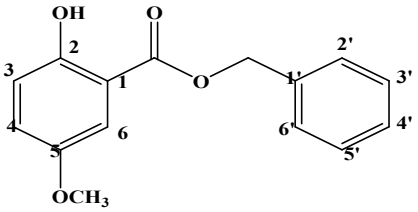

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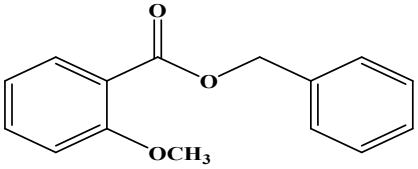

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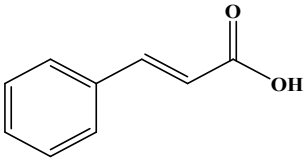

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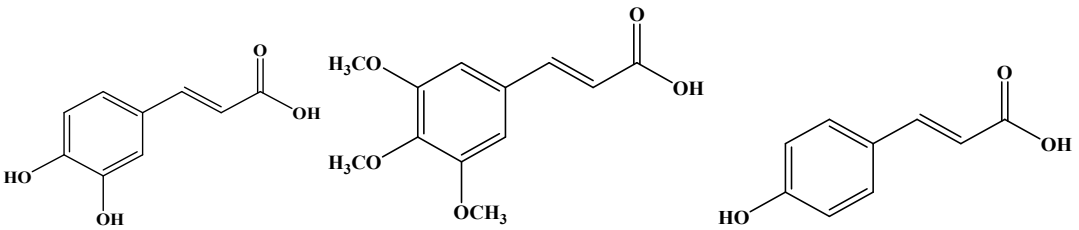
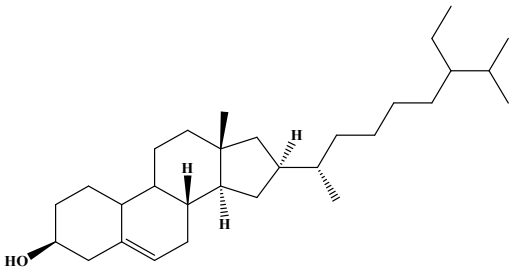


(15) **(16)** **(17)**

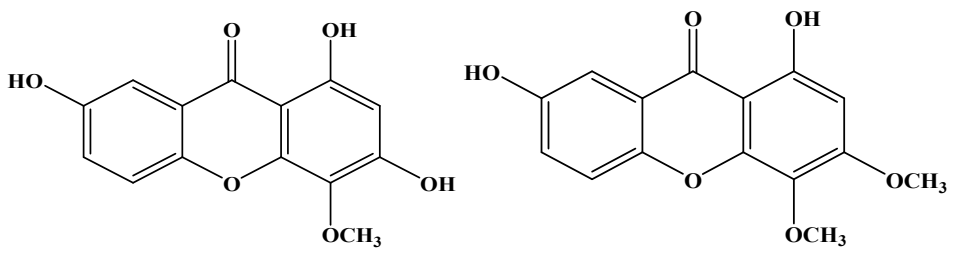


(18)

Figure1: Structures of compounds previously reported from *S. longepedunclata*

2.8. Other species of the genus *Securidaca*

The genus *Securidaca* consists of about 43 species growing in tropical America, a few of them distribute in tropical Asia and Africa [48]. One of the species is *S. inappendiculata*. It is a traditional Chinese herbal medicine which is used as an anti-inflammatory, anti-bacterial and anti-rheumatism agent [49]. Studies on this species have revealed the presence of xanthones, organic acids, hemiterpenic acid glycosides and other compounds [50]. Re-investigation on the stem bark of *S.inappendiculata* led to the isolation of two xanthones namely Securixanthone C (1, 3, 7-trihydroxy-4-methoxy xanthone) **(19)** and Securixanthone D (1,7-hydroxy-3, 4-dimethoxy xanthone) **(20).** [51].



(19) **(20)**

Figure 2: Structures of compounds isolated from *S. inappendiculata*

2.9. Biological activities of *S. longepedunclata*

As reported in different literatures the extracts and the compounds from *S. longepedunclata* have good antimicrobial activities against different pathogens. The crude extract from the leaves and roots of this plant show potent antimicrobial activity on different pathogens. For example it was previously reported that the aqueous leaf extract of *S. longepedunclata* yielded zones of inhibition (ZI) of 15 mm against both *Escherichia coli* and *Salmonella typhi*, while the chloroform leaf extracts exhibited ZI of 18 mm against *Pseudomonas aeruginosa*. The methanol extracts and the chloroform fraction of the root bark exhibited ZI of 28 mm against methicillin resistant *Staphylococcus aureus*, while hexane and ethyl acetate fractions exhibited ZI ranging from 14 to 19 mm against *Streptococcus pyogenes, Pseudomonas fluorescens and Klebsiella pneumonia* [43].

The extracts from the root bark of *S. longepedunclata* show anti bacterial activities, however they show negative result on a particular fungal strain *Candida albicans* as shown in Table 3. [16]

Table 3: The antimicrobial susceptibility test of *S. longepedunculata* root bark fractions [16].

Tested microbials	Hexane extract		Chloroform extr.		Ethyl acetate extr.		Methanol extract	
	Sen/	In/ zon	Sen/	In/ zone	Sen/	In/ zon	Sen/	In/ zone
S. aureus	S	14	S	28	S	22	S	28
S. pyogens	S	17	S	20	S	19	S	20
P aeruginosa	S	16	S	28	S	17	S	18
P.flourens	S	14	S	20	S	17	S	16
K.pneumonia	S	16	S	17	S	16	S	20
S.typhi	S	16	S	27	S	18	S	23
C.albicans	R	0	R	0	R	0	R	0

Key: Sen/= Sensitivity In/ zone= inhibition zone S=sensetive R= resistant

3. MATERIALS AND METHODS

3.1 Plant materials collection and authentication

The fresh stem bark of *S. longepedunclata* was harvested from Tanki hill which is found in Menesibu woreda of Oromia region which is 585 km west of Addis Ababa near Mendi town in November 2018. The plant material was authenticated by botanist Shambel Alemu and a voucher specimen (Voucher no. 002) was deposited in the National Herbarium, Ethiopia, Department of Biology, Addis Ababa University, Adis Ababa. The collected stem bark was washed with distilled water to avoid dusts. Then it was chopped and dried under shade to avoid direct sun light that can affect some of the compounds in the plant. The chopped and dried stem bark of *S. longepedunclata* was grinded by a new local mortar and local grind stone.

(a)

(b

(C)

Figure 3: *S. longepedunclata* (a) Tree (b) Stem (c) chopped stem bark

(Pictures taken by Markos Addisu on November 2017)

3.2 Materials and instruments

3.2.1 Materials

The materials used in this research were digital balance, measuring cylinder, soaking jars, beakers, separator funnels, gravity filtration apparatus, Whatman no.1 filter paper and rotary evaporator.

3.2.2 Instruments

Analytical thin layer chromatograms were run on a 0.2 mm thick layer of silica gel GF 254 (Merck) coated on aluminium plate. Column chromatography was performed using silica gel (200-400 mesh) Merck. ^{1}HNMR, ^{13}CNMR and DEPT-135 NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz in $CDCl_3$ and DMSO-d_6. Infrared (IR) spectra were obtained on Perkin-Elmer 65FT ((IR ν_{max}KBr (4000-400) cm^{-1}) infrared spectrometer using KBr pellets

3.3 Chemicals

Solvents used in this study were n-hexane (Loba chemie PLC, Mumbai, India), ethyl acetate (Loba chemie PLC, Mumbai, India) and methanol (Loba chemie PLC, Mumbai, India). Other chemicals are distilled water, vanillin, sulphuric acid, silica gel (200-400 mesh size), acetone and ethanol. All the chemicals were of analytical grade and were bought from Alchem general trading, Addis Ababa, Ethiopia.

Spots were detected by observation under UV light (254 and 365 nm) and by heating the TLC plate after dipping in vanillin in sulphuric acid.

3.4 Extraction

The powdered stem bark of *S. longepedunclata* (500 g) was soaked in 2.5 L of n-hexane for 72 hrs, filtered and concentrated by rotary evaporator at 40^{o}C to give 2.6 g of greenish yellow solid. The marc was then soaked in 2.5 L ethyl acetate for 72 hr. Then it was filtered and concentrated by rotary evaporator to give 5 g of dark greenish solid. The marc left after extraction with EtOAc was soaked in 2.5 L methanol for 72 hr, filtered and concentrated to give 51 g of dark brown jelly solid. After soaking the plant material with each solvent regular shaking was carried out for efficient extraction of the secondary metabolites. The extracts

were analyzed with TLC to select the best solvent system for isolation. The TLC profiles of each extract are shown under result and discussion part below in Fig.4.

3.5 Isolation of compounds from the crude extract

The ethyl acetate extract (5 g) was adsorbed on equal amount of silica gel and fractionated over silica gel (150 g) column chromatography using n-hexane for column packing. The column was eluted with n-hexane: ethyl acetate: methanol of increasing polarities to give 90 fractions of each 50 mL.

The purity of the compounds from the collected fractions was detected by TLC. Spots were visualized using UV lamp at 254 and 365 nm and by heating the TLC plate on hot plate after dipping in vanillin in sulphuric acid. Fractions with spots of similar Rf value were combined based on their TLC profiles.

The fractions 39-41 eluted with n-hexane: EtOAc (1:1) showed a single spots each with similar Rf values and combined as one fraction. The combined fraction was a yellow crystal with a yield of 8 mg and coded compound 21.

The fractions 23-31 eluted with n-hexane: EtOAc (6:4) showed a single spots each with similar Rf values and combined as one fraction. The combined fraction was a yellowish white crystal with a yield of 7.5 mg and coded compound 22.

Fractions 57-59 eluted with n-hexane:EtOAc (4:6) showed three spots each with similar Rf values. They were combined as one fraction and re-fractionated over column chromatography with n-hexane:EtOAc (3:7) in which 11 fractions were collected. Fractions 2-7 show one spot each with similar Rf value, combined as one fraction and the TLC profile was analyzed and shown one spot. A brown yellow paste with a mass of 7.0 mg was obtained and coded compound 23.

3.6 Phytochemical screening of the crude extracts

The preliminary phytochemical tests were done to test the types of secondary metabolites present in the three successive extracts of the stem bark of *S. longepedunclata.* These qualitative tests were done according to the protocols in the literatures [52, 53].

3.6.1. Phenols

Ferric Chloride Test: Ferric chloride solution (4 drops) was added to the test tubes containing each extract, and a bluish black colour was formed in all the tubes.

3.6.2. Saponins

To the boiling tubes containing 0.5 g of each extract, 5 mL distilled water was added and shaken and then heated in water bath for 2 min. A stable froth was observed in all tubes.

3.6.3 Terpenoids

0.5 g of each extract was added in a boiling tube and 2 mL chloroform was added. Later 3 mL concentrated sulphuric acid was added drop wise. A reddish brown colour was formed at the interface in all boiling tubes

3.6.4 Alkaloids

0.5 g of each extract was added into a boiling tube. 5 mL of 2 % sulphuric acid was added, mixed and filtered. Few drops of Drangedorff´s reagent were added. An orange red precipitate was observed in the EtOAc and MeOH extracts.

3.6.5 Flavonoids

0.5 g of each extract was heated with 10 mL ethyl acetate over a steam bath for 3 min, the mixture was filtered and 4 mL of the filtrate was shaken with 1 mL dilute ammonia. A yellow colour was observed in all tubes.

3.6.6 Anthraquinone

0.5 g of each extract was taken in to test tubes and 5 mL chloroform was added and shaken for 5 min. The extract was filtered to the second test tube and shaken with an equal volume of 100 % ammonia solution. No result was observed in all the test tubes.

3.6.7 Cardiac glycoside.

To 2 mL of each extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric were added. A reddish brown colour was observed at the junction of the two and layers in EtOAc and MeOH extracts.

3.7 Evaluating antibacterial activity of the crude extracts

The anti-bacterial activity of the three extracts and that of the isolated compounds of the stem bark of *S. pedunclata* was done *in vitro* using the American type culture collection (ATCC). Four bacterial strains were obtained from ASTU biology laboratory, Adama, Ethiopia. Two Gram-negative bacterial strains (*Escherchia coli* and *Salmonella typhi*) and two Gram–positive (*Bacillus subtillus* and *Staphylococcus aureus)* human bacterial pathogens were selected for the study. The antibacterial activities were determined using well diffusion method against different strains of bacteria. Chloramphenicol and dimethylsulphoxide (DMSO) were used as a positive and negative control respectively.

3.7.1 Inoculation and Incubation

Antibacterial activity was done on Mueller Hinton agar. 0.1 mL of bacteria suspension was uniformly spread on the sterile Mueller Hinton Agar Petri dish. Standard solutions of 200 μg/mL concentration of the extracts and isolated compounds were prepared and 100 µL solutions from the concentration were loaded to the discs. 6 mm-diameter wells were cut from the agar using a sterile cork-borer and the sample were placed in the wells. The Petri dish was then placed in an incubator for 24 hours at 37^{o}C. At the end of incubation period, the inhibition diameter was measured and expressed in mille meters. Antibacterial activity was determined by measuring the inhibition zone diameter (in mm) against each tested organism.

4. RESULTS AND DISCUSSION

4.1 Extract yield

The stem bark powder of *S. longepedunclata* was successively extracted with n-hexane, EtOAc and MeOH to give 2.6 g (0.52%), 5 g (1%) and 51 g (10.2%) of crude extract yields respectively. The amount obtained from MeOH was much greater than that of n-hexane or (and) EtOAc. This implies that most of the secondary metabolites in the stem bark of *S. longepedunclata* are polar compounds.

4.2 TLC profiles of the crude extracts

The TLC profiles of the three extracts (n-hexane, EtOAc and MeOH) are shown in Figure 4. The mobile phases used to detect the TLC profiles for n-hexane, EtOAc and MeOH extract were n-hexane:EtOAc (1:1), n-hexane:EtOAc (2:8), and EtOAc:MeOH (8:2), respectively. The spots in each case were visualized first under UV lamp and then by heating on a hot plate after dipping the TLC plate in vanillin in H_2SO_4. The EtOAc and MeOH extracts show good TLC profiles than that of n-hexane.

-a- **-b-** **-c-**

Figure 4: TLC profiles of (a) n-hexane, (b) EtOAc and (c) MeOH extracts

4.3 TLC profiles of different fractions

4.3.1 TLC profile of fractions 39-41

Fractions 39-41 which were eluted with n-hexane:EtOAc in the ratio of 1:1 show a single spot each with similar Rf values. The fractions were combined as one fraction and the TLC of their combination was analyzed and observed that it show a single spot with Rf = 0.63.

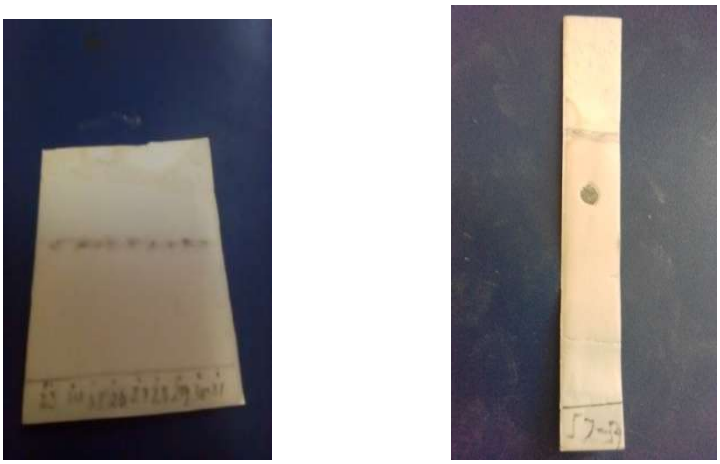
The TLC profiles of each fraction and that of their combination are given below in Fig.5. The mobile phases used to analyze the TLC of each fraction and the combination were, n-hexane:EtOAc in the ratio of (4:6) and (1:1) respectively.

-a- -b-

Figure 5: TLC profiles of fractions 39-41(a) separate (b) combined

4.3.2 TLC profiles of fractions 23-31

Fractions 23-31 which were eluted with n-hexane:EtOAc (6:4) show one spot each with similar Rf values. The fractions were combined as one fraction and the TLC of their combination also show a single spot with Rf = 0.77. The mobile phase used to analyze the TLC of the fractions and their combination was n-hexane:EtOAc in (1:1) ratio. The TLC profiles are shown in Figure 6.

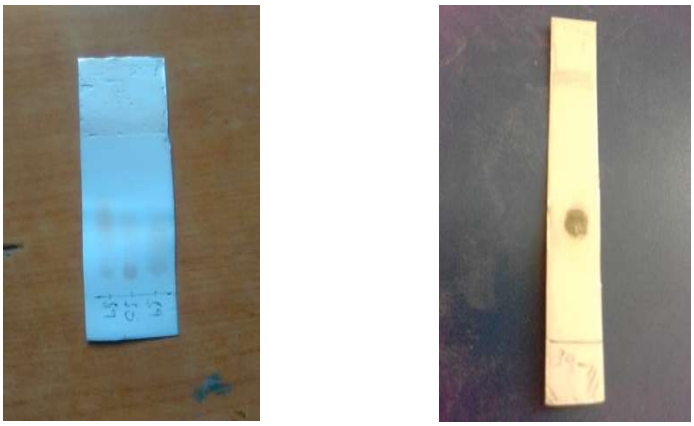


(a) (a) (b)

Figure 6: TLC profiles of Fractions 23-31 (a) separate (b) combined

4.3.3 TLC profiles of fractions 57-59

Fractions 57-59 which were eluted with n-hexane:EtOAc in the ratio of 3:7 show three spots each with similar Rf values. They were combined as one fraction. The combination of the three fractions was re fractionated over a column chromatography with n-hexane:EtOAc in the ratio of 2:8. A total of 11 fractions were collected. Fractions 2-7 show one spot each and they were combined as one fraction. The TLC of the combined fractions was analyzed and observed to be one spot (Rf = 0.44). The TLC profiles of the first three fractions and the one that obtained after re fractionation are shown in Figure 7.



-a- -b-

Figure 7: TLC profiles of Fractions 57-59 (a) before (b) after refractionated

4.4 phytochemical screening of the extracts

Phytochemical screening of n-hexane, EtOAc and MeOH extracts of stem bark of *S. logepedunclata* revealed the presence of secondary metabolites such as phenols, saponins, alkaloids, cardiac glycosides, flavonoids, and tannins. The present study revealed that phenols, saponins and flavonoids exist in the three extracts while anthraquinones were absent. Alkaloids,tannins and cardiac glycosides are found only in EtOAc and MeOH extracts but not in n-hexane extract. The presence of alkaloids and absence of anthraquinones in the EtOAc and MeOH extracts of root bark extract of *S. longepedunclata* were previously reported in [16].

The medicinal value of plants lies in some chemical substances or secondary products that have definite physiological functions in the human body and which may help in protection against various diseases.

The phytochemical screening result of the three crude extracts (n-hexane, EtOAc, and MeOH) are summarized in Table 4.

Table 4: Phytochemical screening of stem bark n-hexane, EtOAc and MeOH extracts of *S. longepedunclata*

No	Phytochemical	n-hexane extract	EtOAc extract	MeOH extract
1	Phenols	+	+	+
2	Saponins	+	+	+
3	Alkaloids	-	+	+
4	Anthraquinones	-	-	-
5	Cardiac glycosides	-	+	+
6	Flavonoids	+	+	+
7	Tannins	-	+	+

Key: (+) present, (-) absent

The use of some plants for medicinal purpose is due to the presence of secondary metabolites [54], which supports the traditional use of *S. longepedunclata* for the treatment of different diseases. For example the alkaloidal compound named papaverine which is reported in [20] has been shown to have a potent inhibitory effect on the replication of several virus including crytomegalo virus, meascles and HIV. Hence the existence of alkaloids in *S.longepedunclata* stem bark extract is an evidence for its medicinal values. Additionally the flavonoids described in [55] were reported to have anti-plasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) *P. Falsiparum*. Also phenolic compounds were reported to have anticarcinogenic, anti-oxidant, anti-malarial and anti-microbial activities. For example the compound named combretastin A-4 is an anti cancer molecule, which has a potent target in cancer chemotherapy in cell division [56]. Hence the presence of such secondary metabolites is significant as they may help to cure various life threatening diseases.

4.5 Structural elucidation of the isolated compounds

In this study three compounds were isolated from the stem bark of *S. longepedunclata* and the structures of the compounds are characterized. The characterizations of the compounds are given below.

4.5.1 Characterization of compound 21.

Compound 21 (8 mg) was obtained as a yellow crystal having melting point of 236 $^{\circ}$C. The IR spectrum (Appendix-1) the absorption band at 3286 cm^{-1} indicates the presence of O-H stretching. The weak band at 2920 cm^{-1} shows the C-H stretching of methoxy group. The absorption band at 1667 cm^{-1} is that of the conjugated carbonyl group. The absorption band at1640 cm^{-1} implies the C=C bond stretching of the aromatic ring. The absorption band at 1482 cm^{-1} with the band at 1238 cm^{-1} show the C-H in plane bend of aromatic ring [57].

The ^{1}H NMR (Table 5, Appendix-2) showed the presence of five protons on the aromatic ring and three on the methoxy group. The existence of a singlet signal at δ 3.88 is evident for the presence of methoxy group and it is consistent with the signal reported in [51] for methoxy proton. Two proton ortho coupled signals with AB multiplicity pattern at δ 6.61 (1 *H, d, J* = 8.8 Hz) and at δ 7.17 (1*H, d, J* = 8.8 Hz) are accounted to the C-H aromatic protons on C-2 and C-3 respectively. Three aromatic protons with ABX multiplicity pattern were observed at δ 7.24 (1*H*, *dd, J* = 2.8 and 9.2 Hz), δ 7.42 (1 *H, d, J* = 9.2 Hz) for H-6 and H-5 respectively, and a signal at δ 7.45 (1 *H, d, J* = 2.8 Hz) for H-8 (meta coupling with the one on C-6.)

The ^{13}C NMR spectrum (Table5, Appendix-3) consists of 14 carbon signals which indicates the compound consists of 14 carbon atoms. This signals in combination with that of DEPT-135 signals suggested the presence of 12 aromatic carbons, one methoxy carbon and one C=O carbonyl carbon in the structure of the compound. The six signals at δ 154.0 (C-1), 107.9 (C-2) 119.8 (C-3), 140.2 (C-4), 145.9 (C-4a) and δ 108.9 (C-8b) stand for the six aromatic carbons on the one aromatic ring while the other six signals at δ 119.3 (C-5), 125.3 (C-6), 153.8 (C-7), 108.1 (C-8), 120.0 (C-8a) and δ 150.1 (C-8b) are for the aromatic carbon on the other aromatic ring. The signals at δ 153.8 and 154.0 are the signals of the carbon atoms that bear the hydroxyl groups. Due to their bonding to the more electronegative oxygen atom, the chemical shifts of the two carbons are down field than that of the others.The carbon that bears the OH group must be para to the methoxy bearing carbon in order to fit with that chemical shift (140.2 ppm) as one reported in [50]. The positions of the two hydroxyl groups are on either of the two aromatic rings. The signals at δ 145.9 and 150.1are accounted to the two quaternary carbons that bonded to the oxygen atom, hence assigned for C-4b and C-4a respectively. Similarly the signals at δ 120.0 and 108.9 are consistent with the other two quaternary carbons. The signal at δ 182.2 is that of C=O which is on C-9. The eight signals

that appear on ^{13}C NMR spectrum at δ 108.9, 120.0, 140.2, 145.9, 150.1, 153.8, 154.0 and δ 182.2 do not appear on DEPT-135. This implies that this compound consists of eight quaternary carbons. The other positive signal that appears at δ 57.2 is the signal of the methoxy group in the structure of this compound.

Table 5: ^{1}H NMR, ^{13}C NMR and DEPT 135 NMR of compound 21

Carbon position	^{1}H NMR δ (ppm), J in Hz	^{13}C NMR δ (ppm)	DEPT 135 NMR
1	_	154.0	Q
2	6.61 (*1H, d, J* = 8.8)	107.9	(CH)
3	7.17 (1 *H, d J* = 8.8)	119.8	(CH)
4	_	140.2	Q
4a	-	145.9	Q
4b	-	150.1	Q
5	7.42 (1 H, d, J = 9.2)	119.1	(CH)
6	7.24 (1 *H, dd,* J = 2.8 and 9.2)	125.3	(CH)
7	_	153.8	Q
8	7.46 (1 *H, d,* J = 2.8)	108.1	(CH)
8a	_	120.0	Q
8b	_	108.9	Q
9	_	182.2	Q
-OCH_3	3.88 (3H, s)	57.2	(CH_3)

By gathering all the above spectroscopic information the compound is identified as 1, 7-dihydroxy-4-methoxyxanthone whose structure is presented in Figure 7.

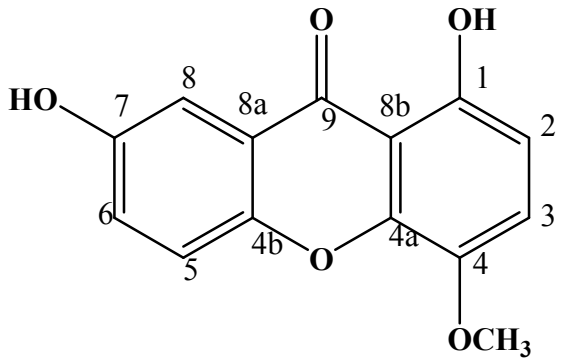


Figure 8: The proposed Structure of 1, 7-dihydroxy-4-methoxyxanthone

Xanthones are secondary metabolites commonly occurring in higher plant families, fungi, and lichens. Their pharmacological properties have raised great interest. Structures of xanthones are related to that of flavonoids and their chromatographic behaviours are also similar. Flavonoids are frequently encountered in nature, whereas xanthones are found in limited number of families. Xanthones are sometimes found as the parent poly hydroxylated compounds but most are mono- or poly methyl ethers or are found as glycosides [58].

Xanthones are generally classified according to substitution on the basic of xanthone skeleton giving 6 main groups: simple xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, bis-xanthones and miscellaneous xanthones. These main groups can be further sub-divided according to the degree of oxygenation into non-, mono-, di-, tri-, tetra-, penta-, hexa and hepta-oxygenated xanthones [59]. Naturally occurring xanthones have emerged as an important class of organic compounds in view of their remarkable pharmacological and other biological activities. It has now been observed that a number of plant products which are in regular use as chemotherapeutic agents contain xanthones as active constituents. Xanthones have been reported to display anti-microbial, anti-carcinogenic, anti-leprosy, anti-oxidant and radio protective effect, anti-parasitic effects, anti-malaria, anti-complement, anti-fungal, anti-HIV, anti-tumoral, anti-bacterial, anti-diabetes, anti-inflammatory, anti-ulcer, anti-viral, anti-diarrheal and anti-protozoal activities [58]. On the basis of this report the compound that isolated above, being a member of tri oxygenated xanthone exhibited good antibacterial activity.

4.5.2 Characterization of compound 22

Compound 22 (7.5mg) was obtained as a yellow white crystal malting at 102^{o}C. The IR spectrum (Appendix 5) showed absorption band at 3394 cm^{-1} attributed to O-H stretching. The strong absorption band at 2920cm^{-1} is that of C-H bond stretching. The band at 1636cm^{-1} along with the band at 1610cm^{-1} implied the C=C bond stretching of aromatic ring. The band at 1478 cm^{-1} showed the C-H in plane bending of the aromatic ring. The band at 1237cm-1 is evident for the existence of C-O stretching.

The ^{1}H NMR (Table 6, Appendix 6) showed the presence of six protons on the aromatic ring. The existence of a singlet signal at δ 3.48 integrated for two protons is evident for the presence of one methylene (CH_2) group. Three aromatic protons with ABX multiplicity pattern were observed at δ 7.54 (1 *H, dd, J* = 2.8, 8.4 Hz), at δ 6.89 (1 *H, d, J* = 8.4 Hz) H-2 and H-1 and at δ 7.48 (1 *H, d, J* = 2.8) H-4 proton. The aromatic protons with ABX multiplicity pattern were observed at δ 7.26 (1 *H, dd, J* = 2.8, 9.2 Hz), at δ 7.41 (1 *H, dd, J* = 7.2, 9.2 Hz) (H-6 and H-7) and at δ 6.72 (1 *H, d, J* = 7.2 Hz) (H-8).

The ^{13}C NMR (Table 6, Appendix 7) consists of 13 carbon signals which indicate that the compound has 13 carbon atoms. These signals in combination with the signals of DEPT-135, suggested the presence of 12 aromatic carbons and one methylene carbon. The signals at δ 136.5 (C-1), 108.2 (C-2), 161.2 (C-3), 107.1 (C-4), 156.4 (C-4a) and at δ 128.1 (C-8b) are those found on one of the two aromatic rings while the other six signals at δ 153.4 (C-5), 109.6 (C-6), 125.3 (C-7), 119.1 (C-8), 150.2 (C-4b) and at δ 128.6 (C-8a) are those found on the second aromatic ring. Among these signals, the six carbon peaks mentioned above on C-3, C-4a, C-4b, C-5, C-8a and C-8b which appear on ^{13}C NMR spectra do not appear on DEPT-135 NMR spectra. This implies that the compound consists of six quaternary carbons. The two signals at δ 161.2 and at δ 153.4 are the signals of carbon atoms that bonded to the oxygen atoms of the carboxyl group. The other two signals at δ 156.4 (C-4a) and δ 150.2 (C-4b) are those attached to the oxygen atom of ether link. Due to their bonding with the more electronegative oxygen atom the chemical shifts of these four carbon atoms are more down field than others.

Table 6: ^{1}H NMR, ^{13}C NMR and DEPT-135 NMR of compound 22

Carbon position	^{1}H NMR δ(ppm), *J* (Hz)	^{13}C NMR δ (ppm)	DEPT-135 NMR
1	6.89 (1 *H, d, J* = 8.4)	136.5	CH
2	7.54 (1 *H, dd, J* = 2.8, 8.4)	108.2	CH
3	-	161.2	Q
4	7.48 (1 *H, d, J* = 2.8)	107.1	CH
4a	-	156.4	Q
4b	-	150.2	Q
5	-	153.4	Q
6	7.26 (1 *H, dd, J* = 2.8, 9.2)	109.6	CH
7	7.41 (1 *H, dd, J* = 7.2, 9.2)	125.3	CH
8	6.72 (1 *H, d, J* = 7.2)	119.1	CH
8a	-	128.6	Q
8b	-	128.1	Q
9	3.48 (2H, s)	29.6	CH_2

By gathering all the above spectroscopic information the compound is identified as 9H-xanthene-3, 5-diol whose structure is indicated in Figure 9.

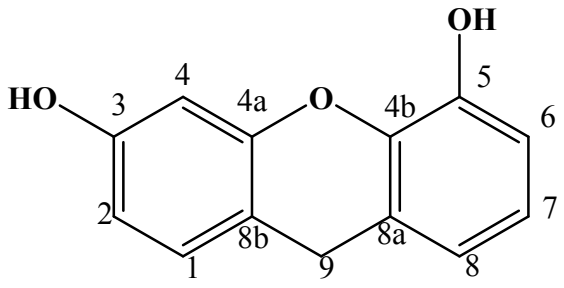


Figure 9: The proposed structure of 9H-xanthene-3, 5-diol

Xanthenes are a very important classes of compounds widely used as leuco-dye in laser technology and in fluorescent materials. Furthermore, these compounds have recently received great attention because of the wide range of their therapeutic and biological

properties such as antibacterial, antiviral and anti inflammatory activities [60]. 9H-xanthene is a yellow organic heterocyclic compound. It is soluble in diethyl ether. Its melting point is 101-102^{0}C and its boiling point is 310-312^{0}C. It is used as a fungicide and it is also a useful intermediate in organic synthesis [61]. From the view point of these reports, the compound that isolated above is a member of xanthenes and it has anti bacterial activity.

4.5.3 Characterization of compound 23

Compound 23 (7.0 mg) was obtained as a brown yellow jelly like paste, melting at 36 ^{o}C. In the FTIR spectra of compound 23 (Appendix-9) the absorption band at 3420 cm^{-1} indicates the O-H stretching of the carboxyl group. The strong band at 2908 cm,$^{-1}$ shows the C-H stretching of alkyl group. The band at1730 cm^{-1} stands for the C=O stretching of carbonyl group. The band at 1616cm^{-1} is accounted to the C=C group. The bands at 1445 cm^{-1} and at 1372 cm^{-1} reveal the presence of C-H bending. This is consistent with the one reported in [58].

The ^{1}H NMR of compound 23 (Table 7, Appendix-10) reveal the presence of 18 proton signals separately and by over lapping one another. The singlet proton signal at δ 0.89 integrated for 3H indicates the presence of terminal methyl group. The triplet proton signal at δ 2.36 integrated for 2*H* stands for the two protons on CH_2 group adjacent to the carbonyl group.

The very intense signal at δ 1.27 (m, 18 *H*) is the overlap of 9 methylene groups. The other proton signal at δ 1.65 (m, 4 *H*) accounted to the overlap of two methylene groups. Also the multiplet proton signal at δ 2.03 (m, 4 *H*) accounted to two methylene groups.

The two triplet signal at δ 5.36 is the proton signal of the two CH=CH protons. The only one singlet signal at δ 12.64 is the proton signal of the O-H on the carbonyl carbon.

The ^{13}C NMR of compound 23 (Appendix-11) in combination with Dept-135 NMR (Appendix 12) indicates the presence of a total of 18 carbons signals. The signal at δ 14.1 (C-18) implies that there is one terminal methyl group. The signal at δ 22.7 (C-17) is the CH_2 signal adjacent to the methyl group. The very intense signal at δ 29.7 (C-5_C-16) is the characteristics of overlapping of many (12) methylene groups. The signal observed at δ 31.9 (C-2) is the signal of methylene carbon adjacent to the carbonyl group. The two carbon

signals at δ 128.3 (C-9) and 128.8 (C-10) are those of C=C, that positioned at the centre of the chain.

Even though the carbon signal at δ 182.2 (C-1) is not observed well as other signals, the presence of proton signal at δ 12.64 of the proton NMR and the presence of O-H IR band at 3420cm^{-1} reveal the presence of carboxylic acid carbon. Thus the small peak at δ 182.2 (C-1) is accepted as a carbon signal of the carbonyl carbon. The positive signal at δ 14.1 on DEPT-135 reveals that the carbon signal on C-18 is a terminal methyl group. The other two positive signals at δ 128.8 and128.3 show that the two carbon atoms are the CH carbons of CH=CH group on C-9 and C-10 respectively. The three negative signals at δ 22.7, 29.7 and 31.9, support that the three signals are of methylene group that mentioned above.

Table 7: ^{1}HNMR, ^{13}C NMR and DEPT 135 data of compound 23 ($CDCl_3$, DMSO, 400MHz)

Position of carbon	^{1}H NMR δ (ppm)	^{13}C-NMR δ (ppm)	DEPT 135 NMR	Literature data [63] ($CDCl_3$, 400MHz)	
				^{1}Hδ(ppm)	^{13}C δ(ppm)
1	_	182.9	Q	-	180.0
2	2.36	31.9	CH_2	2.34	34.03
3-4	1.65	29.7	CH_2	1.61	29.2
5-7	1.27	29.7	CH_2	1.26	29.2
8	2.30	29.7	CH_2	2.18	27.2
9	5.36	128.8	CH	5.34	129.9
10	5.36	128.3	CH	5.34	129.7
11	2.30	29.7	CH_2	2.02	27.1
12	1.27	29.7	CH_2	1.26	29.6
13-16	1.27	29.7	CH_2	1.25	29.6
17	1.27	22.7	CH_2	1.25	22.7
18	0.89	14.1	CH_3	0.88	14.1
OH	12.64	-	-	-	-

By gathering all the above spectroscopic information and by comparing with reported literature the compound is characterized as oleic acid [62, 63] whose structure is presented in figure 10.

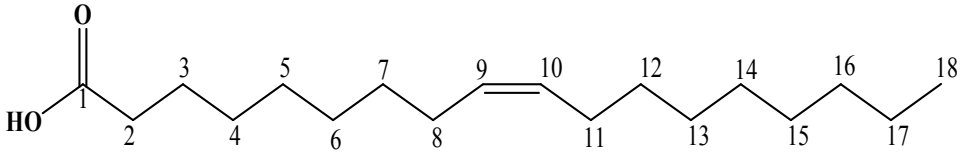


Figure 10: proposed Structure of oleic acid

4.6Antibacterial activity

The target of performing this antibacterial activity was to identify the zone of inhibition at which the three successive stem bark extracts of *S.longepedunclata* and the three isolated compounds from the EtOAC extract do actively. As it can be seen from Figure 11, all the three successive extracts and the isolated compounds show positive result of inhibiting the growth of two bacteria strains namely *E.coli* and *S. aureus*. Additionally the EtOAc extract and the two compounds show positive result on *S.typhi* while the n-hexane and MeOH extracts form no inhibition zone on this specific bacteria strain. The bacteria strain named *B.subtillus* show resistance to all the extracts and the isolated compounds.

The inhibition zone of the extracts and the isolated compounds against *S. aureus*, *E. coli*, S. tayiphi and B. saptillus were determined with the results shown in Figure 11 below.

Table 8: Inhibition zones diameter of three successive extracts, compound 21, compound 22, Chloramaphenicol and DMSO on four bacteria strains**.**

	Inhibition zone in mm on bacteria strains			
Extracts	*E.coli ATCC25922*	*S. aureus ATCC25923*	*S. typhi ATCC13311*	*B.subtillus* ATCC6633
n-hexane (C1)	12	14	-	-
EtOAc (C2)	11	15	10	-
MeOH (C3)	11	12	-	-
Compound 21	9	15	10	-
Compound 22	11	16	8	-
Chloramphenicol	17	27	17	18
DMSO	0	0	0	0

From the above table it is clear that the n-hexane, EtOAc and MeOH extracts show inhibition zones of 14, 15 and 12 mm on *S.aureus*. This result was reported in [16] with some deviations of results in which the three successive extracts shown inhibition zones of 14, 22 and 28 mm respectively on this bacteria strain. On the other hand only the EtOAC extract show positive result on *S. typhi* with 10 mm zone of inhibition. Again this result agrees with the one reported in the above literature as 18 mm.The two isolated compounds exhibited positive results on *S.aureus, E.coli and S. typhi* while *B. Subtillus* resisted the two compounds and also the three successive extracts. For example Dibewe et al. reported in [18] that *S. longepedunclata* is used to treat diseases such as diarrhoea, pneumonia and other inflammatory diseases. By relating this information with the findings in the above table of inhibiting activity of the isolated compounds on *E. coli* and *S. aureus* one can decide that the stem bark of this plant may be recommended as a remedy against bacteria causing diarrhoea, pneumonia and other inflammatory diseases . Similarly Ndamtso et al. reported in [15] that this plant is used to cure diseases like urinary tract infection and this can be related with the inhibiting activity against *E. coli* of the chemical constituents of this plant that mentioned in Table 4.

5. CONCLUSION AND RECOMENDATION

5.1 Conclusion

The dried powder of stem bark of *S. longepedunclata* was successively extracted with n-hexane, EtOAc and MeOH to give 0.52%, 1% and 10.2% respectively.

The EtOAc extract after silica gel column chromatography yielded three compounds namely 1, 7-dihydroxy-4-methoxyxanthone, 9-H xanthenes-3,5-diol and oleic acid. Even though different xanthones were isolated from different parts of *S. longepedunclata*, the first compound (compound 21) is isolated here for the first time from the stem bark of this plant. Also the second compound (compound 22) is not isolated earlier from any part of this plant, and now it is isolated for the first time.

The three crude extracts and the isolated compounds show different inhibition zone on four bacteria strains namely *Escherchia coli*, *Salmonella typhi*, *Bacillus saptilulas* and *staphylococcus aurous.* Therefore the antibacterial activities reported here strengths the traditional use of this plant as anti bacteria agent.

5.2 Recommendation

i. From the TLC profiles of the extracts it is clear that the stem bark of *S. longepedunclata* does not contain only these three compounds. Therefore future work is important to isolate additional compounds from the stem bark of this plant.
ii. The biological activities of the plant extract and the compounds were done only on four bacteria strains. It is recommended that the biological activity of this plant has to be examined on other human bacteria strains, other microbial and also on bacteria of other animals such as cattle and birds.
iii. The biological activities of the plant products are tested only on bacteria pathogens. But many literatures reported that this plant is traditionally used to treat fatal diseases such as cancer and tumour. Future research should be extended to cover other biological activities including anti-cancer, antitumour and antimalarial activity.
iv. The characterizations of the isolated compounds were done by using FTIR, ^{1}H NMR and ^{13}C NMR. It is recommended in addition to do with UV, 2D NMR and MS.

REFERENCES

1. Hanson J. R. (**2003**). Natural products. The secondary metabolite. *Cambridge: Royal society of chemistry*: ISBN 0-85404-490-6..p 1-3
2. Herdugan S. (**2008**). Introducao atituterapia. Utilizando adequa damente as planta medicinais Colombo: Herbarium lab. Bot. Ltda
3. Farnsworth N.R. (**1996**). Biological and phytochemistry screening of plants. *Journal of pharmacological science* **55**, 225-276.
4. Padney R.C. (**1998**). Prospecting for potentially new pharmaceuticals from natural products. *Medical research review* **18**, 33-34
5. Mongalo N.I.,Mafoko, B.J.,(**2013**). Cassia abbreviata oliv. A review of its ethnomedicinal, uses toxology, phytochemistry, possible propagation techniques and pharmacology. *African journal of pharmacy and pharmacology* **7**, 2901-2906.
6. Krishna K. (**2004**). Global health care chalange. Indian experiences and new prescription. Pp 17-34.
7. Hamilton A. (**2003**). Medicinal plants and conservation. Issues and approaches. Panda house, Catteshall lane, London *international journal of remoute sensing*, **21(12)** 2389-2402.
8. Shariff Z. (**2001**). Modern herbal therapy for common ailments. Nature pharmacy series (volume 1). *Ispecrum books limited Ibadan Nigeria in association with Safari books (export) limited,* United Kingdom .pp 9-18
9. Okwu D.E. (**2005**). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. *Int. J. Mol. Med. Adv.Sci*. **1(4)**, 375-381.
10. Anonymous (**2007**). Medicinal, culinary and aromatic plants in the near east-Medic...http:;//www.fao.org /dorep/x5402e16. htm (cited4,August 2007).
11. Grzegorz G. And Maria G. (**2008**). Tropane alkaloids as medicinally useful natural products and their synthetic derivatives as new drugs. Pharmacological reports **60**(4)
12. Christen P. (**2000**). Tropane alkaloids, old drugs used in modern medicine. Bioactive natural products. *Elsevier science and technology* **22**,717-749.
13. Degu Lere Keshebo and Manash Kumar Choudhory **(2015).** Phytochemical investigation of secufridaca longepedunclata (polygalaceae) and structural elucidation of benzyl-2-hydroxy-5-methoxybenzoate. Department of chemistry, Dilla University, P.O.Box 419, Dilla, Ethiopia.
14. Tabuti F. D. (**2012**). Herbal medicine uses in the districts of Nakapiripirit pallisa, Kanungu and Mukono in Uganda. *Journal of ethnobiology and ethnomedicine*.

15. Ndamtso M.M., Mohammed, A., Jimoh, T.O., Idris, S., Oyeleke, S.B., Etsuyangkpa, M.B., (**2013**). Phytochemical and antibacterial activity of *Securidaca longepedunclata* on selected pathogens. *Federal university of technology,* Nigeria.
16. Akpemi A. Musa, Adebayo O. Oyewale, Iloegibulam G. Ndukwe (**2013**). Phytochemical screening and anti microbial activity of solvent fractions of *Securidaca longepedunclata (Fresen*) root bark methanol extract: Department of chemistry, *Federal collage of education*, Zaria-Nigeria.
17. Debella A., Kunert, O., Schmid, M.G., Michl, G., Bucar, F., Abebe, D., Hasliinger, E.(**2000**). A diterpene a flavonon glycoside and a phytosterol glycoside from *Securidaca longepedunclata* and Entada abbyssinica. *Monatshefte fur chemie***131**, 401-408.
18. Dibewe D.F., Awale, S., Kadota, S., Tezuka, Y.(**2013**). Hepatoxygnated xanthones as anti-austerity agents from *Securidaca longepedunclata*. *Bioorganic and medicinal chemistry*, **21**, 7663.
19. Wetungu M. W. (**2007**). Antimicrobial phenylpropanoid esters and sesquiterpene lactones from the leaves of *Tarchonanthus canforatus* (Asteraceae), M.Sc. project, *Egerton University*, Kenya.
20. Irungu Stanley (**2012**). Isolation and characterization of anti-microbial compounds. School of pure and applied science, *Kenyatta University*, Nairobi, Kenya.
21. Eriksen B. and C. Persson (**2007**). Polyglaceae. In: K.Kubtziki (eds), The family and genera of vascular plants IX: 345-363. Doi:10.1007/978-3-540-32219-1_41
22. Christenhusz M.J.M. & Byng J. W. (**2016**). The number of known plant species in the world and its annual increase. *Phytotaxa Magnolia press*. 261(3):; 201-217
23. Paiva J. & J. D. Santos Dias (**1990**). The pollen grain of Polygala fruticosa Berg. (polyglaceae) anales jara. Bot.Madr/d 47(2): 377-385
24. Heywood V.H.(**2007**) Flowering plant family of the world firefly books: Ontario,Canada
25. Ndou, Avhurengwi Phillemon(**2013**). *Securidaca longepedunclata*. *South African national biodiversity institute*, Web 20 Nov. 2013
26. Wallnofer,B. (**1998**). A new species of Securidaca l.(polyglaceae) from Peru. Annalen des naturhistorischen museums in Wien. 100B, 109-114.
27. C.T.Johnson (**2013**). Taxonomy of the African species of Securidaca (polyglaceae), Department of botany, University of western Cape, Bellville, 7535,Republic of South Africa.
28. Van Wyk B.E. (**2009**). Medicinal plants of South Africa, second ed. *Briza publications Pretoria* pp 268.

29. *Securidaca longepedunclata* Wikipedia the free encyclopaedia
30. Hamill F.A. (**2003**). Traditional herbal drugs of southern Uganda, II: literature analysis and antimicrobial assays, *Journal of ethnopharmacology*. **84**,57-78.
31. Maroi A. (**2013**). Traditional use of medicinal plants in south central Zimbabwe. Review and perspective. *Journal of ethnobiology and ethnomedicine* 9.31: http// www.ethnobiomed.com/content/9/1/31.
32. Ahmed A. A. (**2010**). Anti snake venom activity of *Hibiscus aethiopicus L.*against *Echis oscellatus* and *Naja nigiricollis. Journal of toxicology*, 5(3), 123-130.
33. Viol, D. I. (**2009**). Screening of traditional medicinal plants from Zimbabwe for phytochemistry, antioxidant, antimicrobial, antiviral and toxicological activities. M. Phill. Dissertation. University of Zimbabwe.
34. Mustapha A.A. (**2013**). Ethno-medico-botanical uses of *Securidaca longepedunclata Fresen* (family polyglaceae) from Kefi local government. Nasarawa state, Nigeria. *Journal of natural remedies*. **13**, 133-137.
35. Augustino S., Hall, J.B., Makonda, F.B.S., Ishengoma, R.C.(**2011**). Medicinal resources of the Miombo woodlands of Urumva, Tanzania: plants and their use. *Journal of medicinal plants research.* **5**, 6352-6372.
36. Borokini T.I., Clement, M., Dickson, N.J., Edagbo, D.E. (**2013**). Ethnobiological survey of traditional medicine practice for circulatory and nervous system related diseases in Oyo State, Nigeria. Top class *Journal of Herbal Medicine*. **2**, 111-120
37. Das,K. (**2009**). Medicinal plants for snake bite treatment- future focus. *Ethnobotanical leaflets*.**13**, 508-521
38. Hedimbi, M., Chinsembu, K.C. (**2012**). An ethnomedicinal study of plants used to manage HIV/AIDS-related disease conditions in the Ohangwena region, Namibia. *International Journal of Medicinal Plants Research*. **1**, 4-11.
39. Orwa E.Leo (**2009**) *S. longepedunclata*. Agro forestry data base.
40. Ojewole J.A.O. Mawoza, T., Chiwororo, W.D.H., Owira, P.M.O., (**2008**). Analgesic, anti-inflammatory and hypoglycaemic effect of *Securidaca longepedunclata, Fresen* (polyglaceae) root bark aqueous extract. *Inflammopharmacology* **16(4),** 174-181.
41. Booth, F.E.M. Wickens G.E. (**1988**). Non timber uses of selected arid zone trees and shrubs in Africa. FAO conservation guide. No. 19, Rome.
42. Ndou, Avhurengwi, phillemon (**2013**). *Securidaca longepedunclata*. South African national biodiversity institute. Web 20, Nov. 2013.

43. N.I. Mongalo L.J. McGaw, J.F. Finnie, J. Van staden (**2010**). *Securidaca longepedunclata Fresen (polyglaceae*): A review of its ethnomedicinal use, phytochemistry, pharmacological properties and toxicology. University of South Africa, collage of agriculture and environmental science private bag X6 Florida 1710. South Africa pp11-1

44. Auwal, S.M., Atiku, M.K., Wudil, A.M. and Sule, M.S., Phytochemical composition and acute toxicity evaluation of aqueous root bark extract of *S. longepedunclata* (**2012**). Department of biochemistry, Bayero University, Kano, Nigeria. *Bayero journal of pure and applied science* **5(2**): 67-72

45. Joseph C.C., Moshi,M.J.,Sempombe, J., Nkunya, M.H.H.,(**2006**). 4-methoxy-benzo [1,3] dioxol-5-yl phenylmethanone: An antibacterial benzophenone from Securidaca longepedunclata. *African journal of traditional complementary and alternative medicine*. 3,80-86.

46. Lognay G. ., Marlier, M., Seck, D., Haubruge, É. (**2000**).The ocurance of 2-hydroxy-6-methoxy benzoic acid methyl ester in Securidaca longepedunclata Fresen root bark. *Biotechnology and agronomy society environment*. 4, 107-110

47. Jayascara T.K. Stevenson, P.C., Belmain, S.R., Farman, D.I., Hall, D.R (**2002**). Identification of methyl salycylate as the principal volatile component in the methanol extract of root bark of *Securidaca longepedunclata Fresen. Journal of mass spectrometry*. 37, 577-580.

48. Jayasekara T.K. Stevenson, P.C., Belmain, S.R., Farman, D.I., Hall, D.R (**2005**). Effect of volatile constituents from *Securidaca longepedunclata* on insect pests of stored grain. Journal *of chemical ecology* 31:2

49. Beentje H.J. (**1994**). Keniya trees, shrubs and lianas. National museum of Kenya.

50. Yang XD (**2001**). Studies on the organic acids constituents from the stem bark of Securidaca inappendiculata. *China jchin mater med* **26**: 258-260.

51. Yang Xue Dong (**2002**). Two new xanthones from stem bark of Securidaca inappendiculata. Department of chemistry, Tsinghua University Beijing, China.

52. Evans W. (**1996**). Trease and Evans pharmacognosy, 14th edition. *WB Saunders company* , Ltd London, pp 224-228, 290-309, 542-575.

53. Harbone J. (**1998**). Phytochemical methods. A guide to modern techniques of plant analysis 3rd edition. Chappman and Hall Int. Ed. New York.

54. Baker J.T., Borris R.P., Carte B., Cordell, G. A., Soejarto, D. D., Cragg, G. M., Gupta, M. P., Iwu M. M., Madulid D., Rand Tyler, V. E. (**1995**). Natural product drug discovery and development: New perspectives on international collaboration. *J. Nat. Prod*. **58**, 1315 – 1357

55. Abiy Yenesew, Martha Induli, Solomon Derese, Jacob O. Midiwo (**2004**) Anti plasmodial flavonoids from the stem bark of Erythrina abbyssinica. Department of chemistry, university of Nairobi, Chiromo road, Nairobi, Kenya

56. Pettit, G.R. Singh, S.B. Schmidt, J.M. Niven, M.L. Hamel, E.Lin, C.M. (**1988**). Isolation, structure, synthesis, and antimitotic properties of combretastatins B-3 and B-4 from *Combretum caffrum*. *J. Nat. Prod*., **51**(3), 517-527

57. John Coates (**2007**). Interpretation of Infrared spectroscopy, a practical approach. *Coate's consulting*, Newtown USA.

58. Negi J.S.,Bishit V,K.,Singh P. and Joshi d. (**2013**). Naturally occurring xanthones; Chemistry and Biology. Herbal analytical laboratory, herbal research and development institute, Mandal, Gopeshwar, Uttarakhand 246401, India.

59. Hesham R., El-seedi, Dina M.H., Marvat F. Zayed (**2009)**. Naturally occurring xanthones; latest investigation, isolation, structure elucidation and chemosystematic significance. Department of medicinal chemistry, Uppsala University, biomedical centre, Uppsala, Sweden

60. Mino Dabiri, Seyedeh C. Azimi, Ayoob Bazagir (**2007**). One spot synthesis of xanthenes derivatives under solvent free conditions. Department of chemistry, Shahid Behshti university, Tehran 1983963113, Iran

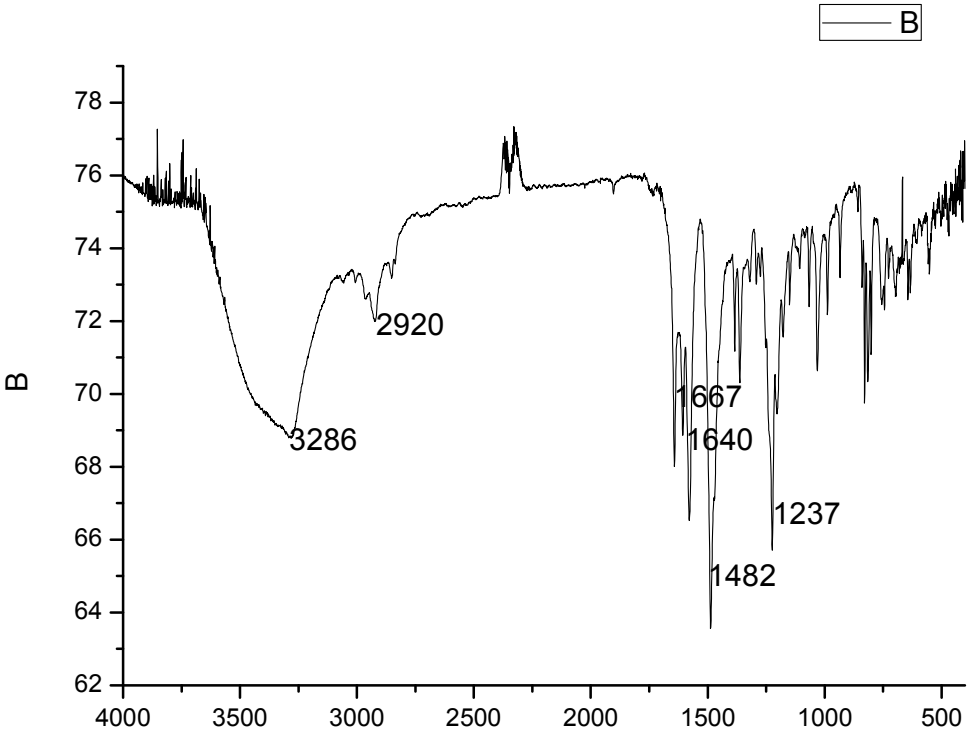
61. Sara Mirpourzadheh (**2007**). Development of new mimetics for the Tyr-pair of the epoxide hydrolase. Department of biochemistry and organic chemistry Uppasala University.

62. Deivasgamani Malarvizhi, P. Anusooriyaa, S. Sanduram (**2016**). Isolation and structural characterization of Oleic acid from *Zelya decandra* root extract, Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, 641021, India

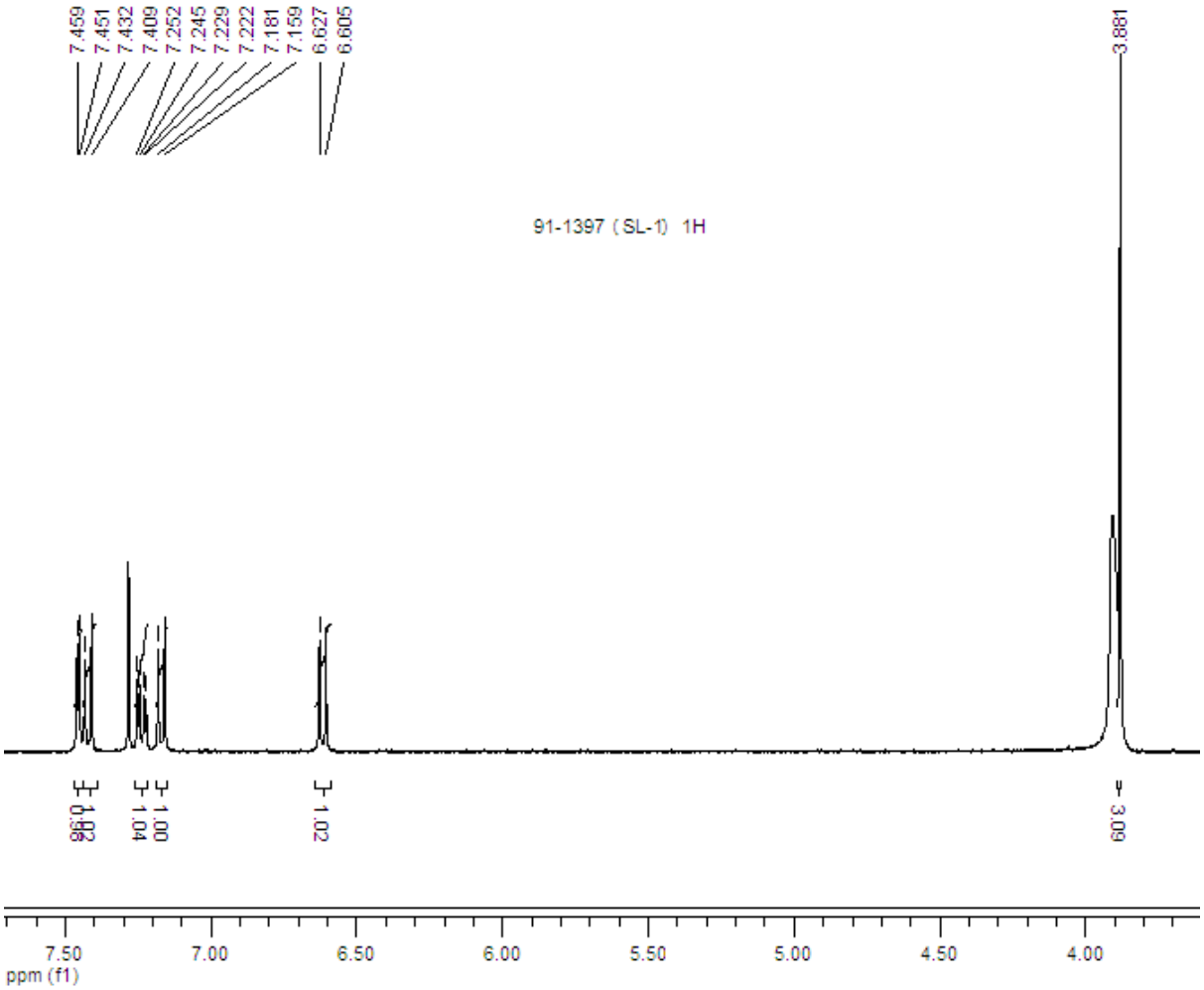
63. Melkamu Fufa, Padmanabhan R. And Gurmessa GT. (**2016**) Phytochemical investigation and in vitro anti bacterial evaluation on root extract of Rumex abyssinicus. Department of chemistry, college of natural science, Jimma university, Jimma ,Ethiopia

APPENDIXES

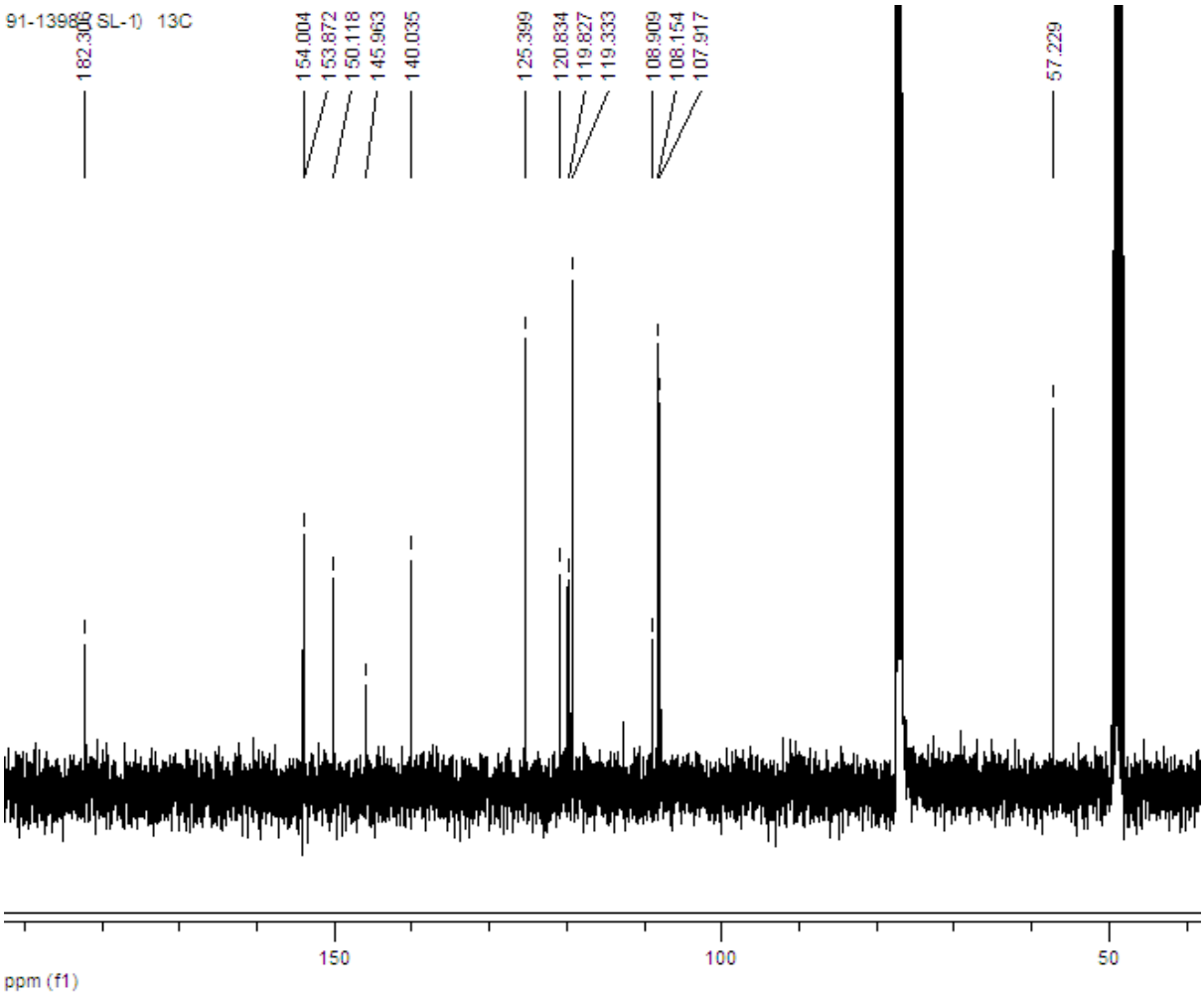
Appendix-1: FTIR of compound 21



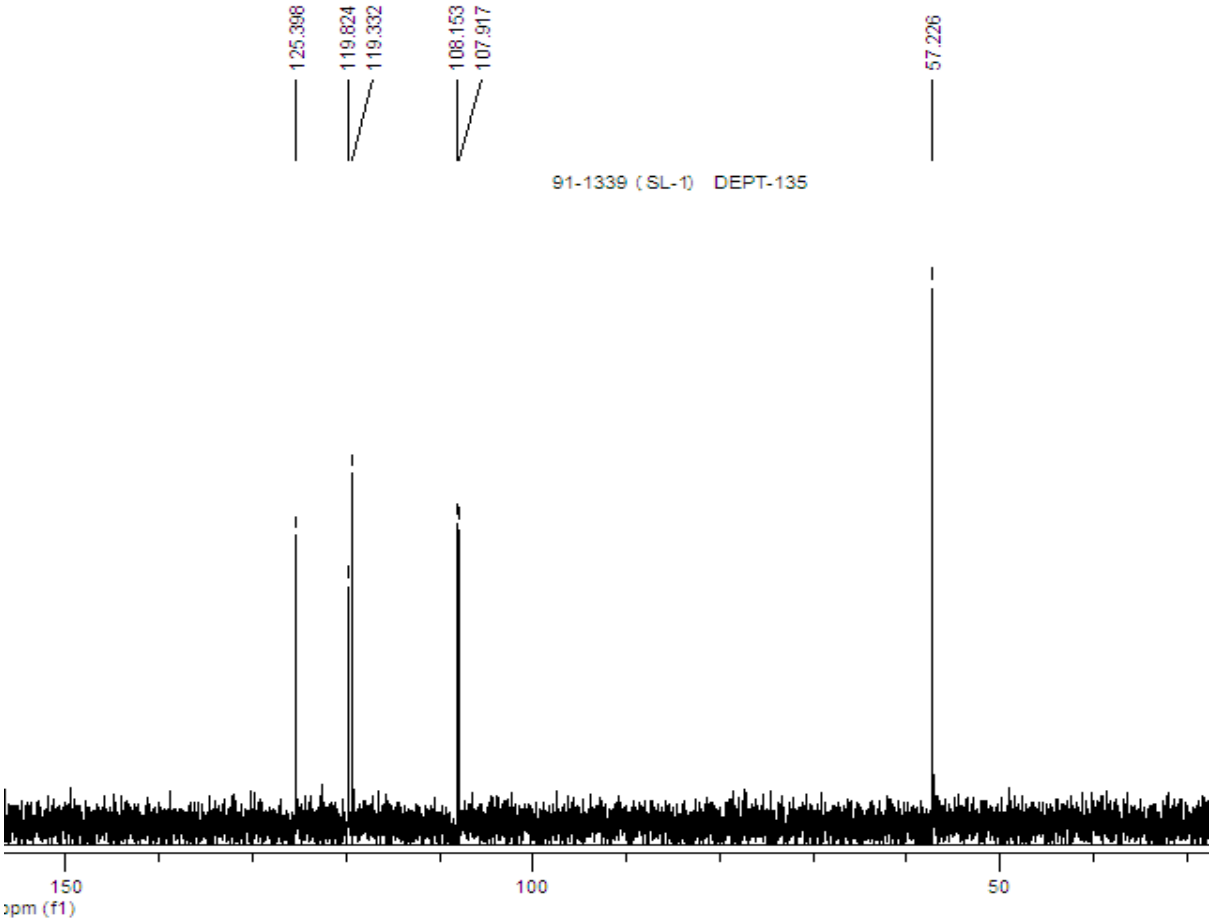
Appendix-2: ^{1}H NMR of compound 21



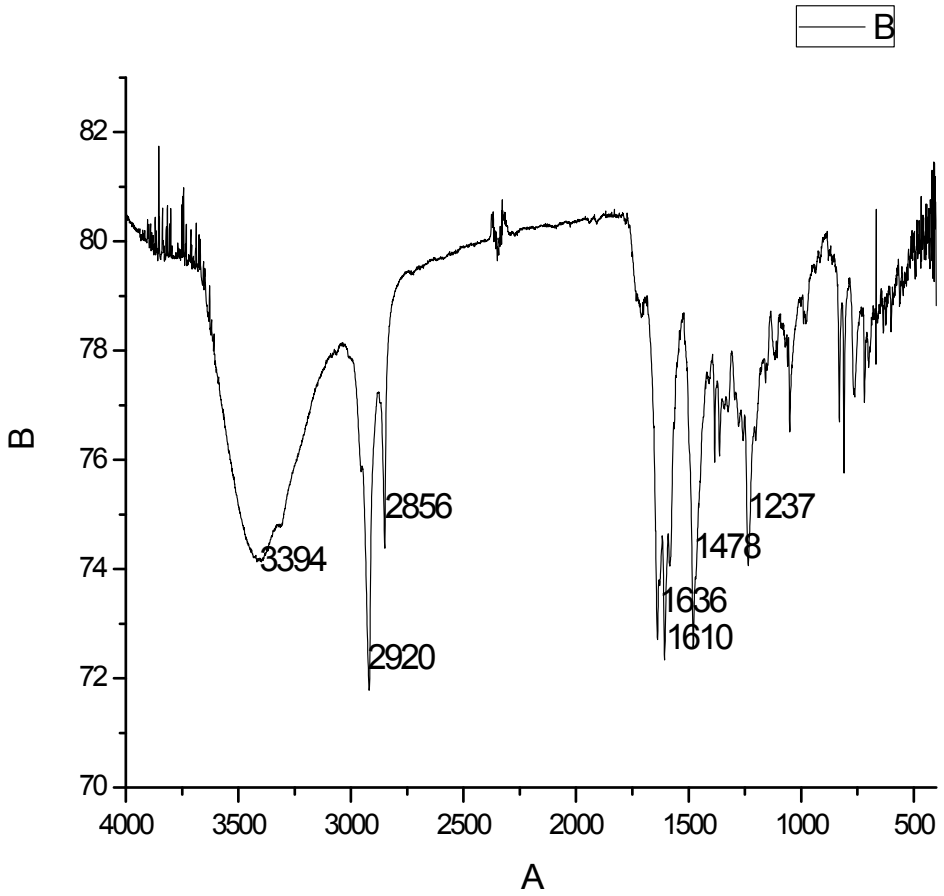
Appendix-3: ^{13}C NMR of compound 21



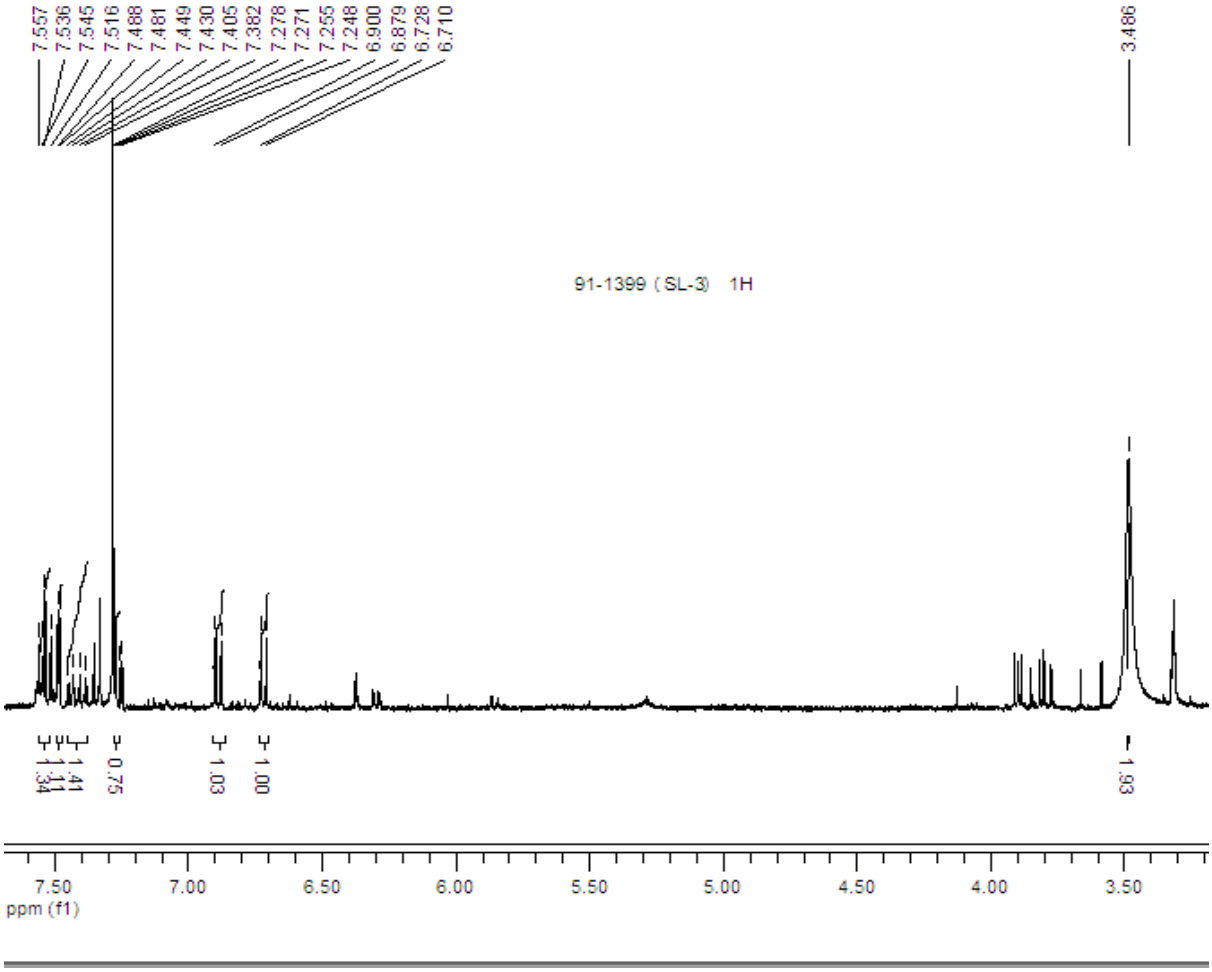
Appendix-4: DEPT-135 of compound 21



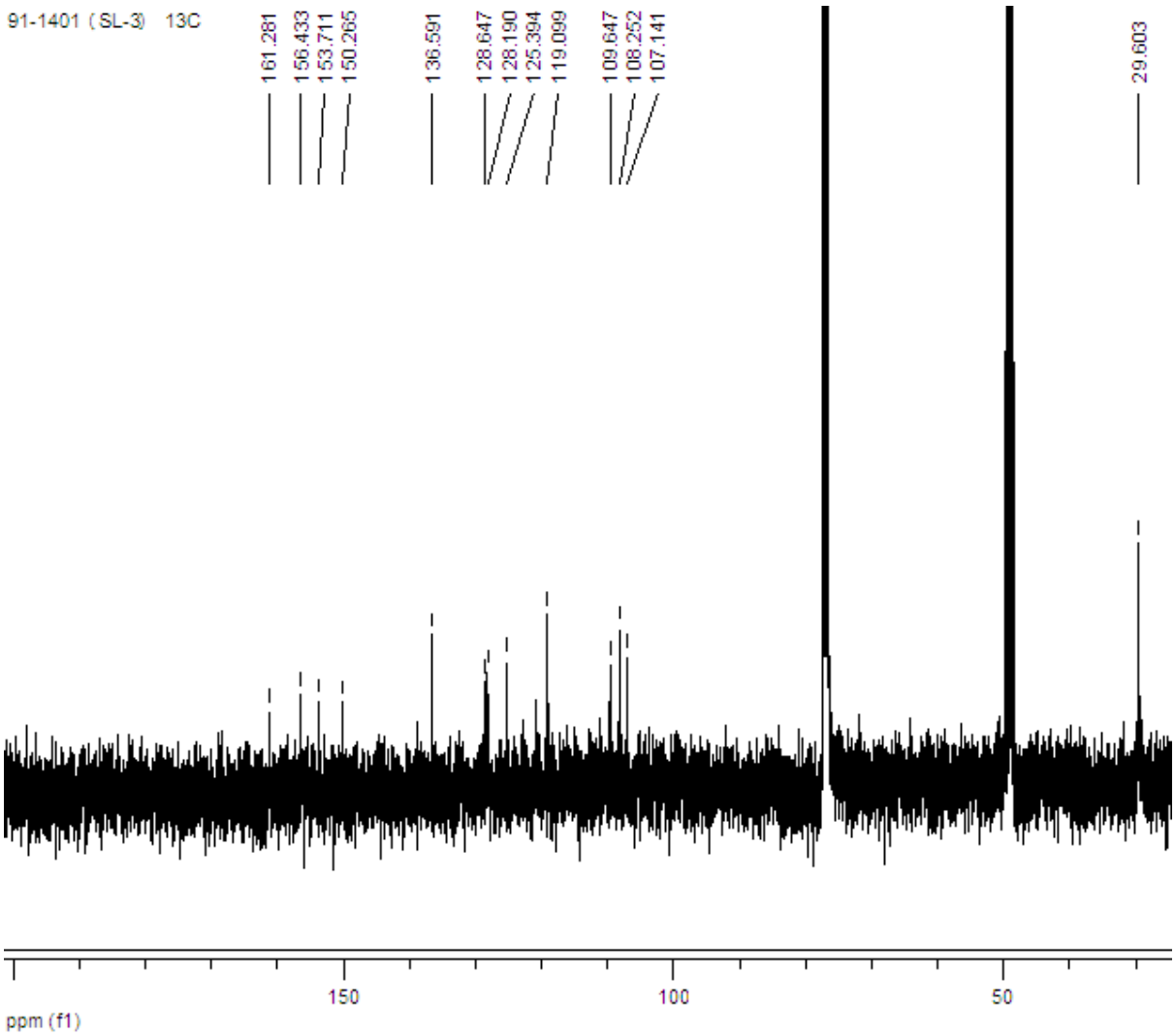
Appendix-5: FTIR of compound 22



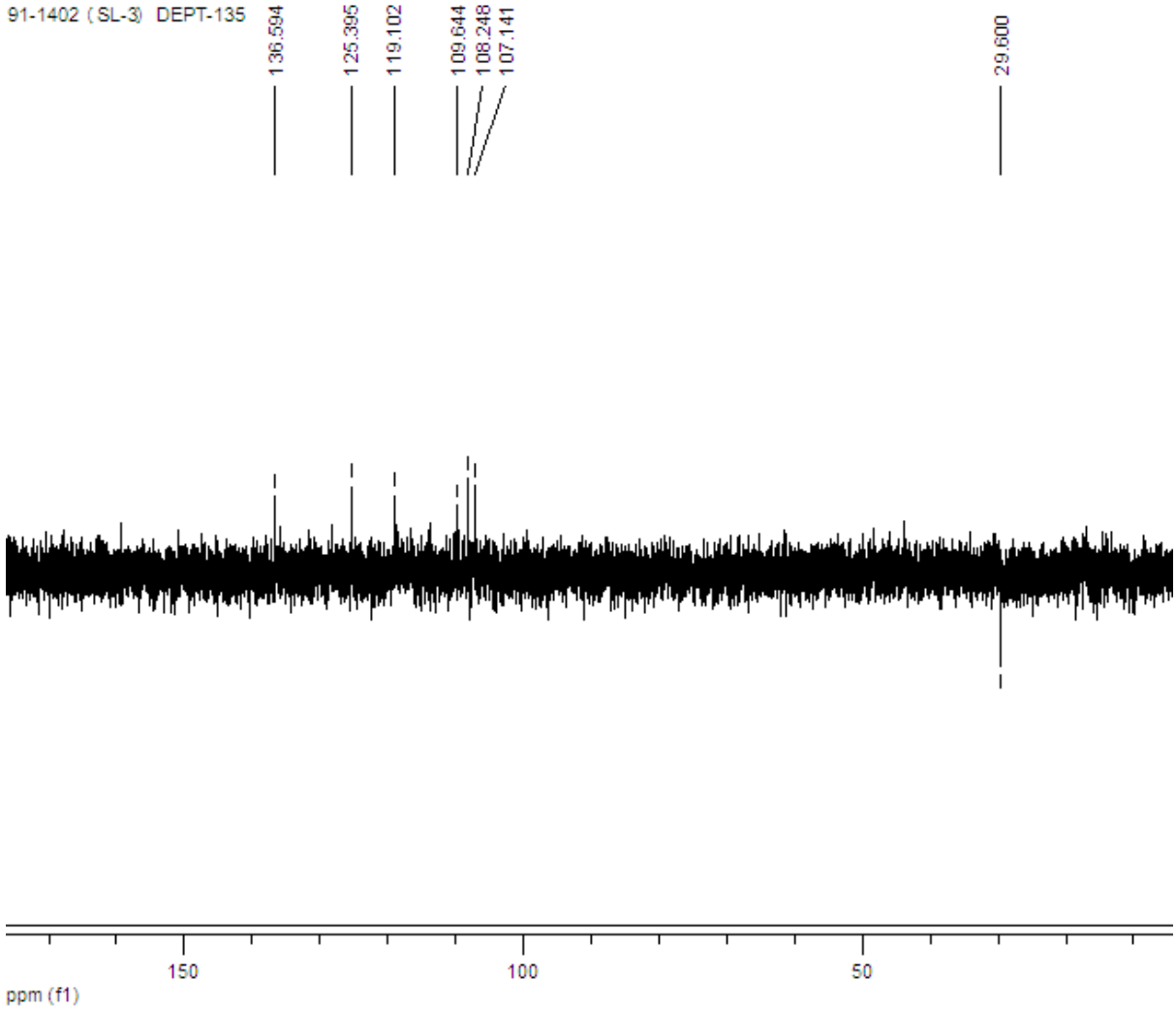
Appendix 6: ^{1}H NMR of compound 22



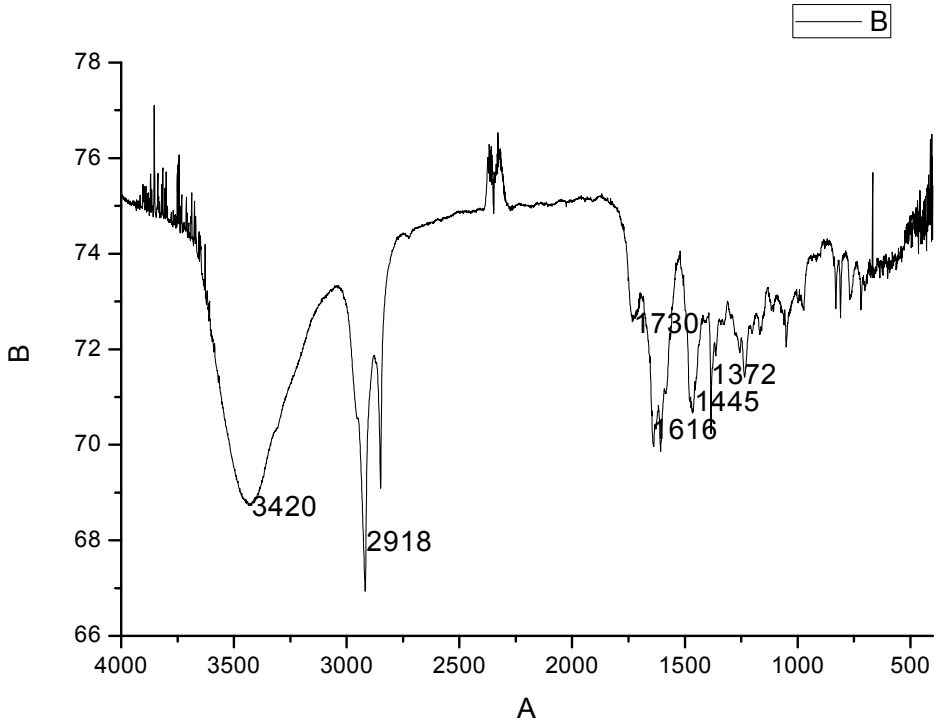
Appendix 7: ^{13}C NMR of compound 22



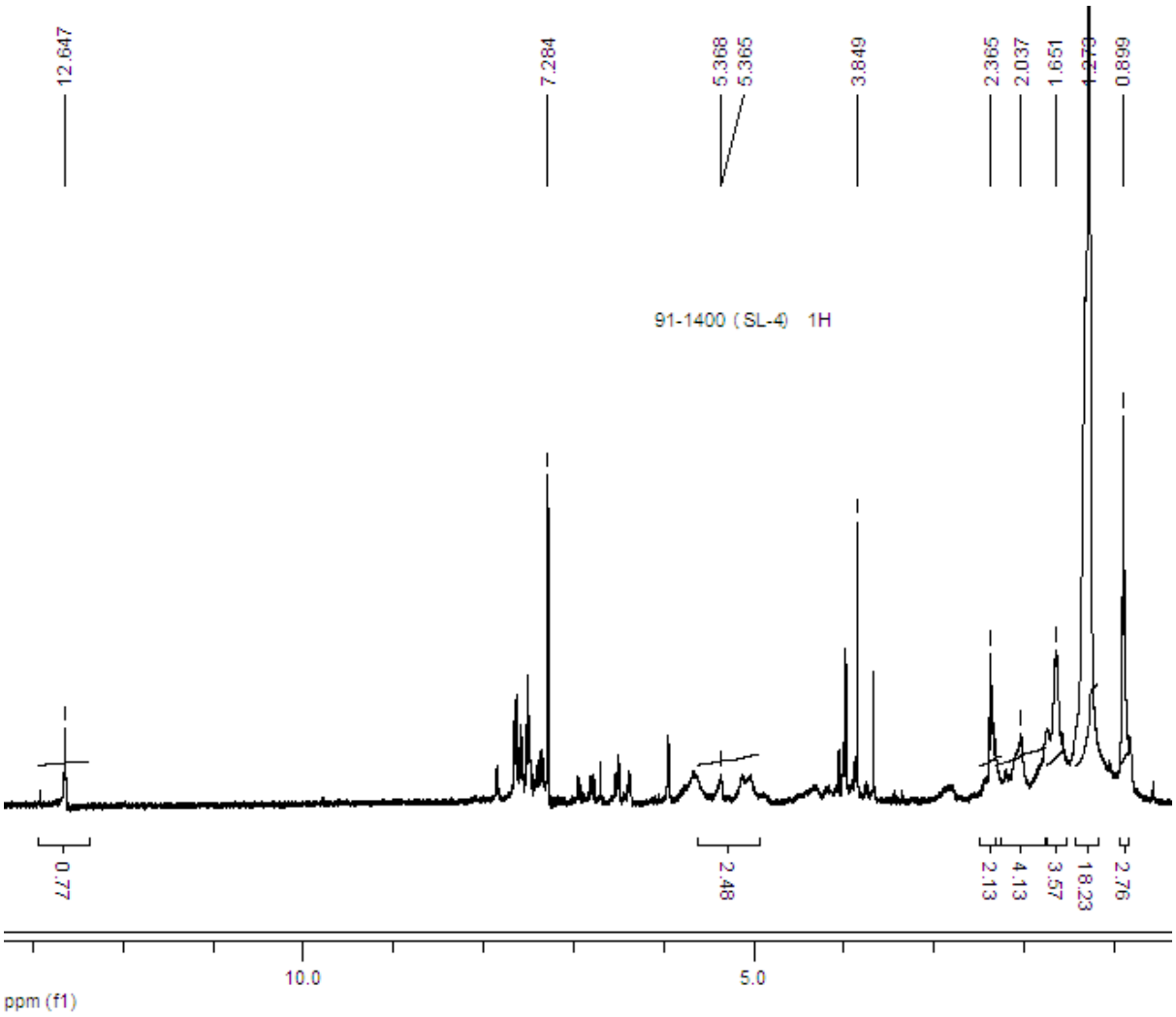
Appendix 8: DEPT 135 NMR of compound 22



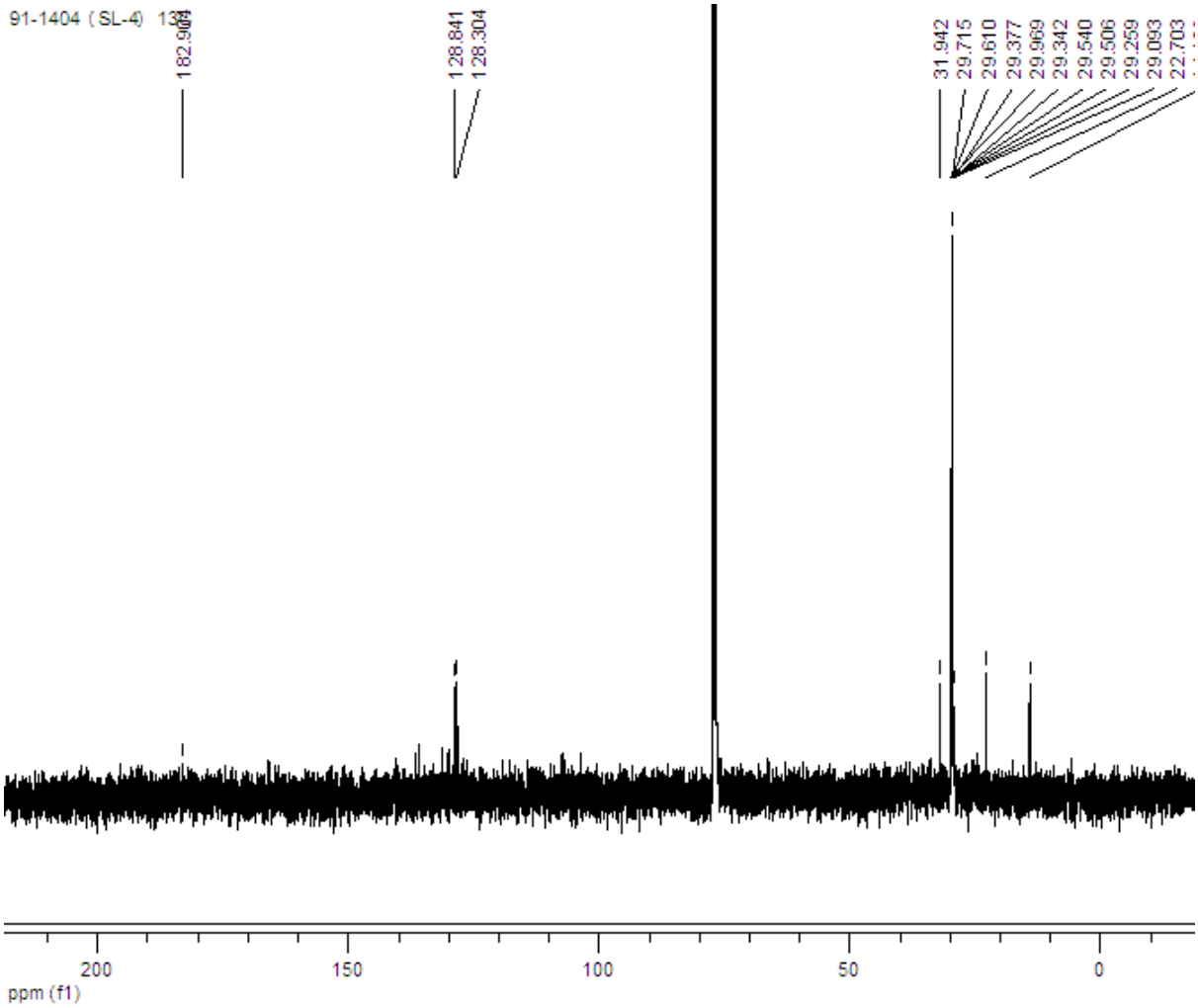
Appendix 9: FTIR of compound 23



Appendix 10: ^{1}H NMR of compound 23



Appendix-11: ^{13}C NMR of compound 23



Appendix 12: DEPT 135 of compound 23