ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

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MScThesis

Phytochemical Investigation on the Leaves of *Securidaca longipedunculata*

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This is to certify that the thesis prepared by Emebet entitled: Phytochemical Investigation of the leaves of *Securidaca longipedunculata*and submitted in partial fulfillment of the requirements for the degree of Master of Science in chemistry complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this thesis, entitled Phytochemical Investigation of the leaves of *Securidaca longipedunculata*, is my original work under the supervision of Dr.Mekonnen Abebayehu, Department of Chemistry, Addis Ababa University, and that all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution, anywhere for the award of any academic degree, diploma, or certificate.

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

Advisor: Dr. Mekonnen Abebayehu

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List of Abbreviations

TLC……………………………..Thin Layer Chromatography

CC………………………………Column chromatography

UV………………………………Ultra-Violent

NMR…………………………....Nuclear Magnetic Resonance

DEPT……………………………Distortion less Enhancement by Polarization Transfer

COSY…………………………...Correlation Spectroscopy

HSQC…………………………..Hetronuclear Single Quantum Correlation

HMBC…………………………..Hetronuclear Multiple Bond Correlation

DMSO …………………………..Dimethyl sulphoxide

δ…………………………………Delta (symbol for chemical shift)

d…………………………………Doublet

dd……………………………….Doublet of doublet

m………………………………...Multiplet

s………………………………….Singlet

t …………………………………. Triplet

∈A (EtoAC) …………………........ Ethylene acetate

CH ……………………………….. Cyclo hexane

Chlo ……………………………….. Chloroform

Me ………………………………….. Methanol

UDP ………………………………… Uridine Diphosphate

UTP ………………………………… Uridine triphosphate

WHO ……………………………… World Health Organization

PPM……………………………..Parts Per Million

MHz……………………………..Mega Hertz

mL……………………………….Milliliter

Km……………………………….Kilometer

RF………………………………..Retention Factor

g………………………………….Gram

mg………………………………..Milligram

nm……………………………….. Nano meter

hr ………………………………… Hour

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Abstract

Securidaca lonpeduculata is a well-known medicinal plant in the family of polygalaceae.The plant is locally known as “Itsemenahi’’ Amharic and “temenayi’’ in Afanoromo. It is traditionally used in headache, fever, epilepsy, asthma and cough. The different parts of *S. longipeduculata* have different purpose, in different countries, for example the root of this plant is used for treatment of fever, malaria, toothache in Nigeria, the leaves are used for treatment of headache skin infection in Nigeria and the steam bark is used for skin disease in Burkina Faso. Phytochemical investigation on the leaves of this plant resulted in the isolation of two compounds (**SLE-6**and**SLE-7**). Compound SLE-6 is flavonoid glycoside, compound SLE-7 is a group of galotannins namely:

1. Quercetine-3-O-L-arabinopyranoside.

2.1,5-Anhydro-D-glucitol

Structural elucidations of those compounds are based on UV, 1D NMR (^{1}H, ^{13}C &DEPT-135) and 2D NMR (COSY, HSQC &HMBC) spectroscopic data obtained in comparison with similar compounds isolated from the plant.

1. Introduction

Natural product is a chemical compound or substance produced by a living organism, Plant-derived substances have recently become of great interest owing to their important role [1]. Plants are used medicinally in different countries and are a source of many potent and effective drugs [2]. Natural product chemistry is a science, which studies different products from dwelling matter, animals or plants. These products are very important for human being. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than20, 000 species [3]. According to the WHO more than 64 % of the world's population relies on traditional herbal medicine for their primary health care [4]. Medicinal plants were used as an exemplary supply for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of energetic ingredients of healing value.

Plants had been used as a supply of conventional remedy in Ethiopia from the historic time to fight unique illness and human sufferings. Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of Ethiopian people [5, 6]. The traditional medicines are prepared in various dosage forms such as liquid, powder and prescribed in a non-formulated form [7]. The majority of traditional medicines used in developing countries have not been evaluated for quality, safety and efficacy to some standards while in developed countries there are some remarkable claims made for their effectiveness [8].The use of traditional medicine among both urban and rural population in Ethiopia could be attributed to cultural acceptability, efficacy against certain type of diseases, physical accessibility and economic affordability as compared to modern medicine [9].

Although they lack precision in determination of doses, traditional healers usually establish doses based on age, physical appearance of the patient, socio cultural explanation of the illness, duration of the illness, diagnosis and experience of individual herbalist. Reported unit of measurement used to establish the dose of traditional herbal remedies in Ethiopia were finger length for roots, barks and stems, pinch for powder, water cup for latex/liquid and numbers for leaves, seeds, fruits and flowers [9, 10].The methods of administration of herbal medicines were 48 (59.2%) internal, particularly oral, followed by 22 (27%) dermal and10 (12.3%) nasal [10].Traditional healers use roots, leaves, barks and other parts of the plant to prepare physiotherapies and in the process they

have developed their own local knowledge, According to [11].The most commonly used plant parts for remedy preparations were roots (35%), followed by leaves (24.6%) [17].But studies conducted elsewhere showed the dominance of leaves in the preparation of traditional remedies [11]. Natural products can be used for distinctive purposes. Some of them are used for pharmaceutical purpose, for foods, insecticides, antioxidants, coloring matters, flavors , extraction of enzymes, and so on [12].

Any biological molecule is a natural product, but in general the term is inter changed for secondary metabolites (carotenoids, phytosterines, saponines, phenolic compounds, alkaloids, glycerinates, flavonoids, terpenes etc.), produced by an organism [13].Secondary metabolites are chemicals derived from residing organisms. Secondary metabolites appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as intraspecific and interspecific attractants [14].

2-Review Literature

2.1. The Genus *Securidaca*

The genus *Securidaca* comprises about 80species, characterized by purplish or blue colored flowers, oblancelate and obtuse broad leaves and the seeds are winged. *S.longipedunculata* an important multipurpose African medicinal plant, and which produce compounds known as secure xanthones with antimicrobial and antioxidant properties [15-18]. *S.longipedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa [19, 20].

2.2. *Securidaca longipeduculata*

2.2.1 Background *of securidaca longipeduculata*

*Securidacalongipedunculata 8*Fresen (synonyms *Securidaca longipedunculata*var,*longipedunculata* or *Elsotalongipedunculata*,Estemenahi in Amharic temenayiin Afan Oromo, family Polygalaceae) is a small tree up to 6 meters high. It has a pale grey, smooth bark and, more or less hairless alternate leaves that are variable in size [21]. Clustered flowers are small, pink to lilac or purple in color, sweet scented and are produced in early summer [22].Fruits are a spherical nut, closely veined, on occasion smooth, oblong, purplish inexperienced whilst younger and own a membranous wing of approximately four cm long [23].

The species is commonly disbursed in numerous tropical African countries, along with Angola, Benin, Botswana, Burundi, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe, Mozambique, in addition to the North West and Limpopo Provinces of South Africa.[24,25].

Figure 1: Securidacalongepedunculata plant

2.2.2 Cultivation and distribution

S.longepedunculata belongs to the family of Polygalaceae widely distributed in Western and Southern Africa and almost all the parts of the plant are reported to be used in disease management [26]. The plant is a savanna shrub with twisted bole or slender erect branches and grows up to 30ft high in Malawi; it is distributed in a wide range of climates ranging from subtropical, hot, and arid climate to summer rainfall and equatorial humid. It grows in different vegetation ranging from semi-arid scrub to dense forest, including bush habitats and gallery forests and woodland [27].It is sensitive to frost and resistant to bush fires [28].

S. longipedunculata grows with inside the Bereha, Kolla and Weyina Dega zones. The climatic conditions in which this plant grows are similar and with sunlight throughout the year. This plant grows abundantly in the low lands and warm areas of Tigray, Amhara, Oromia, Benshangul Gumz, Afar, Gambela and the southern nation and nationalities regions.

2.3 Uses of *Securidaca longipeduculata*

Since ancient times, plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock. The application of medicinal plants is almost as old as the history of mankind. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 B.C.in China, and 1600 B.C. by Syrians, Babylonians, Hebrews and Egyptians. Considerable indigenous knowledge, from the earliest times, is linked to the use of traditional medicine indifferent countries [29].The use of traditional medicine and medicinal plants in third world countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and isolation. [30, 31].

Ethiopia has a long history of traditional medicines due to the cultural acceptability of healers and local pharmacopeias, relatively low cost of traditional medicine, difficult access to modernhealth facilities and due to various side effects caused by modern synthetic medicines [32]. Healing of traditional medicine in Ethiopia is not only concerned with curing of diseases but also with the protection and promotion of human physical, spiritual, social, mental and material wellbeing. It is widely believed in Ethiopia that the skill of traditional health practitioners is ‘given by God’ and knowledge on traditional medicines is passed over orally from father to a favorite child, usually a son or is acquired by some spiritual procedures [33].

Plants produce a good sized sort of natural products with extraordinarily numerous structures. These products are usually termed as number primary metabolites and secondary metabolites. Primary metabolites are accountable for the increase and improvement of plants, for example; sugars, protein, lipids, starch. Secondary metabolites seem to characteristic often in protection towards predators and pathogens and in supplying reproductive advantage. Some of the most important secondary metabolites (bioactive phytochemical) constituents are alkaloids, essential oils, terpenoids, Saponins, flavonoids, tannins and phenolic compounds [34-36]. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, in particular the synergistic effects and poly-pharmacological application of the plant extracts [37-39].
The World Health Organization (WHO) estimated about 80% of the people in the developing countries relies on traditional medicine for primary health care needs, and most of this therapy involves the use of plant extracts or their active component [40] .Herbal treatments had been used for hundreds of years however extra recently, the compounds which are lively had been recognized and artificial natural chemists have then been capable of produce the molecules on large scale along with their synthetic analogues [41]. More than 50% of all modern clinical drugs are of natural product origin and natural products play a dominant role in drug development programs in the pharmaceutical industry [42].

2.3.1 Traditional use of *Securidaca longipeduculata*

This plant is traditionally used for different purposes. *S. longipedunculata* is used in the form of root powder in traditional medicine and sold in markets the root is used by traditional healers to heal different diseases by different ethnic groups. Among the ethnic groups Kunamasin north Tigray use it for treating human and livestock ailments. There are other groups that knew about the use this plant for healing different diseases and animal bites [43].These plant have different purpose in different countries that can be summarized by the following table.

Table 1: Ethno medicinal uses of *S. longipedunculata* in different countries

Plant par	Country	Uses	Reference
Roots	Zimbabwe	Venereal diseases, syphilis, pains, fever, epilepsy, pneumonia, tuberculosis.	[44-46]
	South Africa	Blood purifier, aphrodisiac, psychoactive purposes	[19,48-50]
	Burkina Faso	Malaria	[54]
	Uganda	Fever, malaria, ascariasis	[55]
	Nigeria	Abortion, cough, toothache, tuberculosis	[45,56]
	Botswana	Cough, an aphrosiadic	[58]
Leaves	Nigeria	Headache, skin cancer, skin infections, contraceptive purposes	[45,47]
Stem Bark	Burkina Faso	Skin disease	[54]
	Nigeria	Treat infection, malaria, typhoid, frequent stomach ache	[45,57]
Whole Plant	Kenya	Malaria, tick prevention in animals	[51-53]

Roots

The smoke due to burning the root of *S.longipedunculata* is inhaled to treat malaria and fever [54].A root decoction may also be drunk to treat fever, malaria, ,gonorrhea, palpitations, headaches, rheumatism, diabetes, sexual impotence, toothache, fungal infections , malaria, to treat cancer [44,58 ,60].The root bark is pulverized in water and the resulting mixture is inhaled or used to wash the head, treating excessive headache [59].Roots can also be floor into powder form, dissolved in water and brought orally for constipation, pneumonia, back ache, blood purification, and sexually transmitted infections and as an aphrodisiac [29].

Leaves

Fresh leaves are made into paste with little or no water applied externally twice a day for sixty-three days to treat variety of skin infections and skin cancer [45].Dry leaves also are floor into powder and positioned into the fire and the ensuing smoke is inhaled to treat headache even as the boiled leaves are taken orally for contraceptive purposes [47].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 [61].

Whole plant

One cup from a whole plant decoction may be taken orally three times a day for three to four days to treat malaria [52,53].The decoction of the whole plant may either be drunk or used to wash the mouth and treat infections which include oral candidacies, excessive coughing and other opportunistic infections associated with HIV/AIDS [62].

Stem bark

The dried bark is ground into a powder and taken orally with cow's milk or porridge for fourteen days to treat dysentery [45].A decoction from the stem bark may be taken orally to treat stomach ache, headaches, inflammation, chest complaints, abortion, constipation, snake bites, infertility problems, epilepsy and venereal disease [63-66].The powdered stem bark is also mixed with hot water and taken orally to treat syphilis and gonorrhea [67]

2.3.2 Medicinal uses of *Securidaca longipeduculata*

S longipedunculata has been studied for preparation of medicine for many diseases and anti-microbial. Some studies showed about the use of *S longipedunculata* for treatment of human diseases .It is a crucial plant with the capability blessings with inside the remedy of communicable and transferrable diseases like malaria, tuberculosis, and caused by public acquired microorganisms [68]. The extracts from different parts of *S longipedunculata* have been reported to contain numerous valuable compounds such as xanthones, benzyl benzoates, triterpene, alkaloids, phenols, flavonoids, terpenoids, anthraxquinones, and saponin [70,71]. Some medicinal uses are listed below in Table-2.

Table 2: Some publications on medicinal uses of *S. longipedunculata*

Part	Uses	Reference
Root	Anti-microbial activities, for sexual boost, abortion, tuberculosis, cough, fever and Constipation, for epilepsy, Infertility, placenta expulsion, stomach ache and toothache	[45, 56, 61, 66, 69]
Root extracts	For anti-parasitic activities, or anti-plasmodia activity, for enzyme inhibition	[72,73, 75,]
Root bark extracts	Anti-inflammatory, For the treat infections related to nervous and circulatory system	[57, 74]
Whole plant	For the treatment of malaria	[51-53]
Stem bark	For the treat infections related to nervous and circulatory system, Dysentery, malaria, typhoid and frequent stomach ache	[45, 57]
Leaves	For headache and as contraceptive purpose, For anti-plasmodia activity, Infertility, placenta expulsion, stomach ache and toothache	[47 ,61, 73]
Seeds	for treating headache, fever and rheumatism	[76]

2.4 Chemical Constituents *of Securidaca longipeduculata*

Phytochemicals are certainly taking place materials observed in plants, which offers health benefits. These are known as secondary metabolites and may often be created by modified synthetic pathways from primary metabolite or share substrates of primary metabolite origin [77]..Phytochemical screening refers back to the extraction, screening and identity of the medicinally energetic materials observed in plants. Phytochemical screenings of some of the bioactive substances that can be derived from plants are saponins, flavonoids, alkaloids, carbohydrates, lactones, proteins, tannins, glycosides, sterols, carotenoids, terpenes and phenolic compounds [77, 78]

The review reveals that wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antimicrobial, antioxidant, anti-parasitic, anti-diabetic, anti-inflammatory, anti-malarial, insecticidal, pesticidal, and anticonvulsant properties.

Some of the compounds isolated from *S. longipedunculata* are shown in Fig. 2. The volatile oil of the roots contains large amounts of methyl salicylate [22].The report agrees with those of[79] and [80]which revealed that the major component (over 90%) of the volatile material from the root bark is methyl-2-hydroxybenzoate (methyl salicylate). Furthermore, securitize, presenegenin, 2-hydroxybenzoate esters such as methyl 2-hydroxy-6-methoxybenzoate and its benzyl analogue were also reported. In general, most classes of compounds have been isolated from the roots, using a

variety of solvents (Table- 3). This may well explain the ethno medicinal uses and hence the biologic activity of the plant species.

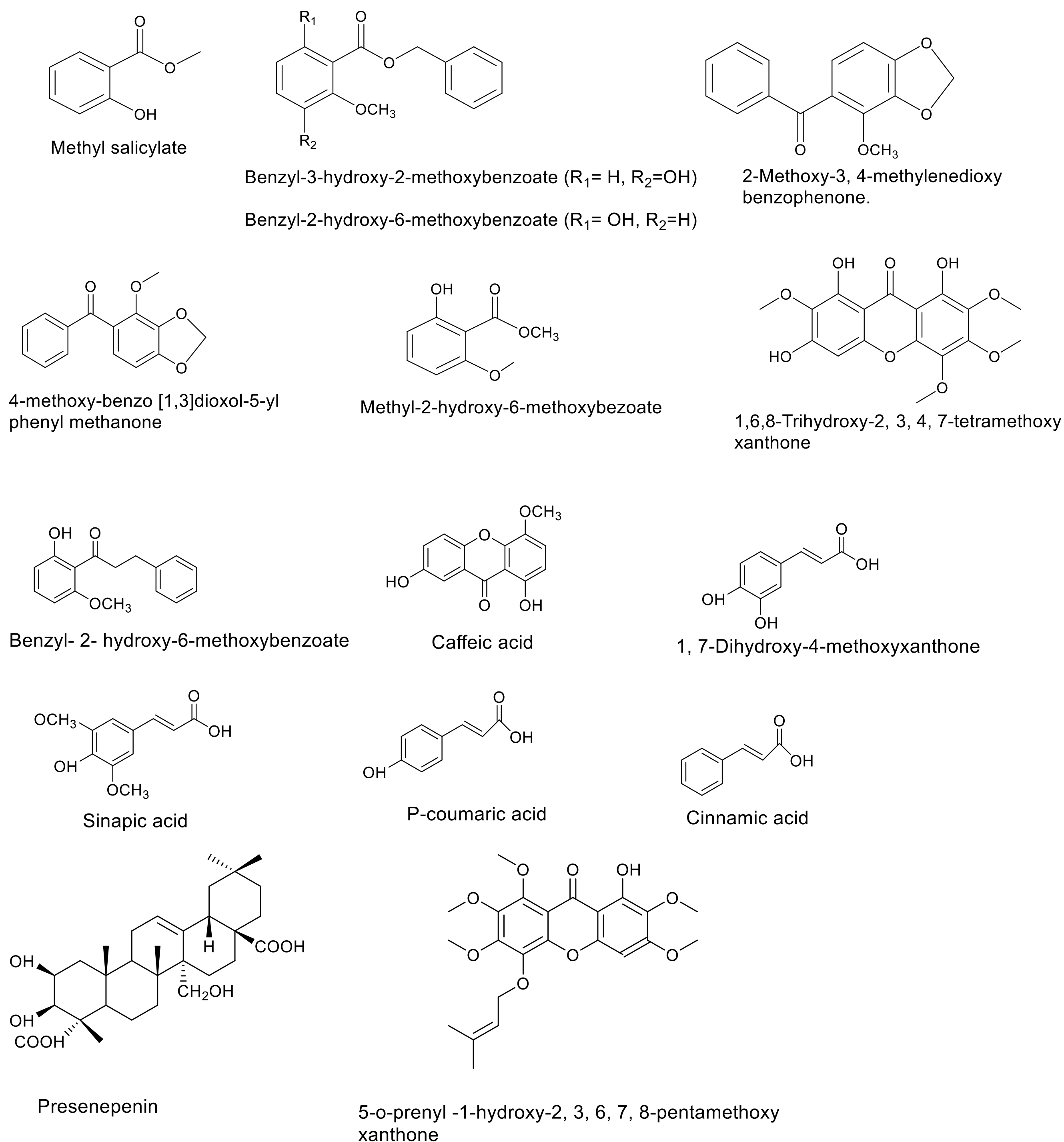


Figure 2: Compounds reported from *Securidaca longipedunculata*

In general, most classes of compounds have been isolated from different parts of this plant by using a variety of solvents as shown below in (Table- 3).

Table 3: Classes of compounds, plant parts investigated and isolated compounds from *S. longipedunculata***.**

Part of plant	Classes of compounds	Examples	Solvent used	References
Roots	**Saponins**	3-o-β-glucopyranosylpresenegenin-28-o-β-D-apiofuranosyl-(1,3)-β-D-xylopyranosyl-(1,4)-[β-D-apiofuranosyl-(1,3)]-α-L-rhamnopyranosyl-(1,2)-{4-O-[(E)-3,4,5-trimethoxycinnamoyl]}-β-D fucopyranosyl ester	70% Methanol	[82]
		Presenegenin	Water	[22]
	Flavonoids	Rutin	Aqueous methanol	[85]
	Alkaloids	Securinine	Water	[22]
	Steroids	β-Sitosterol	Ethyl acetate	[84]
	Phenolic acids	Quercetin, *p*-coumaricacid, cinnamic acid, caffeic acid and chlorogenic acid	Aqueous methanol And water	[85]
Root bark	**Saponins**	Securidaca side A and Securidac aside B	Methanol	[81]
	Flavonoids	1,7-dihydroxy-4- methoxy xanthone.	Dichloromethane and ethyl acetate.	[84,84]
	Volatile oil	Methyl salicylate	Water (Hydro distillation)	[22,79, 80]
Leaves	**Glycosides**	Quercetin-3-O-D-xyloside	Methanol	[86]
Stem bark	**Glycosides**	Δ-Stigmasterol-3-O-D glucopyrano side	Methanol	[86]
	Sucrose derivatives	β-D-(3,4disinapoyl) fructofuranosyl-α-D(6-sinapoy1) glucopyranoside and β-D-(3sinapoyl) fructofuranosyl-α-D (6-sinapoylyl) glucopyranoside	Methanol	[87]
	Phenolic acids	Sinapic acid, 4,5-dicaffeoyl-*D*-quinic acid, caffeic acid and 3,4,5-tricaffeoyl-*D*-quinicacid	Methanol	[87]

Seeds	**Fatty acids and Triacylglycerol**	13-hydroxyoctadeca-cis-9-trans-11-dienoicacid,11-hydroxyhexadeca-cis-7-trans-9-dienoic acid and 9-hydroxytetradeca-cis-5-trans-7-dienoic acid	Light petroleum	[74,88]

The aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids[89-92] while chloroform and ethanol extracts indicated flavonoids, saponins, coumarone, tannins and alkaloids [93]The water and aqueous methanol extracts from the root yielded a variety of compounds in varying amounts, including gallic acid, chlorogenic acid, caffeic acid, epicatechic acid, ruin, p-coumaric acid, cinnamic acid, apigenin, quercetin glucosyl and quercetin dihydrate [85].

.

The genus *Securidaca belongs* to the family Polygalaceae which have been known to contain a large number of chemically complex bioactive compounds such as flavonoids, terpenes, coumarins and steroids. However, the phytochemicals information and bioactivity of the molecules from the leaves of *Securidaca longipedunculata*, which has been widely practiced by the local community for its medicinal role has not been sufficiently reported so far by many researchers in Ethiopia. Therefore this research will provide promising information of the presence of chemicals that can be used as a medicinal value, compounds that can be isolated and motivates for further advanced researchers.

S. longipedunculata has different chemical constituents including methyl salicylate, flavonoids, alkaloids elymoclavine, and dehydro elymoclavine, an ergoline compound and cinnamonic acid and the xanthones: 1,7-Dimethoxy-2-hydroxy-xanthone and 1,4-dihydroxy-7-methoxy-xanthone [94]]. A variety of fatty acids and triglycerols consisting of coriolic acid, trans-9-dienoic acid, and9-hydroxytetradeca-cis-5, trans-7-dienoic acid had been isolated from its seed oil [95]. [95].

The medicinal importance *of S.longipedunculata* has been diagnosed via way of means of the findings of various bioactive metabolites removed from the bark yielding consisting of oleanolic acid, glycoside and alkaloid securiene utilized in treating convulsion in children, increased blood pressure and paralysis following infectious disease [96].It is used for preparation of different medicines for neuromuscular blocking and cardiac effects [97].

Recent research findings showed *S. longipedunculata* used in brain tumor confirming and in cancer management [98].The alkaloid securinine confers activity against Plasmodium falciparum [99].Some flavonoids that were isolated from this plant showed activity against many

microorganisms and methanol extract of the root material against major stored product pests [100,101].

2.5 Glycosides

Glycosides are Organic compounds usually of plant origin that composed of a sugar molecule (such as glucose, fructose, ribose, etc.).And non-sugar molecule bonded via a glycosidic bond. Glycosides are composed of two portions: Glycone portion that refer to the sugar part of the glycoside. The sugar component of glycosides may be mono, di, tri or tetra saccharides and a glycone (genin) portion that refer to the non-sugar part of glycoside.

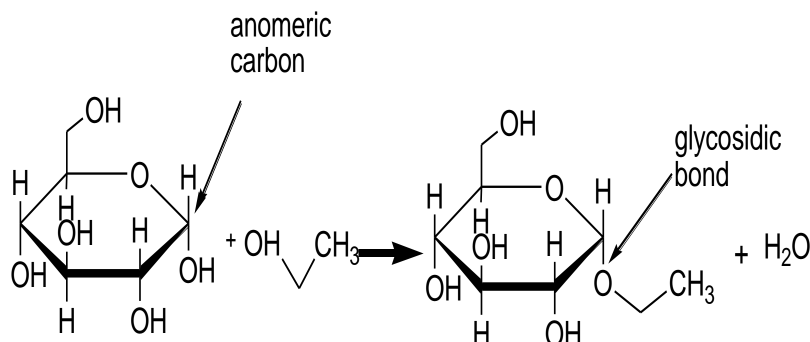
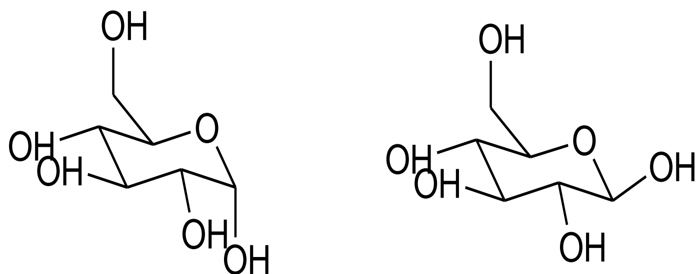


Figure 3: Structure of glycosidic bond

Sugar in glycosides exists in isomeric ά and β forms so both ά and β glycosides are theoretically possible. The two diastereo isomers differ in configuration about the anomeric carbon (c-1) can exist ά and β. If the hydroxyl group on the anomeric carbon is down in relation to the cyclic structure, it is ά anomer while if the hydroxyl group on the anomeric carbon is up in relation to the cyclic structure, it is β anomer.



ά anomer β anomer

Figure 4: structure of alpha and beta anomer

2.5.1Classification of Glycosides

According to the type of glycosides linkage :glycoside is classified in to ά glycosides (ά sugar) andβ glycosides (β sugar).According to the chemical group of the aglycon involved in the formation of glycoside linkage can be classified in: Aglycone-O-sugar:O-glycosides (OH-group) :egsenna and rhubarb.Aglycone-C-sugar: C-glycosides (C-group): eg cascaroside from cascara. Aglycone -S-sugar: S-glycosides (SH-group): egsinigr in from mustard. Aglycone –N-Sugar:N-glycosides (NH-group):eg glycoalkaloid [102].

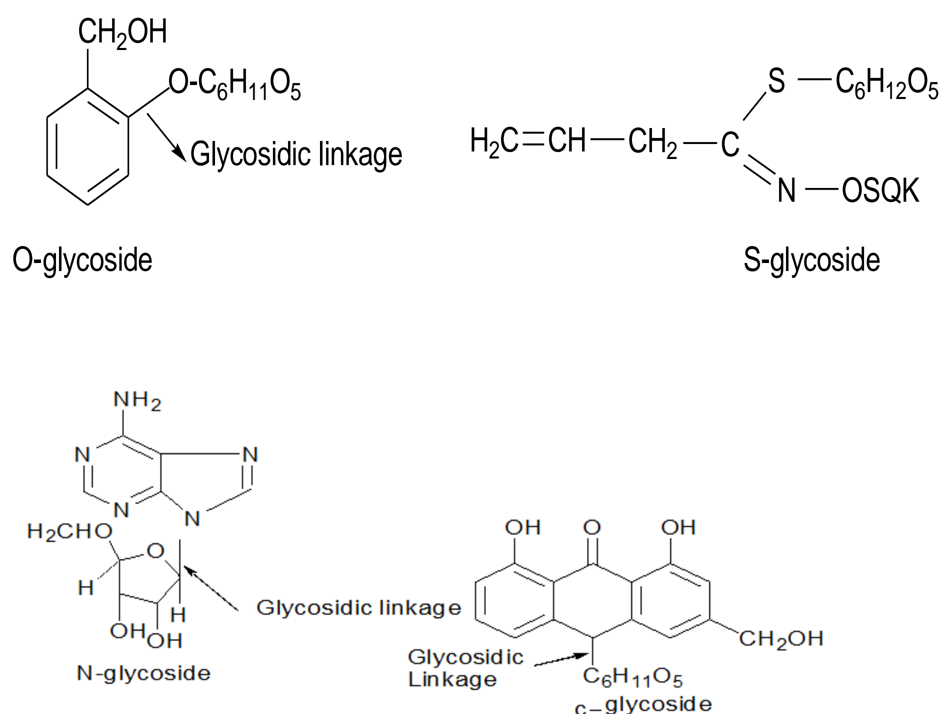
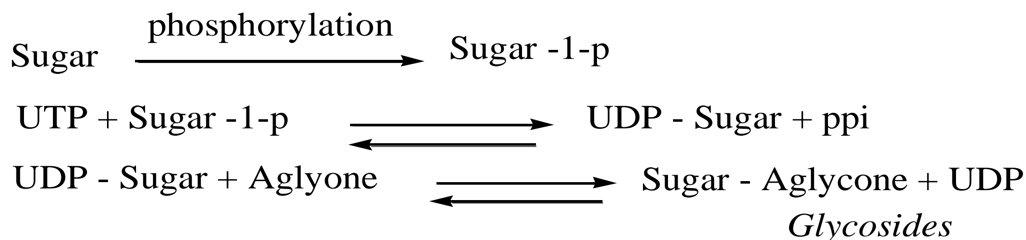


Figure 5: Types of glycoside based on glycosidic bond

2.5.2 Biosynthesis of glycosides

The biosynthetic path ways are widely variable depending on the type of glycone as well as the glycone units. The aglycone and the sugar elements are biosynthesized separately, after which coupled to shape glycoside. The coupling of the two parts occurs via phosphorylation of a sugar to yields a sugar -1-phosphate which reacts with a uridine triphosphate to form auridine diphosphate sugar (UDP-sugar) and inorganic phosphate [102].

This UDP-sugar reacts with the a gycone to form the glycoside and a free UDP.



Scheme 1: Biosynthesis of Glycosides

2.6 Statement of the problem

The plant is selected for this study, because it is used as traditional medicine in Northern Shoa Zone. The people in this area recommend it, especially its fresh leaves for treatment of cough, headache, epilepsy, anti-snakebite and asthma. Few studies were reported on the biological activity of the crude root extracts of this plant, but nothing has been reported about chemical composition and phytochemical constituents from leaves of the plant. Although, the people use this plant as a traditional medicine for treatment of cough, headache, epilepsy and wound. Still now the chemical component in leaves of the plant is not studied. However, up to now many peoples of Northern Shoa Zone, especially Gerbe Guracha town and its areas use the fresh leaves extract of the plant as a drug. As a result, the researchers are intended to perform chemical composition and phytochemical study on the leaves of the plant.

Finally, this study is aimed to extract leaves of *S. longipedunculata* plant with polar, mid-polar and non-polar solvents by TLCM extraction method, to test the phytochemical constituents present in the crude extract and to study the chemical composition of the plant leaves extract.

3. Objective of the study

3.1. General objective

Securidaca longipedunculata is a medicinal plant throughout the world, which is used for many ailments.

Traditionally it is used for epilepsy, headache, anti-snake bites, coughs, asthma etc. Many reports showed that the plant also have different pharmacological activities. The leaves of *securidaca longipeduculata* reported to have diuretic, anti-bacterial and antifungal activities. However, there is no report for the isolation and characterization of the active compounds in the leaves *of securdica longipeduculata* particularly from the species that exist in Ethiopia. Therefore, the main objective of this study is to isolate compounds from the leaves *of securidaca longipeduculata* and characterized them through different spectroscopic techniques.

3.2. Specific objectives

The specific objective of this study is to:

- Prepare extracts from leaves of *securidaca longipeduculata*.
- Isolate major compounds from extract of *securidaca longipedunculata* using different separation techniques.
- Identify and characterize the isolated compounds using a variety of spectroscopic

Techniques.

4. Result and Discussion

Two compounds, SEL-6 and SEL-7 were isolated from methanol extract of leaves *S.longipeducalata.* Structural elucidation of the compound where based on their spectroscopic data and in comparison with data obtained from literature. The characterizations of the two compounds are described below.

4.1Characterization of SEL-6

Compound **SLE-6** was isolated as a yellow powder with the R_f value of 0.45 in EA: MeOH (1:0.5). The molecular formula $C_{20}H_{18}O_{11}$ was assigned for compound **SLE-6** based on its ^{1}H and ^{13}C - NMR data (Table 4). ^{1}H-NMR spectrum (Table 4 and Appendix 1) showed the presence at δ_H7.67*(dd ,J*=8.5,2.3Hz,1H), at δ_H7.51*(dJ*=2.22Hz,1H), *at*δ_H6.84, *(d J* =8. 5Hz,1H *), at δ_H6.41(d, J*=2.0Hz,1H *)and at* δ_H6.20 *(d ,J =2.1Hz,1H)* indicating a methine attached to oliefinic carbon,C-6',C-2',C-5',C-8 and C-6 respectively. On the other hand at δ_H5.28 (d, J=5.2Hz,1H), at δ_H3.75 (1HtJ =6.0Hz,1H),at δ_H3.65 (*dd ,J* =8.5,2.3Hz,1H *),and at* δ_H3.50 (*1HddJ=3.0* 6.8Hz*)* indicating attached to oxymethine carbonsC-1",C-2",C-3"andC-4"respectively.At δ_H3.59*(s, 1H), and* δ_H 3.22 *(dd, J*= 11.4, 2=.4 Hz, 1H*)*indicatingmethyleneatC-5".

In.^{13}C-NMR (Table 4 and Appendix 2) and DEPT-135 spectra (Table 4 and Appendix 3) showed the presence of 20 carbon signals which were attributed to one methylene carbon at δC 64.39,and tenquaternarycarbonsatδC,164.3, δC161.28 δC 64.39, δC 177.8 δC 133.8, δC 156.32, δC 120.8, δC 145.0, δC 148.5, δC 156.3 and four oxymethine at δC 101.4, δC 71.7, δC70.8, δC 66.2 and five methine carbons at δC 122.2, δC 115.8, δC115.6, δC 98.7, δC 93.8.

The COSY spectrum (Appendix 4) showed a proton signal at δ_H6.84(*d J*=8.5Hz,1H*)* correlated with a proton signal at δ_H7*.67 (dd ,J* =8.5,2.3Hz,1H*) (*H-2'*).*The proton signal at δ_H 7.67*(dd, J*=8.5,2.3Hz,1H) correlated with δ_H 6.84*(d. J*=8.5Hz,1H) (H-5'). The proton signal at δ_H 6.41 (*d, J*=2.0Hz*, 1H)* correlated with δ_H 6.20 *(d,J=2.1Hz,1H)* (H-8).The proton signal at δ_H 3.65 (s, 1H) correlated with δ_H 3.22*(dd, J = 11.4, 2.4 Hz, 1H)(3").* The proton signal at δ_H 3.65 (s, 1H) correlated with δ_H7*.67 (dd ,J =8.5,2.3Hz,1H(2").*

HSQC spectrum (Appendix 5) showed an methylene proton signal at δ_H3.59 *(m, 1H*) and 3.22 (m, 1H), correlated with a carbon signal at δ_C 64.39 (C-5"). The proton signal at δ_H 3.65 (m,1H)correlated with carbon signal at δ_C 66.16(C-4").The proton signal at δ_H 3.50(m,1H)correlated with carbon signal at δ_C 70.8 (C-2").The proton signal at δH 3.50 (1HddJ=3.0

and 6.8Hz) correlated with carbon signal at δC 71.7(C-3") The proton signal at δ_H 6.41(m,1H) correlated with at carbon signal δ_C 93.9(C-8).The proton signal at δ_H 5.28 (m,1H) correlated with carbon signal at δ_C 101.4 (C-1").The proton signal at δ_H6.84 (*d J*=8.5Hz,1H) correlated with carbon signal δ_C 115.6 (5`).The proton signal at $\delta_H$ 6.84 ( *dJ*=8.5Hz,1H) correlated with the carbon signal $\delta_C$ 115.8 (2`).The proton signal at δ_H 7.67*(dd, J*=8.5,2.3Hz,1H) correlated with the carbon signal δ_C 122.2 (6`)

The HMBC Spectrum (Appendix 6) of compound SLE-6 showed that the hydroxide proton at δ_H 12.6 *(s, 1H*) (H-5) correlated with methine carbons at δ_C 98.7 (C-6) and at δ_C103.9 (C-10), δ_H 5.28 (dJ=5.2Hz,1H) (H-2") correlated with δ_C 64.4 (C-5"), δ_H 7.51*(d,J*=2.22Hz,1H) (H-3') correlated withδC148.5 (C-4') and δ_C 122.2(C-6'), δ_H 6.20 *(d,J =2.1Hz,1H*) (H-7) correlated withδC93.8 (C-8),δH7.67*(dd,J*=8.5,2.3Hz,1H) (H-2') correlated with156.3(C-2),δ_H 5.28 (dJ=5.2Hz,1H) (H-2") correlated with δ_C 101.4(C-1") and 64.4 (C-5") indicated the hydroxyl group was linked to (C-5,7, 3',4' ,2",3" &4")

Table4:^{1}H-and ^{13}C-NMR data for compound **SLE-6** comparison with reference

Position	DEPT-135	**SLE-6** ^{1}H-NMR (400 MHz, DMSO-d_6) ^{13}C-NMR (101 MHz, DMSO-d_6)		**Lite.**[124][103] ^{1}H-NMR (500 MHz, $CDCl_3$) ^{13}C-NMR (125 MHz, $CDCl_3$)	
		δ_C	δ_H	δ_C	δ_H
2	C	156.3	-	156.2	-
3	C	133.8	-	133.7	-
4	C	177.8	-	177.5	-
5	C	161.3	-	161.1	-
6	CH	98.7	6.20 *(d,J =2.1Hz,1H*)	98.6	6.19 (1H,*d.J*=1.9Hz) (H-6*)*
7	C	164.3	-	164.1	-
8	CH	93.8	6.41 *(d,J*=2.0Hz,1H*)*	93.4	6.39 *(1H,dJ*=1.9Hz) (H-8)
9	C	156.3	-	156.2	-
10	C	103.9	-	103.8	-
1’	C	120.8	-	120.8	-
2’	CH	115.6	7.51*(d,J*=2.22Hz,1H)	115.3	7.49 (1H,*dJ* =2.1Hz) (H-2')
3’	C	145.0	-	144.9	-

4’	C	148.5	-	148.5	-
5’	CH	115.8	6*.84 (d,J*=8.5Hz,1H)	115.7	6.83*(1H,d,J*=8.5Hz) (H-5')
6’	CH	122.2	7.67*(dd, J*=8.5,2.3Hz,1H)	122.0	7.65 (1H,*dd,J*=2.1,8.1Hz) (H-6')
1’’	CH	101.4	5.28 *(d, J* =5.2Hz,1H*)*	101.3	5.27 (1H, *d,J*=5.1Hz) (H-1'*)*
2’’	CH	70.8	3.75 (1H*tJ*=6.0Hz,1H)	71.6	3.74 (1H, *t,J*=6.0Hz) (H-2")
3’’	CH	71.7	3.65 (s, 1H)	70.6	3.64 (1H,brs,H-3")
4’’	CH	66.2	3.50 (1HddJ=3.0 and 6.8Hz)	66.0	3.50 (1H, dd,J=3.0 and 6.8Hz)(H-4")
5’’	CH_2	64.4	3.59 *(s, 1H), 3.22 (dd, J = 11.4, 2.4 Hz, 1H*)	64.2	3.20 *(dd, J* = 11.4, 2.4 Hz, 3.59 1H-*ddJ*=5.4) and 11.4Hz,H-5"
C-5	OH	161.3	12.63*((s, 1H)*		12.64 (1H,s) (OH-5)
C-7	OH	164.3			
C-2’’	OH	70.8			
C-3’’	OH	71.7			
C-4’’	OH	66.2			
C-3’	OH	145.0			
C-4’	OH	148.5			

Table 5: COSY Correlation SLE-6

Carbon No	Proton-Proton correlation
C-2’’(70.8)	H-2’’(3.75)…H-3’’(3.65)
C-1" (101.4)	H-1" (5.28)…H-2"(3.75)
C-5' (115.8)	H-5' (6.84)…H-2'(7.51)
C-6 (99.9)	H-6(6.20) …H-8(6.41)
C-5’(115.8)	H-5’(6.84)….H-6’(7.67)

Table 6: HSQC Correlation SLE-6

Carbon No	Hydrogen No	Remark
C-5" (64.4)	3.59 and 3.22	CH_2
C-4" (66.2)	3.50	CH
C-2"(70.8)	3.75	CH
C-3" (71.7)	3.65	CH
C-8 (93.8)	6.41	CH
C-6 (99.9)	6.20	CH
C-1" (101.4)	5.28	CH
C-5' (115.6)	6.83	CH
C-2' (115.8)	7.51	CH
C-6' (122.2)	7.66	CH

Table 7: HMBC Correlation SLE-6

Proton No	Carbon Correlated
H-5 (12.63)	98.7(C-6),103.9(C-10)
H-1"(5.28)	64.4(C-5") 70.8 (C-2’’),71.7 (C-3’’)
H-2' (7.51)	148.5 (C4'),145 (C-3’), 122.2 (C-6')
H-6 (6.20)	93.8 (C-8),164.3 (C-7).161.3 (C-5)
H-6'(7.67)	156.3 (C-2)
H-5’(6.84)	148.5 (C-4),145.2 (C-3’’)
H-8 (6.41)	164.3 (C-7),98.7 (C-6)

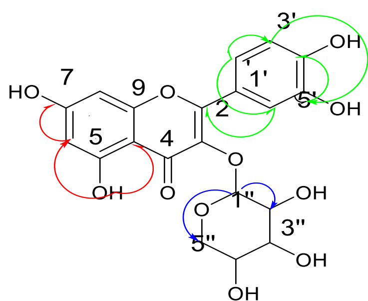


Figure 6: Partial HMBC Correlation of Compound SLE-6

Based on the spectroscopic data obtained and in comparison with literature (124) the structure of compound SLE-6 is proposed to be as Quercetin 3-*O*-L-arabinopyranoside (Figure 7).

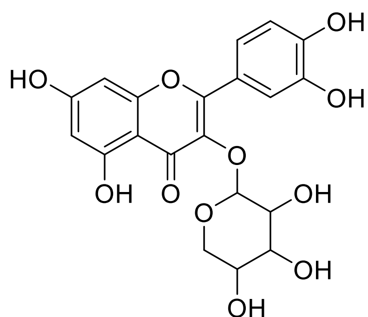


Figure 7: Structure of compound SLE-6

4.2-Characterization of SLE-7

Compound **SLE-7** was isolated as a white crystal with the R_f value of 0.65 in EA:MeOH (1:0.5).^{1}H-NMR(400MHz DMSO) spectrum (Table 8 and Appendix 7) revealed the presence of four methylene signals, which are appeared at δ_H 3.37 *(m,1H,H-1), δ_H 3.68 (dd,J=*11.5,5.5Hz,1H,H-1),3.70 *(dd,J,*11.5,5.5Hz 1H-*6),and* δ_H2.95(m,1H-6),four methine proton signals at *δ_H2.99(m,1H,H-*

2), 2.98 *(m,1H,H-3), and* 3.22 *(m,*1H,H-5*),*and a methine proton signal at position δ_H 3.07 *(dd, J* = 10.9 5.3 Hz, 1H,H-4).

In ^{13}C-NMR(101MHz DMSO (Table 8 and Appendix 8) and DEPT-135 spectra (Table 8 and Appendix 9) showed the presence of 6 carbon signals which were attributed to two methylenes carbon at δ_C 61.5 (C-1) and δ_C 69.6 (C-6).Four methines carbon signal at δ_C 61.5 (C-1), δC70.3 (C-3) δ_C 78.6,(C-4) δ_C 69.9(C-5) could be attributed to oxymethine carbons .

The COSY spectrum (Appendix 10)showed proton signal at δ_H3.68 *(dd, J* = 11.5, 5.5 Hz, 1H,H-1) correlated with a proton signal at δ_H4.47 *(t, J*= 5.9 Hz, 1H*) and*δ_H3.37 (*m,1H) H-1)* thenδ_H3.37*(m,1H,H-1)*correlated with δ_H4.47 *(t, J* = 5.9 Hz, 1H*,)and*,3.7*(dd ,J*=11.5,5.5Hz1H-*6).*The proton signal at δ_H 2.98 (m,1H,H-3)correlated with 3.22 *(m,1H,H-5),3.70, (dd,J=11.5,5.5Hz1H,H-6),3.07 (dd, J* = 10.9 5.3 Hz, *1H) (H-4).* The proton signal atδ_H2.99 *(m,1H,H-2*) correlated with 3.22 *(m,1H,H-5),*3.70 *(dd,J*=11.5,5.8Hz1H,H-6*) and,* 3.07*(dd, J*= 8.5, 4.6 *Hz, 1H)(H-4).*The proton signal at δ_H 3.07 *(dd, J* = 8.5, 4.6 Hz, 1H,H-4),correlated with2.98 *(m,1H,H-3*),3.22 *(m,1H)(H-5).* The proton signal *at* δ_H 3.22 *(m, 1H,H-5*) correlated *with* 3.07 *(dd, J* = 10.9 5.3 Hz,1H,H-4)and2.98 *(m,1H)(H-3).* The proton signal at δ_H 3.70 (*dd,J=11*.5, 5.8Hz1H,H-6) correlated *with 2.95 (m, 1H,H-6),*3.22 *(m,1H) (H-5).*

HSQC spectrum (Appendix 11) showed an oxymethylene proton signal at δ_H3.37 (m, 1H) and δ_H3.68 *(dd, J* = 11.5, 5.5 Hz, 1H)correlated with a carbon signal at δ_C 61.5 (C-1). The proton signal at δ_H2.99 (*m, 1H*) correlated with carbon signal at δ_C 81.7(C-2)).The proton signal at δ_H 2.98 (*m, 1H*)correlated with carbon signal atδ_C70.3(C-3).The proton signal at δH3.07 (*dd, J* = 10.9,5.3 Hz, 1H)correlated with at carbon signal δ_C 78.5(C-4). The proton signal at δ_H3.22 (*m, 1H*)correlated with carbon signal at δ_C69.9(C-5).The proton signal at δ_H2.95 (m, 1H)and 3.70 (*dd*, J=11.5,5.5,Hz 1H) correlated with carbon signal at δ_C 69.6(C-6).

The HMBC Spectrum (Appendix 12) of compound **SLE-7** showed that the oxymethylene proton at δ_H4.47 (t, *J* = 5.9 Hz, 1H) correlated with methylene carbons at δ_C 61.5 (C-1) and δ_H 2.98 (m,1H)correlated with δ_C 81.7 (C-2), δ_H 4.88(d, *J* = 4.9 Hz, 1H), δ_H 3.70 (dd, *J* = 8.5, 4.6 Hz, 1H), δ_H3.22 (m, 1H) ,correlated with δ_C 69.6 (C-6).δ_H4.88 (d, *J* = 4.9 Hz, 1H), δ_H 2.95 (m, 1H) and δ_H 3.22 (m, 1H), correlated with δ_C 69.9 (C-5). δ_H 3.37 (m, 1H) ,4.91 (m, 1H) correlated with δ_C 70.3 (C-3).δ_H 4.91 (m, 2H), δ_H 3.70 (*dd,J*=11.5,5.5,1H),δ_H 2.98 (*m,1H*) correlated with δ_C 78.5 (C-4).δ_H4.91(m, 1H),4.90 (m, 1H)),δ_H3.63 (*dd, J* = 11.5, 5.5 Hz, 1H) and δ_H 2.99 *(m,1H*) correlated

with δ_C 81.7 (C-2) indicated the hydroxyl group was linked to (C-1,3,4,5).The above explanation mentioned below by the following tables.

Table 8 :^{1}H-and ^{13}C-NMR and DEPT-135 data for compound SLE-7 comparison with reference

Carbon No	(H NMR) (400 MHz, DMSO)	Reference [lit.[19b]] **104** (^{1}H NMR) (CD_3OD300 MHz)	^{13}CNMR (101 MHz, DMSO)	Ref[lit.[19b]]^{13}CNMR (CD_3OD 75MHz)	δ_HDEPT$_{135}$	Remark
1	δ_H3.68*(dd, J* = 11.5, 5.5 *Hz, 1H)*,3.37 (m, 2H*)*	δ_H,4.27*(dd,1H12.1,5.5), 3.46(m,1H)*	δ_C 61.5	δ_C 62.7	61.5	CH_2
2	δ_H 2.99 *(m, 1H)*	δ_H3.28(m,1H)	δ_C 81.7	δ_C 80.9	81.7	CH
3	δ_H 2.98 *(m, 1H)*	δ_H3.25(m,1H)	δ_C70.3	δ_C72.8	70.3	CH
4	δ_H 3.07*, (dd, J = 8.5, 4.6 Hz, 1H)*	δ_H,3.30(m,1H*)*	δ_C78.5	δ_C77.7	78.5	CH
5	δ_H3.22*, (m, 1H)*	δ_H,3.4(m,1H)	δ_C 69.9	δ_C71.6	69.9	CH
6	δ_H3.70*(dd, J* = 10.9, 5.3 Hz, 1H) and 2.95(*m, 1H*)	δ_H4.47*(dd,1H,*12.1,1.8*), 3.16(t,1H,10.3,11.0)*	δ_C 69.6	δ_C71.0	69.6	CH_2
1-OH	4.47(t, *J* = 5.9 Hz, 1H					
3-OH	4.91 (m, 1H),					
4-OH	4.91 (m, 1H),					
5-OH	4.88(d,*J* = 4.9 Hz, 1H					

Table 9: COSY (Proton-proton Correlation of SLE-7)

Carbon No	COSY
C-1 (61.5)	H-1(3.37)…. H-1(3.68)
C-6 (69.6)	H-6(2.95)…. H-6(3.70)
C-5 (69.9)	H-5(3.22)…. H-6(2.95)
C-4 (78.5)	H-4(3.07)….H-3(2.98)
H-1(61.5)	H-1(3.68)….H-2(2.99)

Table 10: HSQC Correlation of SLE-7

Carbon No	Hydrogen No	Remark
C-1 (61.5)	3.37 *(m,1H) and* 3.68 *(dd,J* =11.5, 8.5Hz1H*)*	CH_2
C-2 (81.7)	2.99 *(m,1H*)	CH
C-6 (69.6)	3.70 *(dd,J* =11.5, 5.8Hz1H*) and* 2.98 *(m,1H)*	CH_2
C-5 (69.9)	3.22 *(m, 1H)*	CH
C-3 (70.3)	2.98 *(m,* 1H)	CH
C-4 (78.5)	2.99 *(m, 1H*)	CH

Table11:HMBC Correlation of SLE-7

Proton No	Carbons Correlated
H-1 (3.37 *(m,1H) and* 3.68 *(dd,J* =11.5, 8.5Hz1H	H-1…. C-3(70.3)
H-2 (2.99 (*m,1H)*	H-2….C-6(69.6) and C-1(61.5)
H-3 (2.98 *(m,* 1H)	H-3…. C-1(61.5)
H-4(3.07 *(m, 1H)*	H-4….C-6(69.6)
H-5(3.22 *(m, 1H*	H-5…. C-6(69.6)
H-6(3.70 *(ddJ* =11.5, 5.8Hz1H*) and* 2.98 *(m,1H*	H-6…. C-1(61.5)

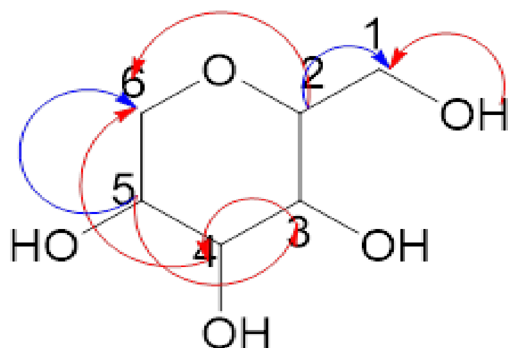


Figure 8: Partial HBMC Correlation of compound SLE-7

Based on the spectroscopic data obtained and in comparison with literature from online, the structure of compound SLE-7 is proposed to be 1,5-Anhydroglucitolas (Figure 9)

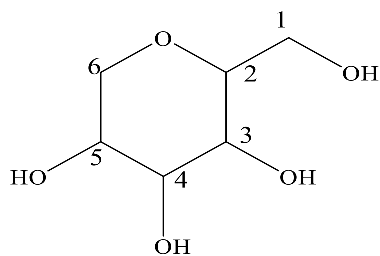


Figure 9: Structure of compound SLE-7

5. Experimental

5.1. General

1H, 13C, and 2D NMR spectra were recorded on a Bruker advance 400MHz spectrometer with TMS as internal started. Solvents were removed using the Buchi type rotary evaporator under reduced pressure at 30^0C. Mixtures of compounds were separated using chromatoron (model 79247), Column Chromatography and TLC. TLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 F254 coated on aluminum foil. Compounds on TLC were detected using UV light on the wave length of 254&365 nm and spraying with 1% vanillin in sulfuric acid.

5.2. Collection and identification of the plant material

The fresh leaves of the plant were collected from North shoa**,** capital City of Fiche Debre Libanos Wereda Oromia regional state of Ethiopia, which is 112 km from Addis Ababa. The leaves were collected and the collected leaves were dried at room temperature.

Figure 10: The dried leaves

5.3. Isolation and analysis

Air dried and finely powdered leaves of the plant (150 g) were soaked in (1000ml) chloroform and (1000ml) methanol with in a percolator at room temperature for 72hr. After the extract was filtered, the solvent free powder was exhaustively extracted with methanol and chloroform in a percolator and the solvent was removed using Rotary evaporator to obtain a greenish gum (24.7 g). The crude methanol extract was taken and dried.

Figure 11:The solvent was removed by rotary evaporator and the dried extract sample

The column was prepared from cyclohexane and silica jell and 24.7g of sample was added. Nine fractions were collected using a 200ml Erlenmeyer flask, each 200ml.Each fraction was checked in TLC plate using different solvent system. After TLC was checked fraction 4, 5,7and 9 were selected. Fraction 4 was evaporated and concentrated it gives white powder and marc-SL-E1.
The TLC of compound SL-E1 is run with pet ether: EtOAc (1:0.5) solvent system and it is UV active and showed a dark blue color seeing in UV lamp at 254 nm. After spraying with 1%vanillin in H2SO4 and heated in hotplate a purple color was observed. The compound obtained (SL-E1) is white powder with RF value 0.8.

Fraction 5 was evaporated and concentrated and the sample was dried and the mass was measured 0.68g. The column was prepared and 0.68g sample was added and 16 test tubes were collected from this test tube 10-13 have similar Rf value 0.51 mixed together and concentrated Mark SL-E2.Test tubes 3-8 were collected together and dried the mass was 0.32g and prepared another column and 24 test tubes were collected, from this test tube 1-3 have Rf value 0.8 markSL-E3.Test tube 7-19 mixed together and goes to sephadex and 29 test tubes were collected. Test tube 7 was pure with Rf value 0.54 mark SL-E4.Test tube14-18 mixed together and concentrated with Rf value 0.7 mark SL-E5.
Fraction 7 were concentrated and got yellow powder with Rf value 0.45 have 0.25g mass and mark SL-E6.The TLC of compound SL-E6 is run with ethyl acetate with methanol (1:0.5) solvent system and it is UV active and showed a dark blue color seeing in UV lamp at 254 nm. After spraying with 1%vanillin in H2SO4 and heated in hotplate a purple color was observed.

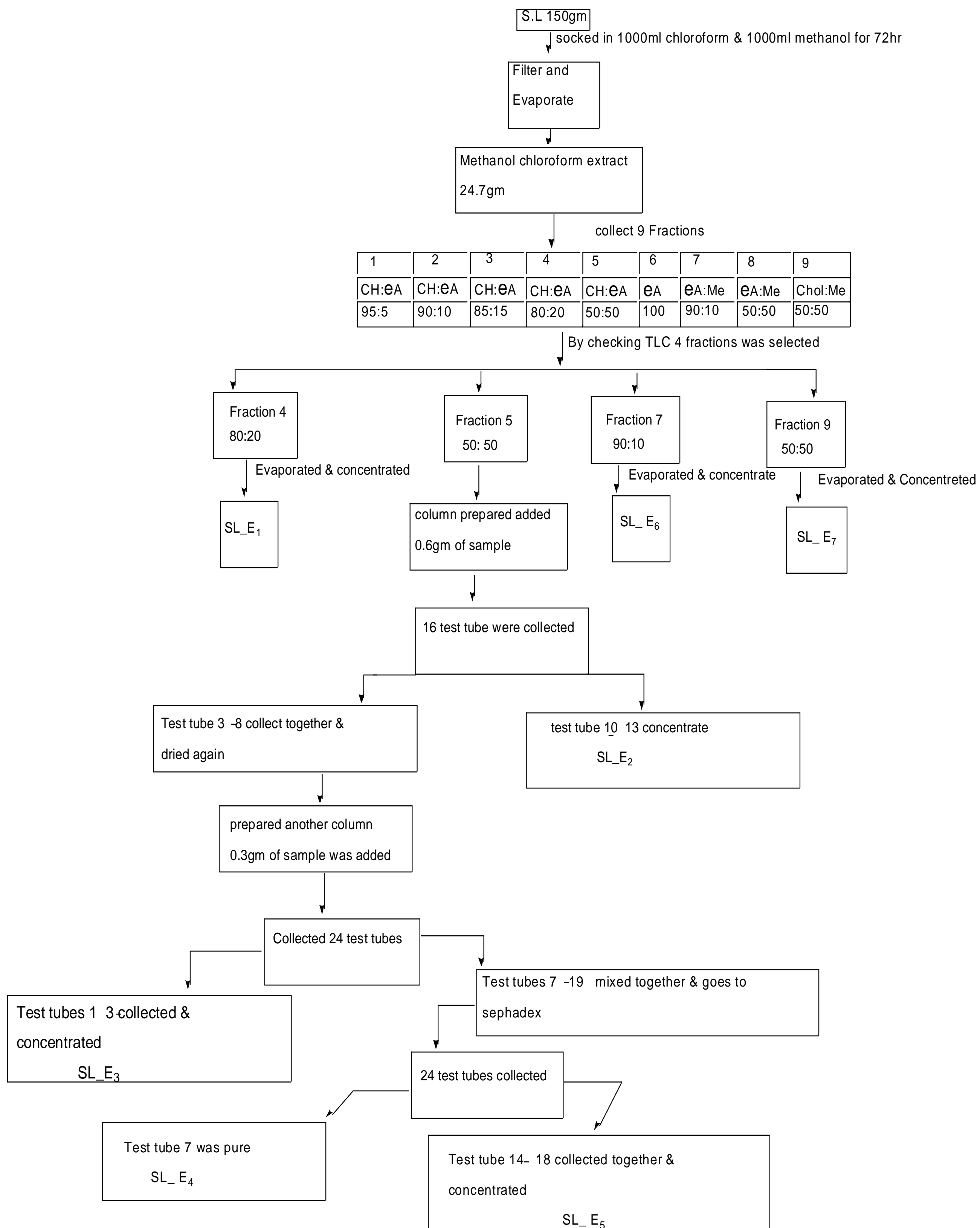
Fraction 9 were concentrated and got white solid with Rf value 0.65, have 0.16g mass and mark SL-E7**.**The TLC of compound SL-E7 is run with ethyl acetate with methanol (1:0.5) solvent system and it is UV active and showed a dark blue color seeing in UV lamp at 254 nm. After spraying with 1%vanillin in $H2SO4$ and heated in hotplate a purple color was observed.

Figure 12 The prepared column and the collected fraction

Figure 12: The Isolated compounds

The isolated compounds are SLE-1-SLE-7.From this isolated compounds SLE-1-SLE-5 was discarded because the amounts are very small to run 1D and 2D Spectra. The SLE-6 and SLE-7were selected.

The method of isolation is summarized below (scheme-2Scheme 2: Method of isolation of components of Securidaca longipeduculata



6. Conclusion

Securidaca longipeduculata a medicinal plant found in the family of polygalaceae. Pharmacological Studies on this plant reports to have antimicrobial, anti-parasitic, anti plasmodial and anti-inflammatory. Phytochemical investigation on this plant reports the plant is rich in, saponin, flavanoid, steroid, glycosides, alkaloid, sucrose derivatives, phenolic acid etc. In this study two compounds were isolated compounds SLE-6, SLE-7. In the ^{1}HNMR the literature(Guaijaverin) the anomeric proton signal was recorded at 5.27ppm (doublet J=5.1),while two proton at C-5" of arabinose gave separate signal at3.59 ppm (*dd*) and3.20ppm (*dd*) , which indicated alpha-pyranose form of sugar unit [105] the suggested structure was confirmed ^{13}C-NMR analysis, recording the chemical shifts of carbon atoms almost identical with SLE-6 .The above spectroscopic data allowed for the identification of compound **SLE-6** as (Figure 6). Comparison of the NMR spectral data of with those reported in the literature for (Guaijaverin) revealed a very close agreement.This compound is Quercetin 3-O-L-arabinopyranoside.).

The^{1}HNMRand^{13}C-NMR data analysis of [lit19b] (Acerginnala) 1,5anhydro- D-glucitol is closer to SLE-7.From the data the compound SLE-7is1,5-anhydro-D-glucitol .

7.Spectral data

SLE-6

^{1}H NMR (400 MHz, DMSO) δ 12.65 (s, 1H), 7.67 (*dd*, *J* = 8.5, 2.3 Hz, 1H), 7.51 (d, *J* = 2.2 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.41 (d, *J* = 2.0 Hz, 1H), 6.20 (d, *J* = 2.1 Hz, 1H), 5.28 (d, *J* = 5.2 Hz, 1H-1′),3.75(1HtJ6.0Hz1H), 3.65 (s, 1H),3.59(s,1H),3.50(1HddJ3.0and6.8Hz), 3.22 (dd, *J* = 11.4, 2.4 Hz, 1H)

^{13}C NMR (101 MHz, DMSO) δ 177.8(C-4), 164.28(C-7), 161.28(C-5), 156.32(C-9) , 148.47(C-4́), 145.04(C-3), 133.8(C-3), 122.15(C-6′), 120.8(C-1́), 115.80(C-2′), 115.6(C-5́), 103.97(C-10), 101.44(C-1′́), 99.9(C-6), 93.8(C-8), 71`.71(C-3′́), 70.79(C-2′́), 66.16(c-4′́), 64.38(c-5′́).

SLE7

^{1}H NMR (400 MHz, DMSO) δ 4.91 (m, 2H), 4.88 (d,*J* = 4.9 Hz, 1H), 4.47 (t, *J* = 5.9 Hz, 1H), 3.70 (dd, *J* = 10.9, 5.3 Hz, 1H), 3.68 (dd, *J* = 11.5, 5.5 Hz, 1H),3.37 (m, 2H), 3.2 (m, 1H), 3.07 (dd, *J* = 8.5, 4.6 Hz, 1H), 2.99,2.98,2.95 (m, 3H).

^{13}C NMR (101 MHz, DMSO) δ 81.67, 78.53, 70.34, 69.94,69.58, 61.51.

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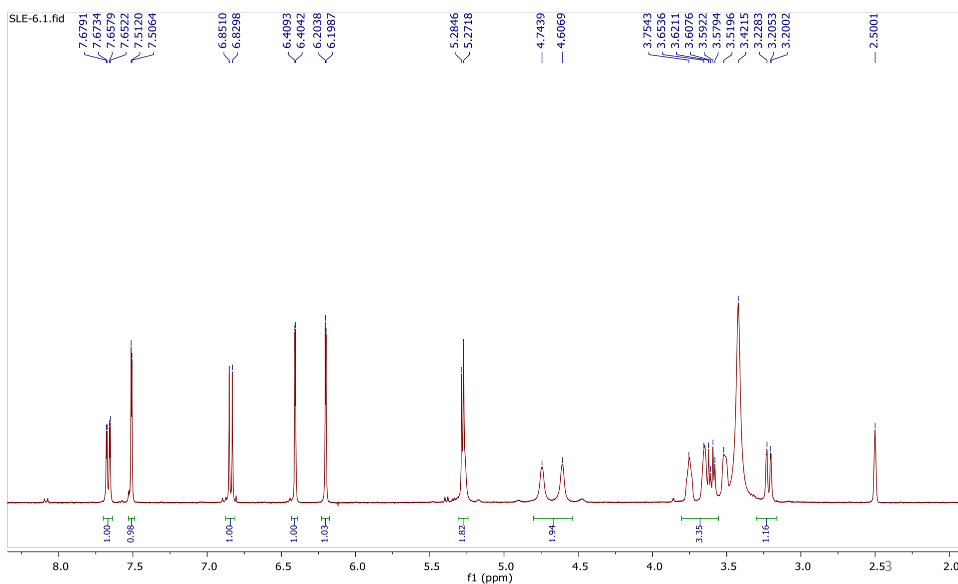
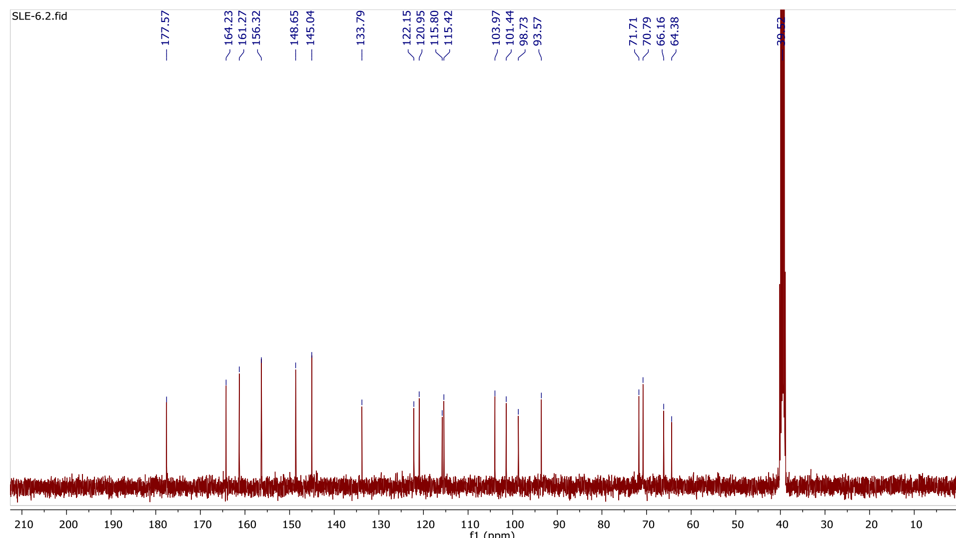
Appendices

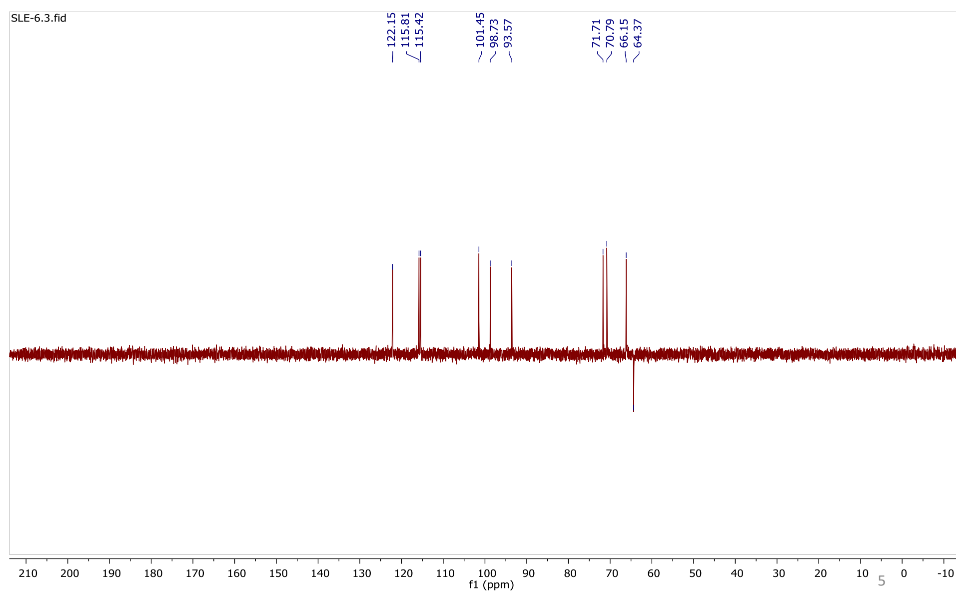
Appendix 1: SLE-6 ^1H- NMR



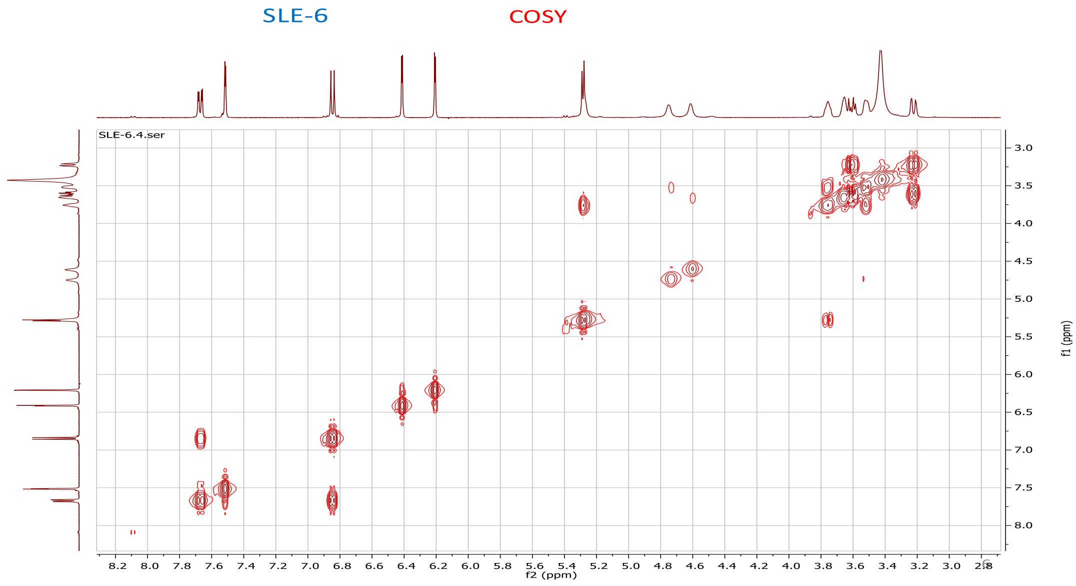
Appendix 2: SLE-6 ^{13}C-NMR



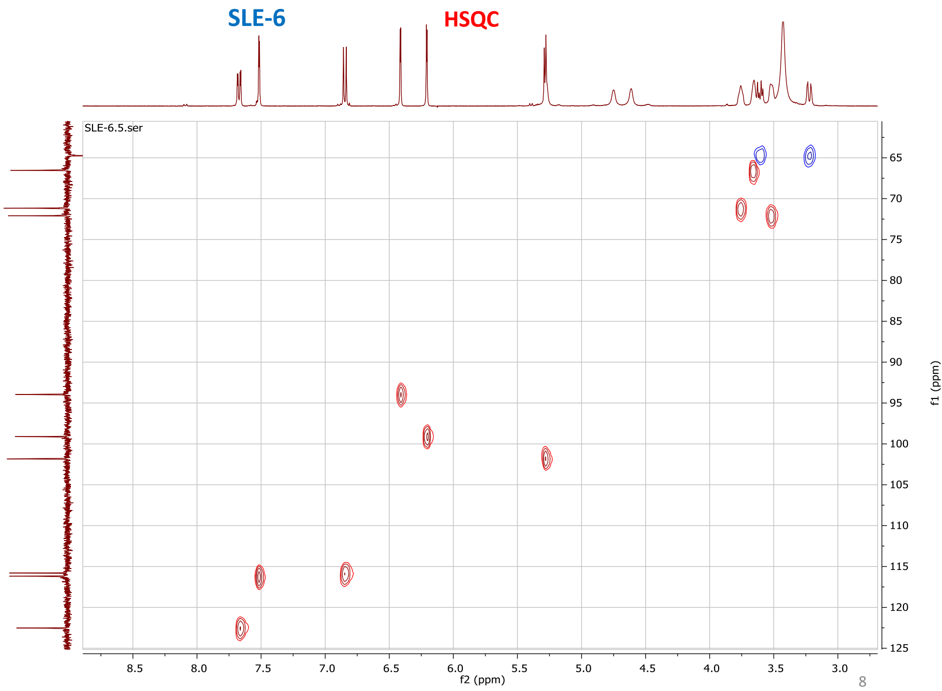
Appendix 3:SLE-6 DEPT- 135



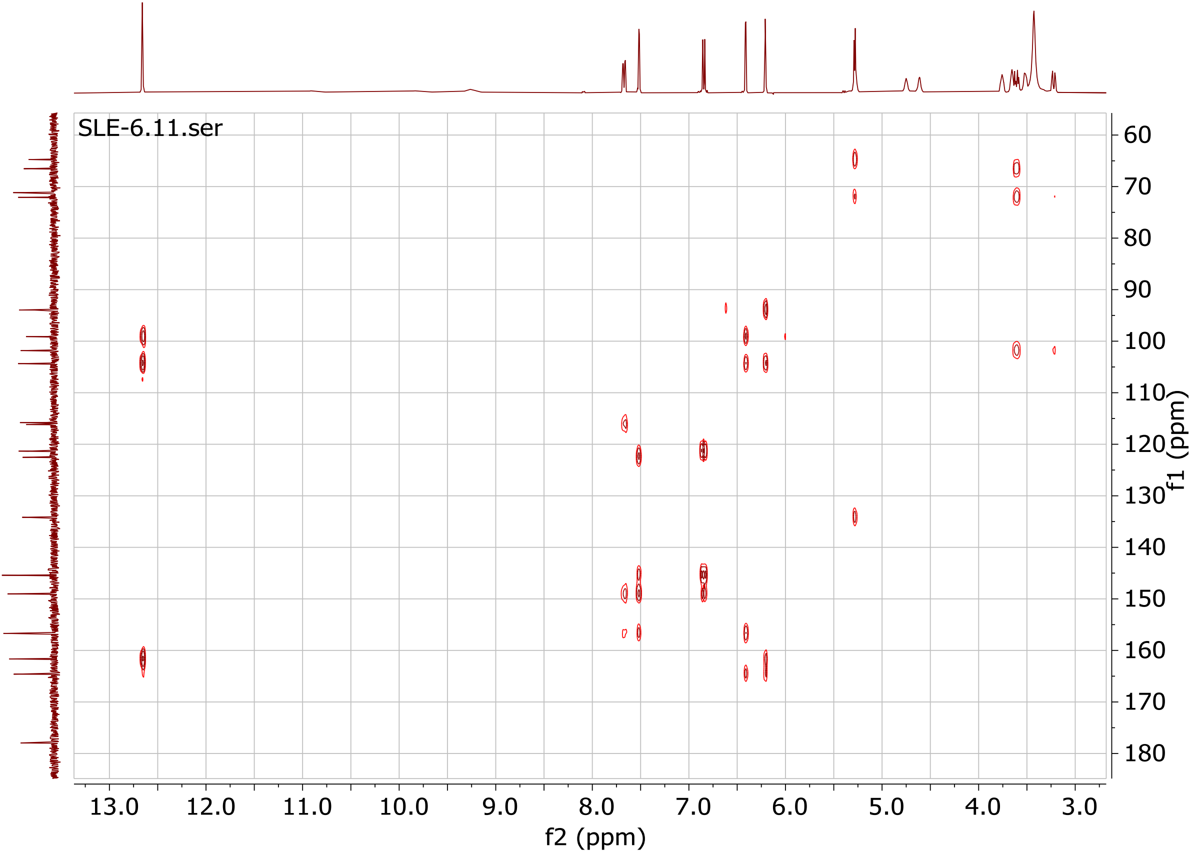
Appendix 4: SLE-6 COSY



Appendix 5: SLE-6 HSQC



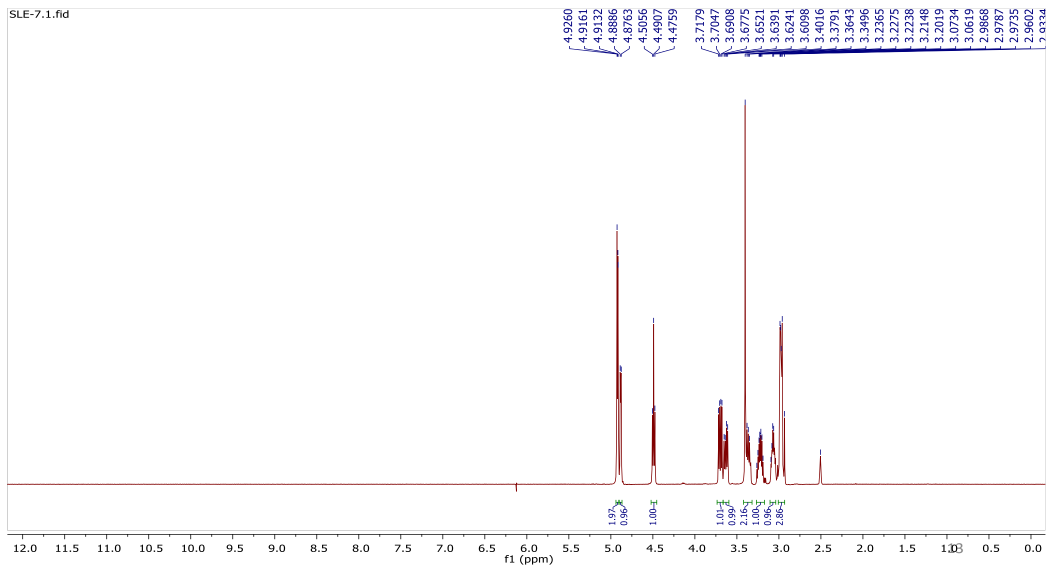
Appendix 6: SLE-6 HMBC



Appendix 7: SLE-7 ^{1}H-NMR

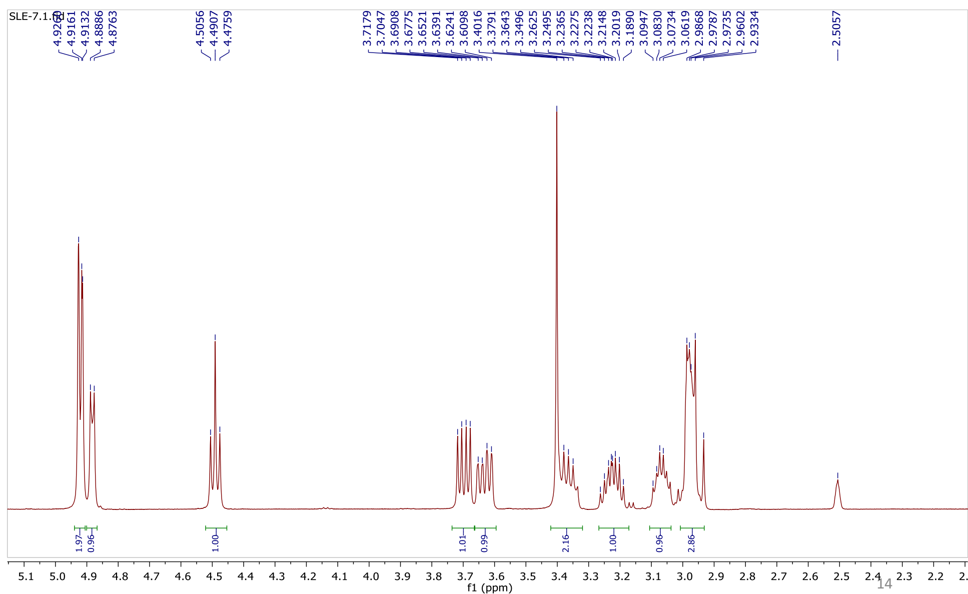
SLE-7

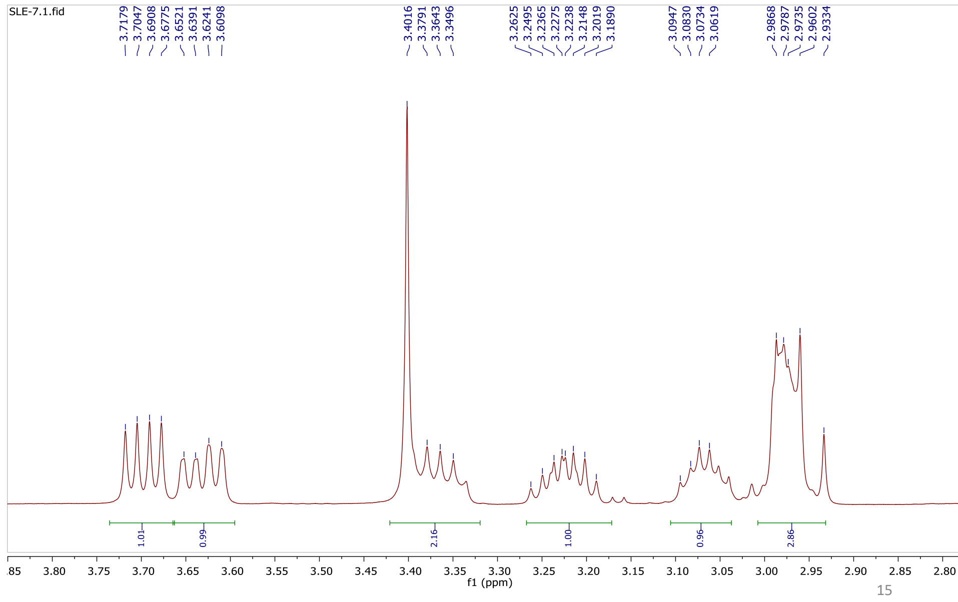
^{1}H-NMR



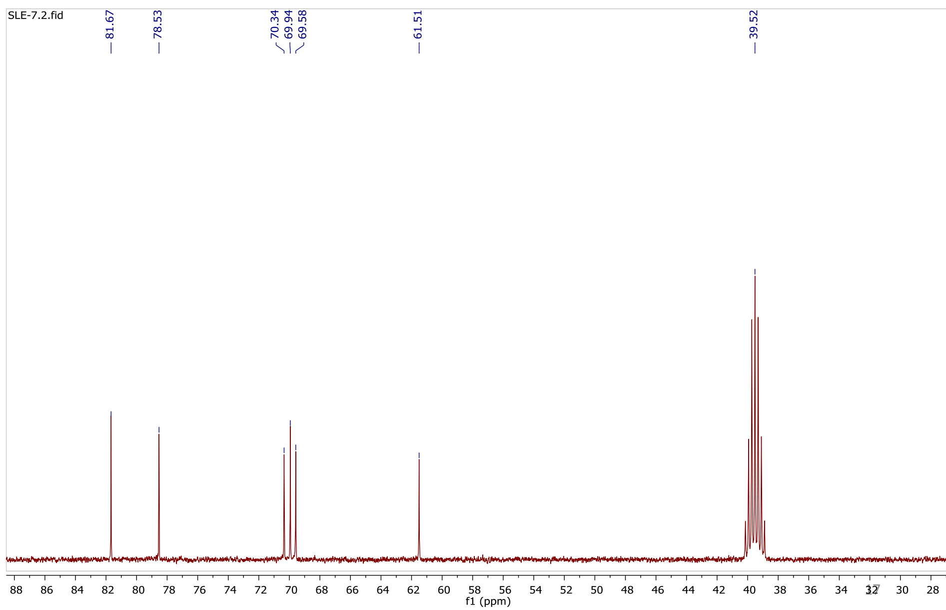
SLE-7

^{1}H-NMR

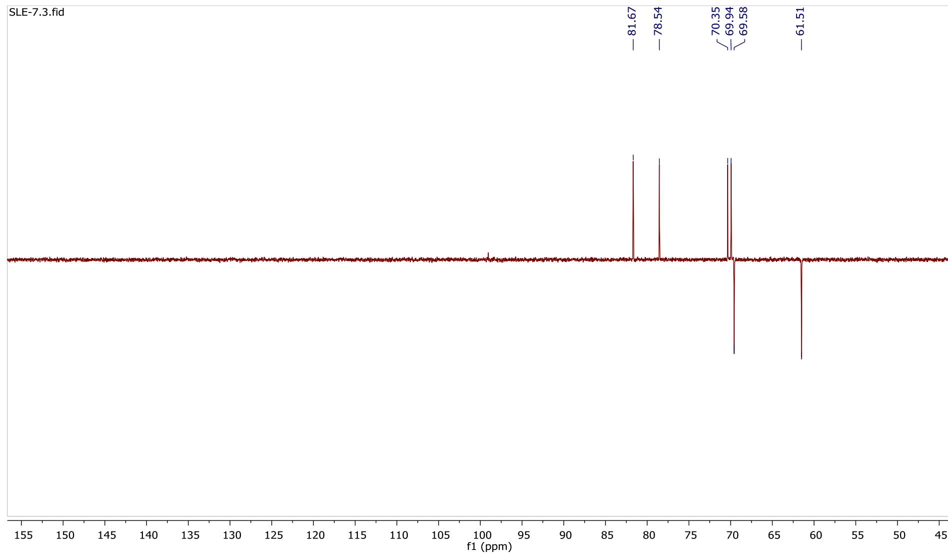




Appendix 8: SLE-7 ^{13}C-NMR



Appendix 9:SLE-7 DEPT-135



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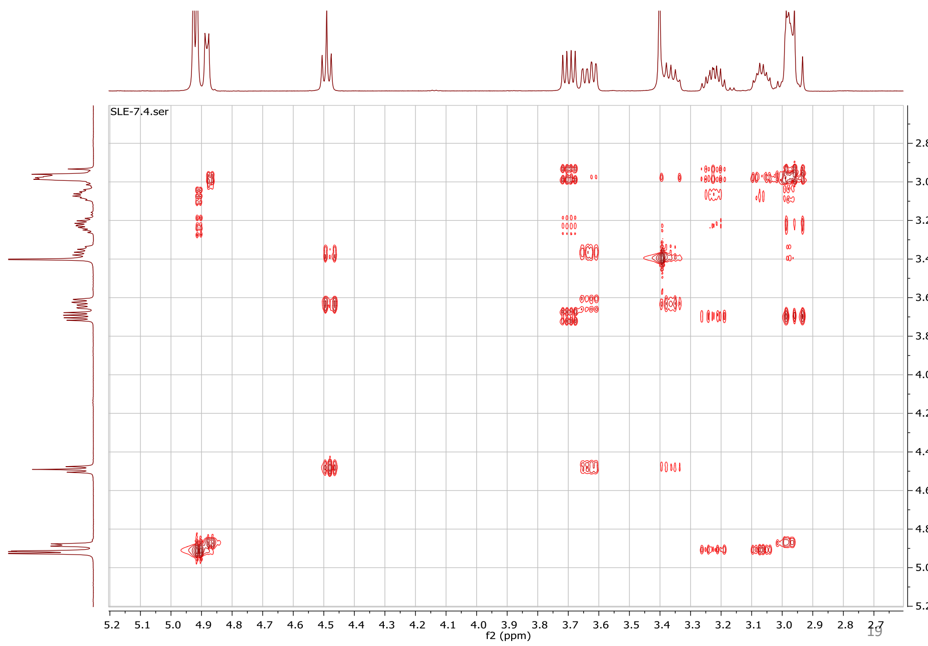
Appendix 10: SLE-7 COSY



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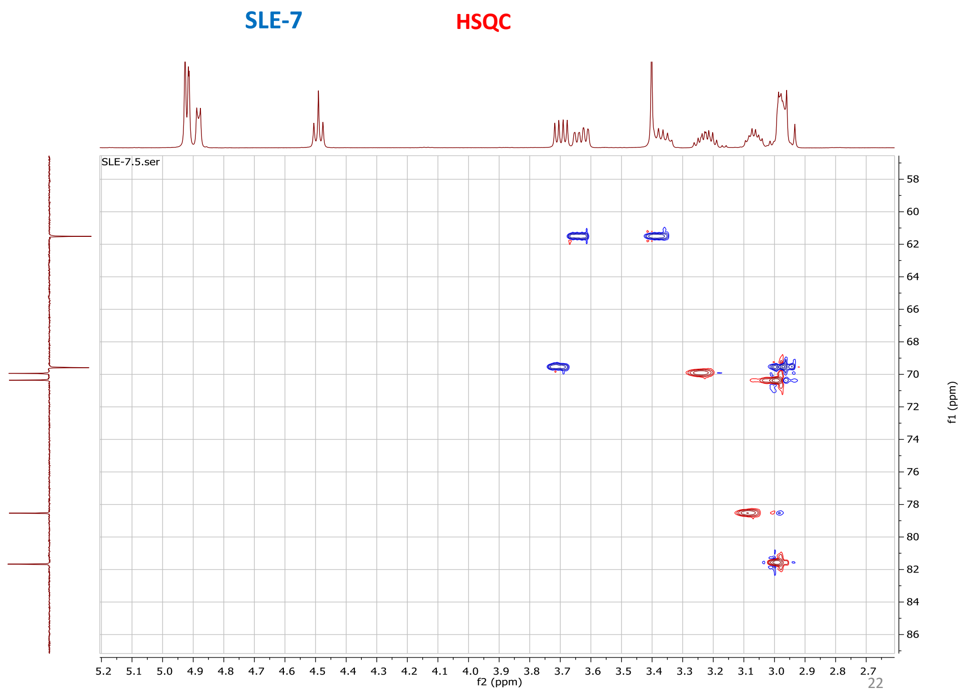
Appendix 11: SLE-7 HSQC



Appendix 12: SLE- HMBC