**ADDIS ABABA UNIVERSITY**

**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE**

**DEPARTMENT OF CHEMISTRY**

MSc Thesis

# Phytochemical Investigation of the Stem Bark of *Securida longipedunculata*

By: Mamite Atlaw G/Tsadik (ID.NO GSK/0588/07)

Advisor: Dr Kibrom G/Hiwot (PhD)

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**ADDIS ABABA UNIVERSITY**

**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES**

This is to certify that the thesis prepared by Mamite entitled: Phytochemical Investigation of the Stem Bark 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.

Approved by the examining board:

| Name | Signature | Date |
|---|---|---|
| 1. Dr Kibrom G/Hiwot (Advisor) | _____ | _____ |
| 2. Dr Mekonnen Abebayeh (Examiner) | _____ | _____ |
| 3.Dr Weldegebriel Yohannes (Examiner) | _____ | _____ |

# Declaration

I declare that this thesis, entitled Phytochemical Investigation of the Stem Bark of *Securidaca Longipedunculata*, is my original work under the supervision of Dr Kibrom G/Hiwot (PhD), 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.

Name: Mamite Atlaw

Signature: _____

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

Advisor: Dr Kibrom G/Hiwot (PhD)

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## Acronyms

| | |
|---|---|
| $^{13}$C-NMR | Carbon Nuclear Magnetic resonance |
| CC | Column Chromatography |
| DMSO | Dimethyl Sulfoxide |
| DEPT | Distortion less Enhancement by Polarization Transfer |
| EA | Ethyl Acetate |
| HMBC | Hetronuclear Multiple Bond Correlation |
| HSQC | Hero-nuclear single quantum correlation |
| HPLC | High performance liquid chromatography |
| NMR | Nuclear Magnetic Resonance |
| Ppm | parts per million |
| $^{1}$H-NMR | Proton nuclear Magnetic resonance |
| Rf | Retention Factor |
| S. longipedunculata | *Securidaca longipedunculata* |
| TLC | Thin layer chromatography |
| WHO | World health organization |

## Abstract

Plants traditionally used in Ethiopian medicine for various ailments including venereal diseases, stomachache**,** dislocated jaw, headaches, skin cancer, skin infections, contraceptive purposes. *Securidaca longipedunculata* is an important plant species with potential benefits in the treatment of various ailments. In this study, two compounds were obtained (**SLM**-1 and **SLM-2**) from the stem bark of *Securidaca longipedunculata* by using solvent extraction and column isolation. The solvents were ethyl acetate, cyclohexane, methanol, and chloroform. The compounds that were obtained from stem bark of *Securidaca longipedunculata* SLM-1 is a group of galotannins, which were 1,5-anhydro-D-glucitol and **SLM-2** is ketone group which were 2,3-dimethoxy-4-hydroxy benzophenone .From this, 1,5-Anhydro-D-glucitol was the major one, which accounts for 3.46%; on the other hand, 2,3-dimethoxy-4-hydroxy benzophenone accounts for a for a small amount, which was (0.027%) from the plant material. The isolated compounds were characterized by the spectroscopic techniques, which are $^{1}$H NMR, $^{13}$C NMR, DEPT-135, HMBC and HSQC.

# 1. Introduction

## 1.1. Background

Plants are majorly used as herbal medicines, besides providing ecological, economic, and cultural services. The plant parts used for herbal medicine are roots, stems, leaves, flowers, fruits or seeds, stems, barks and rhizomes. [1] They contain biologically active phytochemicals in the plant tissue considered as primary and secondary metabolites. Primary metabolites are organic compounds that contain glucose, starch, polysaccharides, proteins, lipids, and nucleic acids. Secondary metabolites are small molecules involved in plant defense mechanisms, including alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc. [2]

Globally, about 64% of the total world population is depending on traditional medicine for their primary healthcare needs. [3] Around 3.5 billion people in developing countries, including Ethiopia, believe in the efficiency of plant remedies and use them regularly, as stated by the World Health Organization (WHO). [4] Ethiopian's plants have shown very effective medicinal value for some ailments of human “human and livestock health care needs throughout the country. [5] The use of medicinal plants in the treatment of diseases in Africa is an ancient tradition that has co-existed with human habitation. [6] Medicinal plants contain a wide range of bioactive compounds that have biological activity, such as anticancer, antimicrobial, antioxidant, anti-diarrheal, analgesic, wound healing, etc., and can also be used to treat chronic as well as infectious diseases. [7]

To promote the use of medicinal plants as potential sources of bioactive compounds, it is important to find out their phytochemicals thoroughly by isolation, analysis and evaluation of their compounds. [8] Various extraction methods are used to extract the desired bioactive compounds from the plant materials, e.g., solvent extraction, distillation method, and sublimation. Solvent extraction is the most widely used extraction method when extracting plant material. And also chromatography techniques are used to separate molecules based on their size, shape, and charge. The techniques are paper chromatography, tine layer chromatography, and column chromatography. [9]

The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as High Pressure Liquid chromatography (HPLC), Column Chromatography (CC) and, Tin layer Chromatography (TLC) as well as non-chromatographic techniques such as immunoassay and Fourier Transform Infra-Red (FTIR) .[10]

The aim of this study was to investigate phytochemicals from the stem Bark of *Securidaca Longipedunculata*.

### 1.2. Statement of the problem

Many cultures in Ethiopia primarily use medicinal plants in different ways, like fumigation, for religious celebrations and ceremonies, air purification and cosmetics special women's for treatments of skin care. In a traditional way, these plants are used for the treatment of different diseases and also for pharmacological purposes. Despite the potential medicinal properties attributed to *S. longipedunculata*, there is a lack of comprehensive phytochemical studies on its stem bark. This hinders the identification and understanding of the bioactive compounds present in the plant, which could have significant implications for the development of new pharmaceuticals. In Ethiopia, there is no enough literature information regarding the chemical composition of the Stem bark of *S.longipedunculata*. Therefore, there is a need to conduct a thorough phytochemical investigation of the stem bark of *S. longipedunculata* to elucidate its chemical composition and potential bioactive properties. This study focuses on the isolation of chemical compounds and characterization of that isolated compounds.

### 1.3. Significance of the study

The Stem bark of *S.longipedunculata* is a medicinal plant used traditionally for treating different diseases in different parts of Ethiopia and for pharmacological purposes. This research finding will give information about the chemical composition of the stem bark of the plant that are used by humans.

## 1.4. Objectives of the study

### 1.4.1. General objective

The main objective of this study is to isolate, and characterize the phytochemical constituents from the Stem Bark of *S. longipedunculata* by using TLC, Column chromatography and NMR spectroscopy.

### 1.4.2. Specific objectives

- To extract compounds from stem bark of *S. longipedunculata*
- To isolate the specific phytochemical constituents present in the stem bark *S. longipedunculata*
- To identify and characterize the chemical structure of the isolated compounds from the Stem Bark of *S. longipedunculata*.

## 2. Literature review

### 2.1. Origen of *Securidaca longipedunculata*

*Securidaca longipedunculata* is a plant species belonging to the genus *Securidaca* of the family *Polygalaceae* commonly known by its local names as 'Etse-manahi' (Amharic) in Ethiopia. [11] The genus *Securidaca* comprises about 80 species. The species is threatened by various anthropogenic and environmental conditions including seasonal fires, droughts, and debarking. [12] The plant is widely distributed in tropical and subtropical areas of Africa, this include Angola, Benin, Botswana, Cameroon, Chad, Eritrea, Ethiopia, Gambia, Ghana, Kenya, Malawi, Namibia, Niger, Nigeria, Rwanda, Senegal, South Africa, Sudan, Tanzania, Uganda, Zambia etc. In Ethiopia it grows at altitude between 0 to 1800m, in arid and semi-arid zones of Tigray region (Tekeze River), Amhara region (Quara), Oromia region (Gibe River) and Abay Bereha .[13]

It is a small tree with a pale grey, smooth bark and hairless alternate leaves which are variable in size and shape. Its flowers are small, pink or purple in color, sweet scented and are usually produced in early summer, while its fruits are heavily veined, smooth, oblong and purplish-green when young. [14] Its stem bark and roots are still found amongst the most traded medicinal plants in Africa. The species is threatened by various anthropogenic and environmental conditions including seasonal fires, droughts, and debarking. [15]

### 2.2. The Chemistry of *Securidaca longipedunculata*

Qualitative Phytochemical screening of the Aqueous and Methanol extracts of *S. longipedunculata* Root bark, alkaloid, flavonoid, saponins, cardiac glycoside, tannins, steroid, triterpenes, phenol, anthraquinones, and carbohydrates. [16]

From the methanol and acetone extract of the root bark of *S. longipedunculata* three compounds **1 - 3** (Fig. 1), 4-Hydroxybenzoic acid (**1**)**,** vanillic acid (**2**) and 2-hydroxy-5,6-dimethoxy-9H-xanthen-9-one (**3**) were reported. [17]

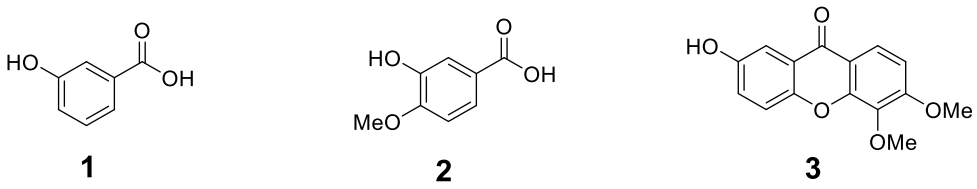



Figure 1: Solvent extracted compounds

Phytochemical investigation of the stem bark of *S. longipedunculata* by LCMS/MS Analysis reported the identification of compound **4 - 11**: 2-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]benzene-1,4-diol **(4),** 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1- 4-one **(5),** 4-hydroxy-3,4-dihydro-1H-isoquinoline-2-carboximidamide **(6),** 5,7-dihydroxy benzopyran -6-methyl-3-(2,4-dihydroxybenzyl)-chroman-4-one **(7),** 5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)-chroman-4-one **(8),** 3-(4-hydroxybenzyl)-5,7-dihydroxy-6-methylchroman-4-one **(9),** methyl 2-((2R,3S,4S,5R,6S)-6-(2-(hydroxymethyl)phenoxy)-tetrahydro-3,4,5-trihydroxy-2H-pyran-2-yl) benzoate **(10),** (R)-3,4-dihydro-1-isopropyl-6-methoxy-4,4,7-trimethylnaphthalen-2 (1H)-one **(11)** [18] .

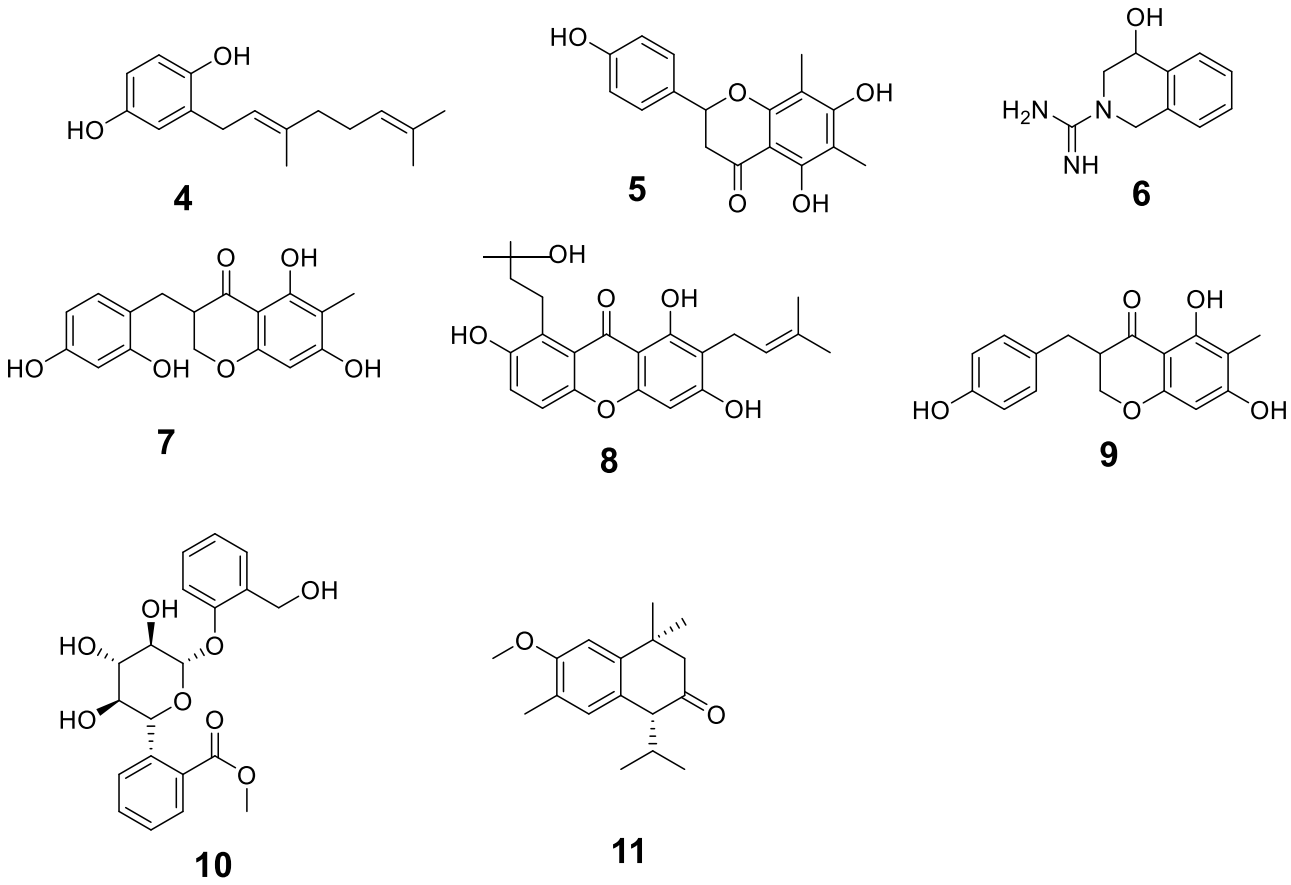



**Figure 2: Structures of the compounds** 4 – 11

### 2.3. Biological activity of *S. longipedunclata*

*S. longipedunculata* is known with its vernacular name "king of medicines" as it is used for almost every conceivable ailment. It is commonly visited by traditional healers for the treatment of sexually transmitted disease, fungal infection, diabetes, and wounds in Ethiopia. [19] The root of *S. longipedunculata* is traditionally used to manage fungal infections, fever, malaria, gonorrhea, headaches, cancer, rheumatism, tuberculosis, diabetes, venereal diseases, syphilis, sexual impotence, toothache, pains, epilepsy, convulsions, constipation, pneumonia, backache, blood purification, sexually transmitted infections, and skin infections .[20]

In Nigeria, the leaves of this plant used for dislocated jaw, headaches, skin cancer, skin infections, contraceptive purposes .[21] And also traditionally, the root and bark are taken orally either powdered or as infusion for abortion, infertility, venereal diseases, headache among other diseases .[22] The powdered stem bark is also mixed with hot water and taken orally to treat syphilis and gonorrhea .[23]

## 2.4. Extraction method

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further isolation and characterization .[24] For this work methanol –chloroform solvent extraction method were used in the first step .

## 2.5. **Types of chromatography**

### 2.5.1. **Thin Layer Chromatography**

There are different types of chromatography such as Thin layer chromatography, Column chromatography, Paper chromatography etc. among them Thin layer chromatography (TLC) is a broadly used for laboratory technique. Compare to other chromatographic methods, TLC takes advantage of the different affinity of the analyte with the mobile and stationary phases to achieve the separation of complex mixtures of organic molecules. [25]

Principle of thin layer chromatography (TLC) is performed on a sheet of glass, aluminum foil, plastic, or, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. The components with more affinity towards stationary phase travels slower and components with less affinity towards stationary phase travel faster. [26]

### 2.5.2. Column Chromatograph

Both solid and liquid samples can be separated and purified by column chromatography. Column chromatography consists of a stationary solid phase (such as silica, alumina, calcium phosphate, calcium carbonate, starch, and magnesia, and different solvent compositions based on the nature of compounds to be separated and isolated) that adsorbs and separates the compounds passing through it with the help of a liquid mobile phase. On the basis of their chemical nature, compounds get adsorbed and elution is based on differential adsorption of a substance by the adsorbent. [27]

## 3. Experimental

### 3.1. Plant materials, chemicals and reagents

#### 3.1.1. Plant material collection

The stem bark of *Securidaca longipedunculata* used in this study was collected from Debire Libanose Monastery about 132 Km away from Addis Ababa, the capital city of Ethiopia, on August 30, 2014 E.C. The plant materials were washed with tap water in the organic Lab section in Addis Ababa University. The stem bark was air dried for four weeks (one month) and then ground into a fine powder using a high-speed multifunctional grinder. The powder form was prepared for solvent extraction.

Figure 3 :Securidaca longipedunculata

#### 3.1.2. Materials and Apparatus

In this study high-speed multifunctional grinder, mortar and pestle were used for grinding and homogenizing the samples, digital balance, measuring cylinder were used for measuring sample and solvents respectively, test tube for collection of sample from the column, capillary tube for transferring very small sample in to TLC plat, spatula to transfer powdered sample, beaker to hold solvent, what man

number one filter paper to filter the crud extract. TLC plate (pre-coated aluminum sheet 20 x 20 silica gel 60 F254) to detect the presence of spot on it, column chromatograph to isolate compounds based on its Polarity, UV chamber to identify the spot which appear on TLC, oven for drying the sample material .The rotary evaporator was used for remove volatile solvent and concentrating the sample.

### 3.1.3. Chemicals and reagents

The chemicals used to conduct this experiment was, chloroform (500 mL), methanol (500 mL) for socking and extracting ,hexane, silica gel, acetone, sulfuric acid and ethyl acetate, DMSO (dimethyl sulfoxide), were used.

### 3.1.4. Instrumentation

NMR instrumentation, all the proton NMR (1 H NMR) experiments were performed on a BRUKER ACQ 400 AVANCE spectrometer operating at 400 MHz equipped with a5 mm proton probe and running topspin 2.1 software at 298K on 600 µL samples.The spectra were recorded in a neutral deuterium oxide (D2O) media, with all chemical shifts (δppm) recognized relative to an internal TMS reference. Typical acquisition parameters for 1 H NMR experiments were: acquisition time (320 s), spectral width (8278.146 Hz), and number of scans (64).The spectral data processing used for the post acquisition included Fourier Transformation (FT) of the Free Induction Decay (FID) data using MestRe-C software, phase correction (performed using automatic phase correction button after FT) and baseline correction for the entire spectral range. In all instances, the baseline was additionally corrected over the integral regions. Areas of peaks were determined by electronic integration of expanded regions of selected resonances.

### 3.2. Sample preparation

The dried plant material was ground using high-speed multifunctional grinder. 150 g of powder were soaked for 24 hour in an equal ratio of chloroform (500 mL) and methanol (500 mL) (1:1) at room temperature. The crude extract was filtered with what man number one filter paper, the solid part was discarded and then the filtrate obtained was partially concentrated using a rotary evaporator at 50℃, in this case white solid material was appeared before completely concentrated by rotary evaporator .The solid white crystal was separated from the filtrate by decantation (as shown in Figure 4). After that, the white material was washed with chloroform and stored in the beaker until it is analyzed by NMR for

further characterization. This compound was the first compound and was labeled as **SLM-1**and stored in vial at room temperature until it was characterized by NMR. The remaining filtrate was adsorbed by 30 g of silica gel. It was allowed to dry in a rotary evaporator, and re-dried in an oven at 50 ℃ for 5 minutes and converted into powder form by using a mortar and pestle (as shown in the figure 5) and then the powder was weighed at 52.76 g (52.76 - 30 g), from which 22.76 g sample were obtained.


Figure 4: First compound

Figure 5: Sample with silica gel

### 3.3. Column Chromatography

#### 3.3.1. Isolation of Compounds from *Securidaca longipedunculata*

Column chromatography was performed on silica gel, to isolate the active compound from the crude extracts. The crude extract (22.76 g) was chromatographed by applying on top of column chromatography packed with silica gel (132 g). Elution was carried out using cyclohexane: ethyl acetate (95:5 ⟶ 50:50, v/v) from fraction one up to fraction five, Ethyl acetate (100:0, v/v) for fraction six and Ethyl acetate: methanol (90:10,v/v) for fraction seven and eight, a total of eight fractions were collected. Based on the TLC profile fraction 1-5 had no spot. Only fraction six was showed spot on TLC ,then dried with rotary evaporator and obtain 0.2 gram sample ,this also further investigations were done on column chromatography elution was carried out using cyclohexane: ethyl acetate (80:20, v/v) (1-12) fractions were collected and examined on the TLC using cyclohexane: ethyl acetate(1:0.5) each had two different Rf value spots. Based on the these TLC profile (1-12 fractions) added together and dried by rotary evaporator and obtain 0.16gm sample and examined further by column chromatography (28:72, v/v) Ethyl acetate with cyclohexane as eluent then collected 1-20 fractions from these only fraction 13 and 14 had Rf value the same then collected to gather leveled as ‘’8’’ and checked by TLC that shows only one pure blue black spot . These sample was the second sample dried and labeled as **SLM-2** and stored in vial at room temperature until it was characterized by NMR. (As shown the figure 7).

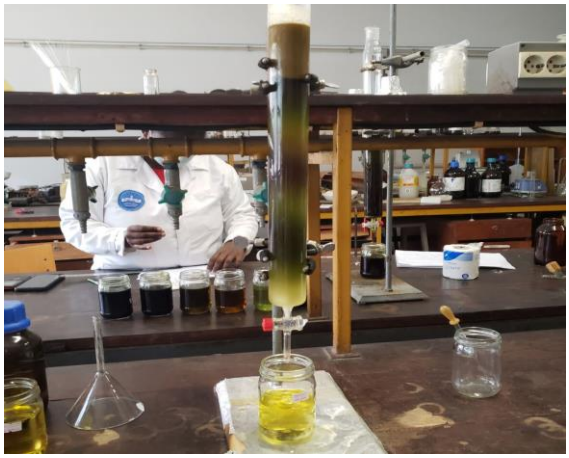
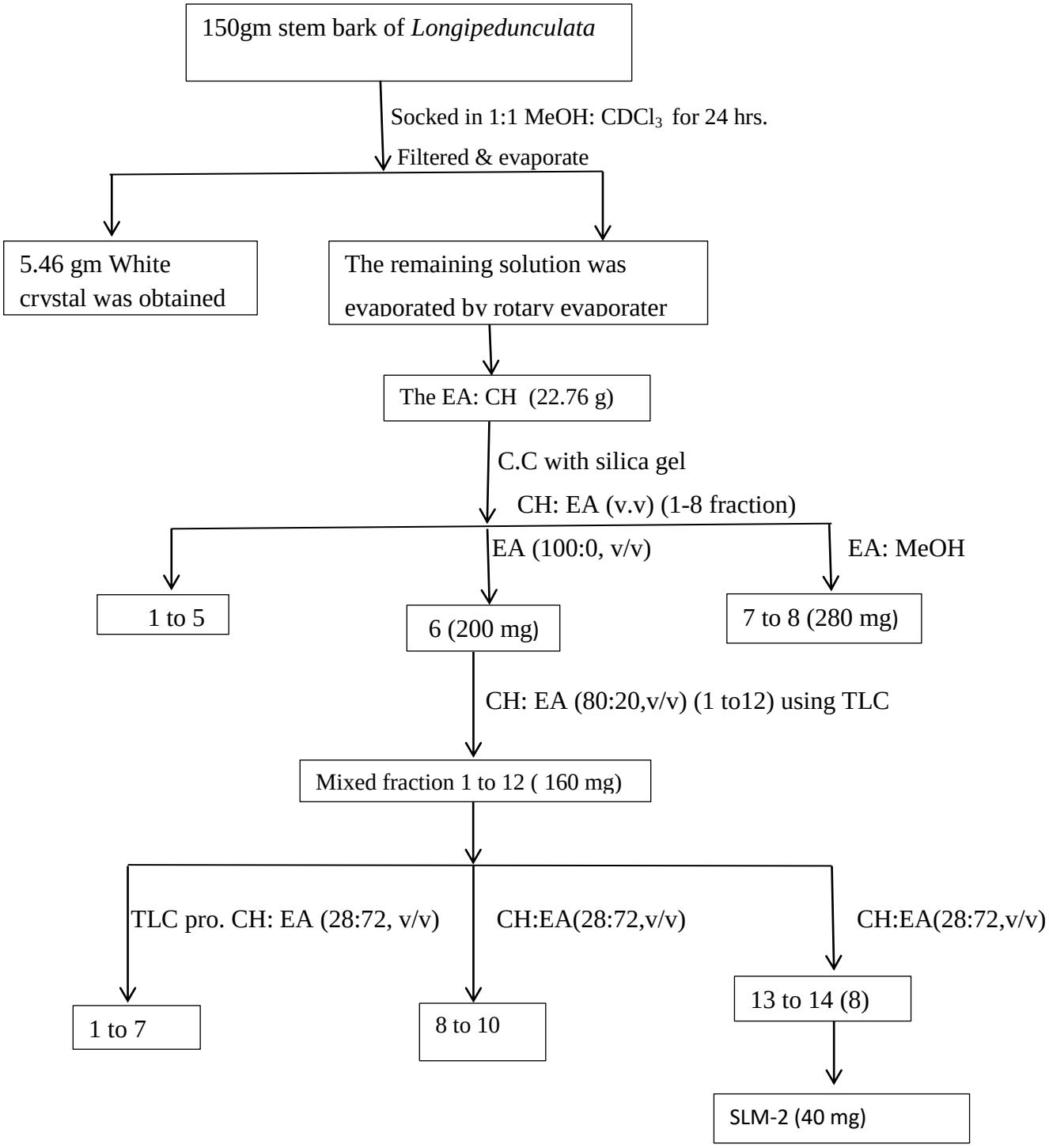


Figure 6: Sample separation in Column chromatography




Scheme for the procedure of isolation of compound from stem bark of *S. Longipedunculata*

### 3.3.2. Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was carried out on acid-washed silica gel with 254 nm fluorescent indicator (Sigma-Aldrich, Germany) pre-coated on aluminum (20 × 20 cm). Thin-layer chromatography (TLC) was performed with Merck Kieselgel 60F254 pre-coated plates which were visualized under UV light. Glass jars were used by the solvent system cyclohexane: ethyl acetate (1:0.5, v/v). The silica gel sheet allowed to dry and then developed in an ascending order for few minutes until solvent front about 4 cm lengths. Produced spots were located by their fluorescence under short and long wave UV light (254 and 365 nm respectively).

Briefly, the solvent front was marked on the TLC plates immediately after removing it from the chamber, and insert into vanillin then allowed to dry before visualizing the bands relating to different compounds. The $R_f$ value was calculated by the following equation:

$$Rf = \frac{distance\ moved\ by\ the\ component\ from\ the\ origin\ to\ spot\ center}{distance\ moved\ from\ origin\ to\ solvent\ front}$$

$$Rf = \frac{2cm}{3.5cm}\ by\ CH1{:}\ EtoAc\ \frac{1}{2} = 0.57$$

From fraction 13 and 14 were combined together and condacted on TLC(as shown the figure 7) it had one spot on TLC that showes pure compound and the Rf valuewas 0.57 . And also concentrated by rotary evaporator then collected by vial and labeled as second compound SLM-2. It was dissolved by solvent DMSO, and analysis by NMR spectrometer.

Figure 7: TLC profile of fractions of CH:EA extract of the Stem Bark of *S. longipedunculata*

# 4. Result and Discussion

## 4.1. Extraction Yield

Extraction yield is the ratio of mass of crude extract to mass of drained sample multiplied by 100.

$$\%\text{yield} = \frac{\text{weight of crude extract (g)}}{\text{weight of draied sample(g)}} \times 100 \ .$$

The percentage yield of compound **1** was:

$$\frac{5.46\ g}{150\ g} \times 100 = \underline{3.64\%}$$

The percentage yield of compound **2** was:

$$\frac{0.04\ g}{150\ g} \times 100 = \underline{0.027\%}$$

## 4.2. Structural elucidation of the Isolated Compounds

### 4.2.1. Characterization of Compound 1

Compound **1** was isolated as a white crystal with Rf value 0.65 in EA:MeOH (1:0.5) .$^{1}$H NMR (400 MHz, DMSO) spectrum (Table 1 and Appendix 1) revealed the presence of 4 methylene signals, which are appeared at δ 3.36 (*t, J*=11.6,5.7Hz,1H-1), δ 3.63 (*dd, J*=11.5,6.2Hz,1H-1), δ 2.95 (m, 1H ,H-6 ), δ 3.70 (*dd*, *J*=10.95,5.3,1H ,H-6); four methine proton signals at δ 2.99 *(m*, 1H, H-2), 2.98 (*m*,1H, H-3), δ 3.07 (*dq*, *J* = 8.7,4.6 Hz, H-4) and 3.22 (*m*,1H,H-5). In HMBC spectrum, none carbon attached protons were detected at δ 4.93 (*t, J*=4.7Hz,2H), δ 4.90 (*d, J*=4.7Hz,1H), δ 4.52 (*t, J*=5.9Hz,1H).

$^{13}$C-NMR(101 MHz, DMSO) (Tables 1 and Appendix 2) and DEPT-135 spectra (Table 1 and Appendix 3) showed the presence of 6 carbon signals which were attributed to two methylene at δ (61.6 ) C-1 and δ (69.6) C-6 ; the carbon signal at δ(81.7) C-2, δ (70.4) C-3, δ (78.6) C-4 and δ (70.0 ) C-5, showed methines and oxymethine carbons signals. The COSY spectrum (Appendix 3) showed at δ 3.63 correlated

with a proton signal at δ 3.36 (H-1) and δ 3.36 (H-1) correlated with δ 3.70 (H-6).The proton signal at δ2.98 (H-2) correlated with δ3.22 (H-5) δ 3.70 ( H-6), δ 3.07) (H-4). The proton signal at δ 2.98 (H-3) correlated with δ 3.22(H-5), δ 3.69 (H-6), δ 3.07 (H-4) . The proton signal at δ 3.07 (H-4) correlated with δ2.95 (H-6), δ 3.22) (H-5). The proton signal at δ 3.22(H-5) correlated with δ3.07 (H-4), δ 2.95(H-6) The proton signal at *δ* 3.69 (H-6) correlated with δ 2.95 (H-6), δ 3.22) (H-5).

HSQC spectrum (Appendix 4) showed an oxmethylene proton signal at δ 3.36 ( *dt, J* = 11.6, 5.7 Hz, 1H) and 3.63 (*dd, J* = 11.5, 6.2 Hz, 1H), correlated with a carbon signal at δ 61.6 (C-1). The signal at δ 2.99 (*m,* 1H) correlated with at carbon signal δ 81.7 (C-2), δ 2.98 (*m,* 1H) correlated with at carbon signal δ 70.4 (C-3) .The signal at δ 3.07 (*dq, J* = 8.7, 4.6 Hz, 1H), correlated with at carbon signal δ78.6 (C-4) .The signal at δ 3.22 (*m,* 1H) correlated with at carbon signal *δ* 90.0 (C-5). The signal at δ 2.95 (*m,* 1H) and 3.70 *(dd, J*=10.9, 5.3 Hz, 1H) correlated with carbon signal at *δ* 69.6 (C-6).

The HMBC Spectrum (Appendix 5) of compound **1** showed that the oxymethylene proton at *δ* 4.52 , *δ* 2.98 correlated with methylene carbons at δ 61.6 (C-1) ; the proton signal at δ 2.98, δ3.70, δ3.37, 4.52 ,4.93 correlated with the carbon signal at δ 81.7(C-2), δ 4.92, δ 3.70, δ 3.22, correlated with carbon signal at δ 69.6 (C-6). δ 4.93, *δ* 3.70, *δ* 3.22, *δ* 3.22, 2.98) correlated with the carbon signal at δ 70.3 (C-5). δ3.22, δ 4.93, δ2.98, 3.70 correlated with the carbon signal at δ 78.6 (C-4). δ 4.92, δ3.22, δ2.98) correlated with the carbon signal at δ 78.85 (C-3). The above explanation was illustrated in the following tables.

**Table 1:**DEPT ,$^{1}$H and $^{13}$C-NMR data for compound 1

| | Experimental | | | Literature .[29] | | |
|---|---|---|---|---|---|---|
| Position | DEPT-135 | $^{1}$H-NMR(400MHz,DMSO),$^{13}$C-NMR(400MHz,DMSO) | | DEPT-135 | $^{13}$C NMR($CD_3OD$,75MHz) $^{1}$H-NMR($CD_3$ OD 300MHz) | |
| | | $\delta c$ | $\delta_H$ | | $\delta_C$ | $\delta_H$ |
| 1 | $CH_2$ | 61.6 | 3.36 (*t*,*J*=11.6, 5.7Hz, 1H), 3.63 (*dd*, *J*=11.5,6.2Hz,1H) | CH2 | 62.7 | 3.47 (*m*,1H) 4.27 (*dd*,1H,12.1,5.5) |
| 2 | CH | 81.7 | 2.97 (*m*,1H) | | 80.9 | 3.28 ( *m*,1H) |
| 3 | CH | 70.4 | 2.98 (*m*,1H) | | 72.8 | 3.25(*m*,1H) |
| 4 | CH | 78.6 | 3.07 (*dq*, J=8.7,4.6 Hz,1H) | | 77,7 | 3.30(*m*,1H) |
| 5 | CH | 70.3 | 3.22 (*m*, 1H ) | | 71.6 | 3.4(*m*,1H) |
| 6 | $CH_2$ | 69.6 | 2.95 (m,1H) 3.70 (*dd*, *J*=10.95,5.3,1H) | CH2 | 71.0 | 3.16(*t*,1H,10.3,11.0) 4.47(*dd*,1H,12.1,1.8) |

**Table 2**: COSY $^{1}$H -$^{1}$H correlations of compound 1

| | |
|---|---|
| C-1 ( δ 61.6) | H-1 (3.63) ↔H-1(3.36) |
| C-3 (δ70.4) | H-3 (2.98)↔ H-5 (3.22) |
| C-4 ( δ 78.6) | H-4 (3.07)↔H-3 (2.98) |
| C-5 (δ 70.3) | H-5 (3.22) ↔ H-6 (2.95) |
| C-5 (δ70.3) | H-5 (3.22) ↔ H-6 (3.70) |
| C-6 (δ 70.0) | H-6 (2.95)↔ H-6 (3.70) |

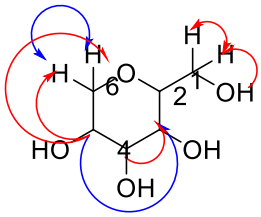



Figure 8 : COSY Relations on 1,5 anhydro-D-Glucitol

**Table 3**: HSQC correlation of compound 1

| Carbon No | Hydrogen number |
|---|---|
| C-1(δ 61.6*)* | *δ* 3.36 (*t, J*=11.6, 5.7Hz, 1H),<br>δ 3.63 (*dd, J*=11.5, 6.2Hz, 1H) |
| C-2 (δ 81.7) | δ 2.97 (*m*, 1H) |
| C-3 (δ 70.4*)* | δ 2.98(*m*, 1H) |
| C-4 (δ 78.6*)* | δ 3.07(*m*, 1H) |
| C-5 (δ 70.3) | δ 3.22 (*m*, 1H) |
| C-6 (δ 70.0) | δ 2.95 (*m*, 1H),<br>δ 3.69(*dd, J*=10.95,5.3,1H) |

**Table 4: The HMBC correlation of compound 1**

| Proton No | Proton with carbon correlation |
|---|---|
| H-1(δ4.52),H-3 (δ2.98) | H-1,H-3↔ C-1 (δ 61.56) |
| H-1 (δ3.36,4,52), H-6 (δ3.7),<br>H-3 (δ 2.98) | H-1,H-3,H-6↔C-2 (δ 81.670 |
| H-2 ( δ2.98), H-5 (δ3.22), H-4 (δ3.07) | H-2,H-5,H-4↔ C-3(δ 78.55) |
| H-5 (3.22), H-3 (2.98) | H-3, H-5↔ C-4(δ70.40) |
| H-6 ( δ 3.7), H-3 (δ2.98) | H-6,H-3↔ C-5(δ 69.98) |
| H-5 (δ 3.22), H-6 (3.7) | H-6,H-5↔ C-6(δ 69.61) |

Based on the above information of hydrogen and carbon NMR, COSY,DEPT and HSQC. I concluded that the chemical formula of the first compound was C6H12O5 and its expected structure were given below in figure 8 . And its structure name also could be 1-5 an hydro -D-glucitol.

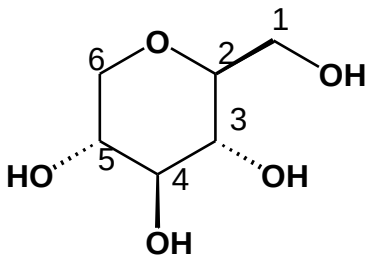



Figure 9: Structure of compound 1

### 4.3. Structural elucidation and characterization of Compound 2

#### 4.3.1. Characterization of Compound 2

Compound 2 was isolated as a yellowish powder. The compound gave a dark blue spots on TLC with vanillin spraying with the $R_f$ value of 0.57 in CH:EA (1:0.5). $^1$H NMR (400 MHz, $CDCl_3$) spectrum (Table 2 and Appendix 6) revealed the presence an AB aromatic system at δ 7.83 ( *m,* H-2'/6',2H ), $\delta_H$ 7.58 (*d, J* = 7.4 Hz, 1H ) , and δ 7.47 (*t, J* = 7.7 Hz, H-3'/5', 2H) as a 1-substituted benzene ring and δ7.11(*d, J*= 8.5 Hz, 1H) (H-6) and δ 6.79 (*d, J* = 8.5 Hz, 1H) (H-5) , as a 1,2,3,4-tetrasubstituted benzene ring with 2 methoxy at δ 3.99 (3H, H-OCH3 at C-3) and δ 3.76 (3H, H-OCH3 at C-2).

The $^{13}$C NMR (101 MHz, $CDCl_3$) spectrum (Table 2 Appendix 7 ) showed a characteristic non-chelated ketone carbon at δ 195.3 (C-7), 6 quaternary carbons at δ 152.4 (C-4), δ 152.0 (C-2), δ 139.5 (C-3), $\delta_C$138.2 (C-1'), δ 125.4 (C-1), δ 195.1 (C-7); 5 methine carbons at δ 132.8 (C-4'), δ 126.0 (C-6), δ 110.0 (C-5) including 2 overlapping signals at δ 129.8 (C-2'/6'), δ 128.2 (C-3'/5'), and 2 methoxy signals at δ 61.1and δ 61.5 .This spectroscopic data implied that the compound was a trioxygenated benzophenone with a 1-substituted benzene ring and a 1,2,3,4-tetrasubstituted benzene ring. An olefin methine signal at δ7.12 (H-6) showed cross peaks with a non-chelated ketone signal at δ 195.3 (C-7) and 2 olefin quaternary carbon signals at δ 151.9 (C-2) and $\delta_C$ 152.4 (C-4). Another olefin methine signal at δ 6.70 (H-5) showed cross peaks with 2 olefin quaternary carbon signals at δ 125.6 (C-1) and δ 139.3 (C- 3). Two methoxy protons at δ 3.99 and δ 3.76 showed correlations with 2 olefin quaternary carbons at $\delta_C$ 139.5 and δ 152.0, respectively. The former correlation indicated 1 methoxy was at C-3 and the latter correlation indicated another methoxy was at C-2 or C-4. *J*2 correlation was observed only between H-5 and an olefin quaternary carbon at δ 152.4 (C-4), which showed no correlation with any methoxy proton, leading to the conclusion that another methoxy was at C-2. Thus, the compound was identified as 2,3-dimethoxy-4-hydroxybenzophenone. The 2,3-dimethoxy-4-hydroxybenzophenone was reported to have been previously isolated compound .[30]

Table 5: $^{1}H$ (400 MHz, CDCl3) & $^{13}C$ NMR (101 MHz, $CDCl_3$)

| Carbon no. | From *securdaca longipedunculata* | | Literature.[30] From *lindera fruticosa* | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 125.6 | | 124.0 | |
| 2 | 152.0 | | 152.5 | |
| 3 | 139.5 | | 140.4 | |
| 4 | 152.4 | | 154.4 | |
| 5 | 110.0 | 6.79 *(*1H,*d*,*J*=8.5Hz) | 110.1 | 6.74 (1H, *d*, *J* = 8.4 Hz) |
| 6 | 126.0 | 7.11 *(*1H,*d*,*J*=8.5Hz*)* | 125.8 | 7.06 *(*1H, *d*, *J = 8.4 Hz)* |
| 7 | 195.1 | | 195.2 | |
| 1' | 138.3 | | 138.2 | |
| 2'/6' | 129.8 | 7.83 *(m*, 2H*)* | 129.7 | 7.78 *(*1H, *dd*, *J* = 8.4, 2.0 Hz*)* |
| 3'/5' | 128.2 | 7.47 *(*1H,*dd*,*J*=7.7Hz*)* | 128.1 | 7.41 *(*1H, *dd*, *J* = 8.4, 8.4 Hz) |
| 4' | 132.8 | 7.59 *(*1H,*dd*,*J*=7.4*)* | 132.6 | 7.53 *(*1H, *dd*, *J* = 8.4, 2.0 Hz*)* |
| OCH3 (C2) | 61.1 | 3.76 *(*3H,*s)* | 61.5 | 3.71 (3H, *s)* |
| OCH3 (C3) | 61.5 | 3.99 (3H,s) | 61.1 | 3.92(3H,s) |
| OH | - | - | | - |

Table 6 **COSY $^{1}H$ -$^{1}H$ correlations of compound 2**

| Carbon position | $^{1}H$ -$^{1}H$ correlations |
|---|---|
| C-3'(δC 128.2) | H-3'(δ 7.47)↔ H-2'(δ 7.83) |
| C-5'(δC 128.22) | H-5'(δ 7.47) ↔ H-6'(δ 7.83) |
| C-5(δC 110.04) | H -5(δ6.79)↔ H-6(δ 7.11) |
| C-6(δC 125.99) | H-6(δ7.11) ↔ H-5(δ 6.79) |

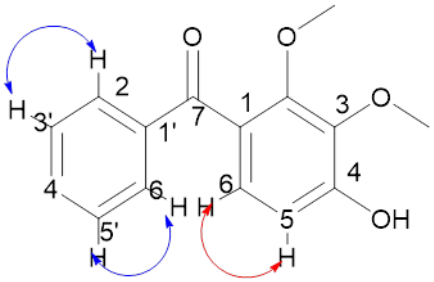



Figure 10 : COSY Correlation

**Table 7** : HSQC correlation of compound 2

| **$^{13}$C** NMR (101 MHz, $CDCl_3$) | **$^{1}$H**(400 MHz, $CDCl_3$) |
|---|---|
| C-2 ( δ 61.1) | δ 3.76 (3H,*s*) |
| C- 3 (δ 61.5) | δ 3.99 (3H,*s*) |
| C-5 (δ110.0) | δ 6.79 (1H,*d*,*J*=8.5Hz) |
| C-4 δ126.0) | δ 7.12 (1H,*d*,*J*=8.5Hz) |
| C-3'/5' (δ 128.2) | δ 7.47 (1H,*dd*,*J*=7.7Hz) |
| C-2'/6' (δ 129.8) | δ 7.83 (*m*,2H) |
| C-4'(δ132.8) | δ 7.59 (1H,*dd*,*J*=7.4) |

**Table 8: The HMBC correlation of compound 2**

| Proton number | $^{13}$C correlation |
|---|---|
| H-5' ( δ 7.58) | H-5'↔ C-6' (129.8) |
| H-3 (δ 3.99) | H-3 ↔ C-139.5 |
| H-5 ( δ 6.8) | H-5 ↔ C-4(152.4), C-1(138.3), C-6 (126.0) |
| H-5' (δ 7.47) | H-5' ↔C-3'(128.2) |
| H-6 (δ7.11) | H-6↔ C-2(151.4) |
| H-6' (δ 7.83) | H-6'↔ C-4'(132.8) |

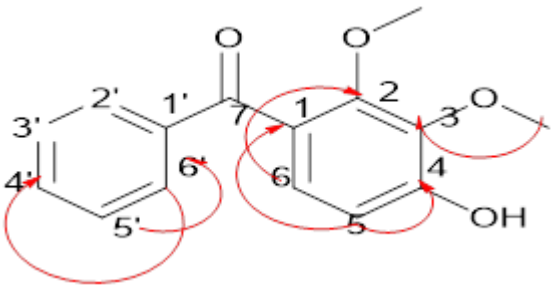



**Figure 11**:HMBC Correlation

Based on the above information of hydrogen and carbon NMR, COSY,DEPT and HSQC. I concluded that the chemical formula of the second compound was $C_{15}H_{14}O_4$ and its expected structure were given below in figure 9 . And its structure name also could be 2,3 dimethoxy-4-hydroxy benzophenone.

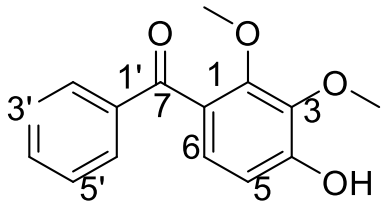



**Figure 12**: structure of compound-2

## 5. Conclusion

*Securida Longipedunculata* is a plant species belonging to the genus *Securidaca* of the family *Polygalaceae*.Medicinal plants contain a wide range of bioactive compounds that have biological activity, such as anticancer, antimicrobial, antioxidant, anti-diarrheal, analgesic, wound healing, etc., and can also be used to treat chronic as well as infectious diseases.

In this study two compounds were isolated, SLM-1 and SLM-2. Based on the $^{1}H$ and $^{13}C$ NMR data the compound SLM-1 was 1,5-anhydro-D-glucitol. The compound 1, 5-anhydro-D-glucitol was previously extracted from a woody plant *Acer ginnala*, but it is not extracted from *S.longipedunculata, this* shows it is the first compound in *S.longipedunculata*. Compound 2 was isolated as a yellowish powder. The $^{1}H$ and $^{13}C$ NMR data comparison from previously isolated from *Lindera fruticosa* [30]; the reported dates were very close agreement with SLM-2. Based on the spectroscopic data $^{1}H$ NMR,$^{13}C$ NMR, HSQC, HMBC and closely related previously isolated compound data *Lindera fruticosa,* the compound-2 molecular formula was $C_{15}H_{14}O_4$. Generally from the above information the expected isolated compound from *S.longipedunculata* was also 2,3 dimethoxy -4-hydroxy benzophenone .This compound is belongs to the class ketone. This finding could be beneficial in the future to complete a further study of biological activity and other possible therapeutic uses of the plants under study.

## 6. Spectral data of SLM-1 and SLM-2

### The SLM-1 $^{1}$H NMR (400 MHz, DMSO)spectral data

$^{1}$H NMR (400 MHz, DMSO) δ 4.93 (*t, J* = 4.7 Hz, 2H), 4.90 (*d, J* = 4.7 Hz, 1H), 4.52 (*t, J* = 5.9 Hz, 1H), 3.70 (*dd, J* = 10.9, 5.3 Hz, 1H), 3.63 (*dd, J* = 11.5, 6.2 Hz, 1H), 3.36 (*dt, J* = 11.6, 5.7 Hz, 1H), 3.28 – 3.19 (m, 1H), 3.07 *(dq, J* = 8.7, 4.6 Hz, 1H), 3.01 – 2.92 (m, 3H).

$^{13}$C NMR (101 MHz, DMSO) δ 81.67, 78.55, 70.40, 69.98, 69.61, 61.56.

### The SLM-2

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.80 (m, 2H), 7.58 (*d, J* = 7.4 Hz, 1H), 7.47 (*t, J* = 7.7 Hz, 2H), 7.28 (*s*,1H), 7.11 (*d, J* = 8.5 Hz, 1H), 6.79 (*d, J* = 8.5 Hz, 1H), 3.98 (s, 3H), 3.76 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 195.33, δ 152.36, 151.97, 139.54, 138.34, 132.77, 129.81, 128.22, 125.99, 125.58, 110.04, 61.49, 61.11.

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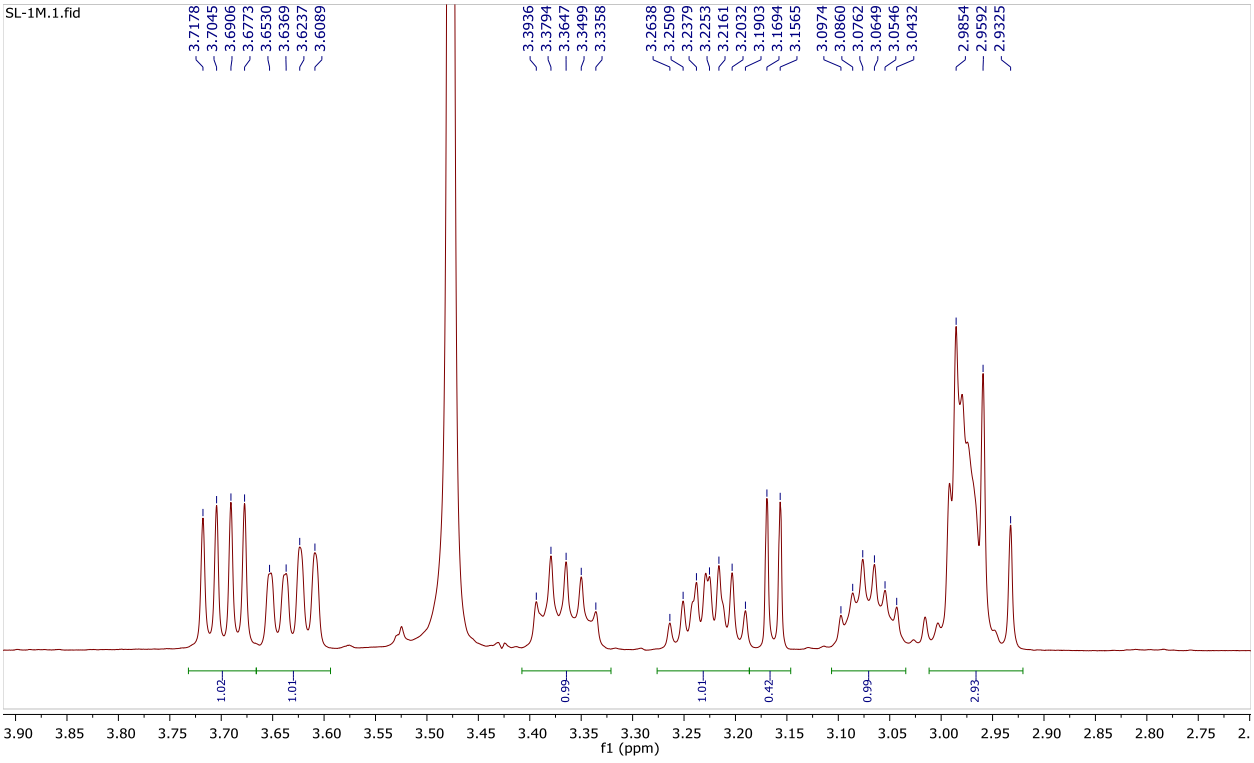
# 7. Appendix

## Appendices 1

SLM-1

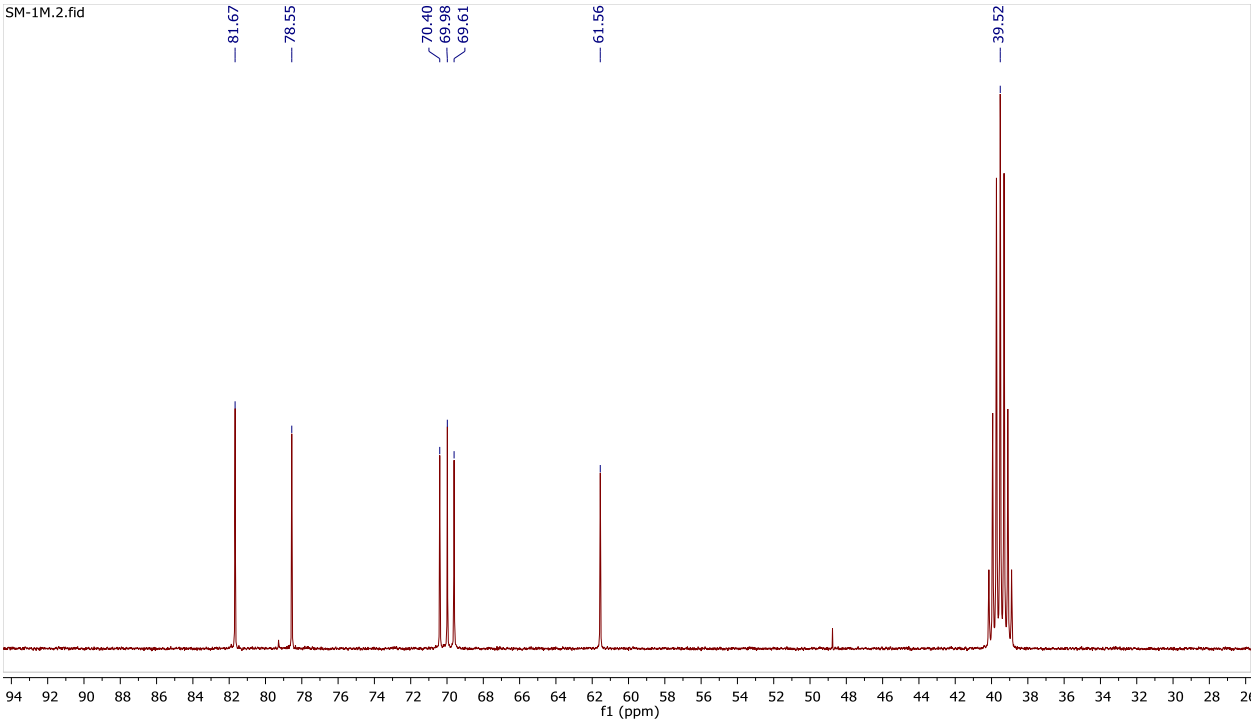
$^{1}$H-NMR



## Appendices 2

SLM-1

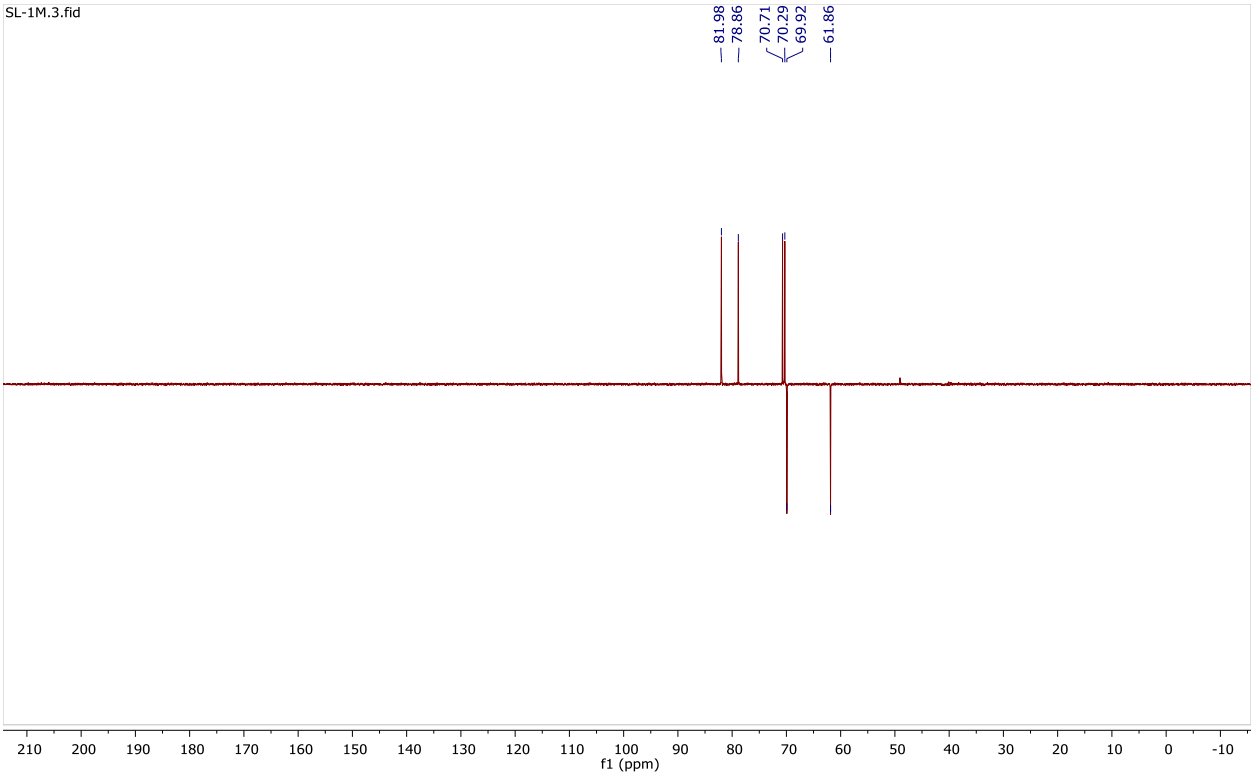
$^{13}$C-NMR



## Appendices -3

SLM-1

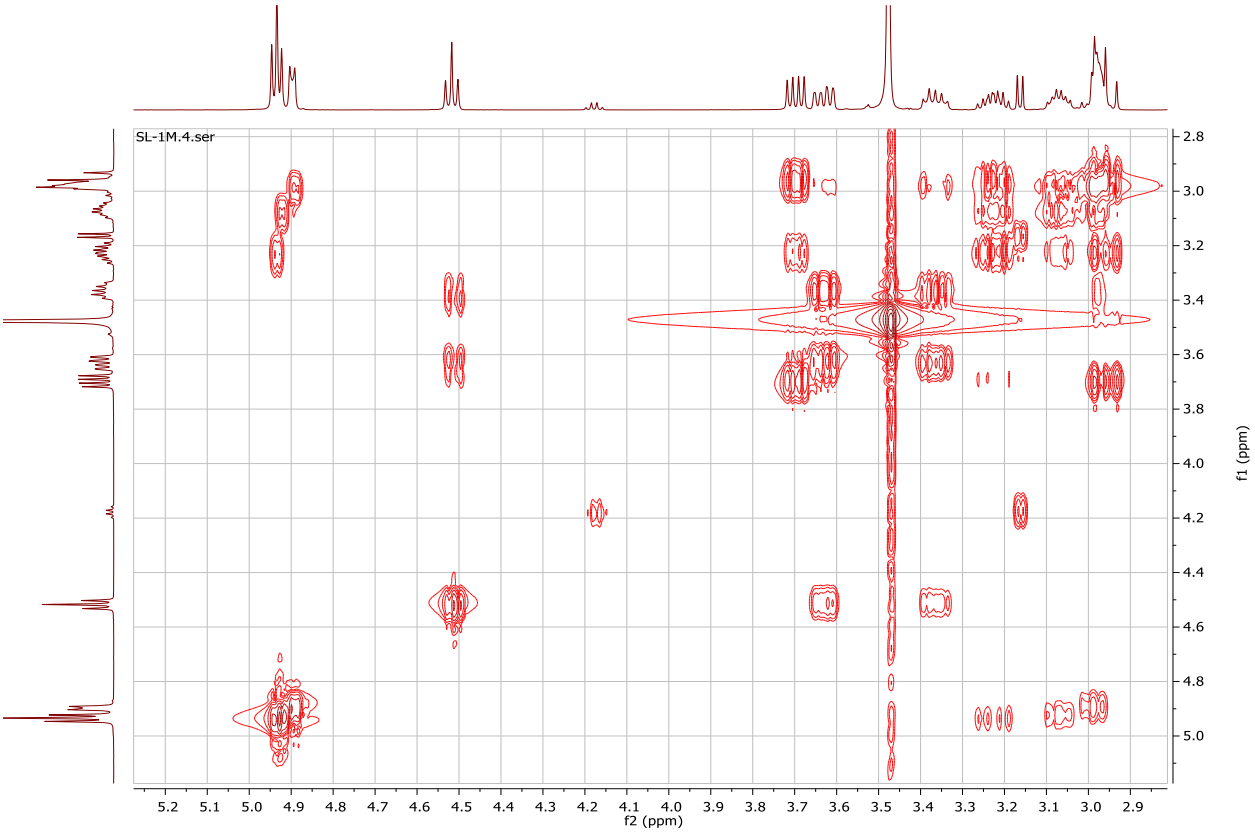
DEPT_135



## Appendices -4

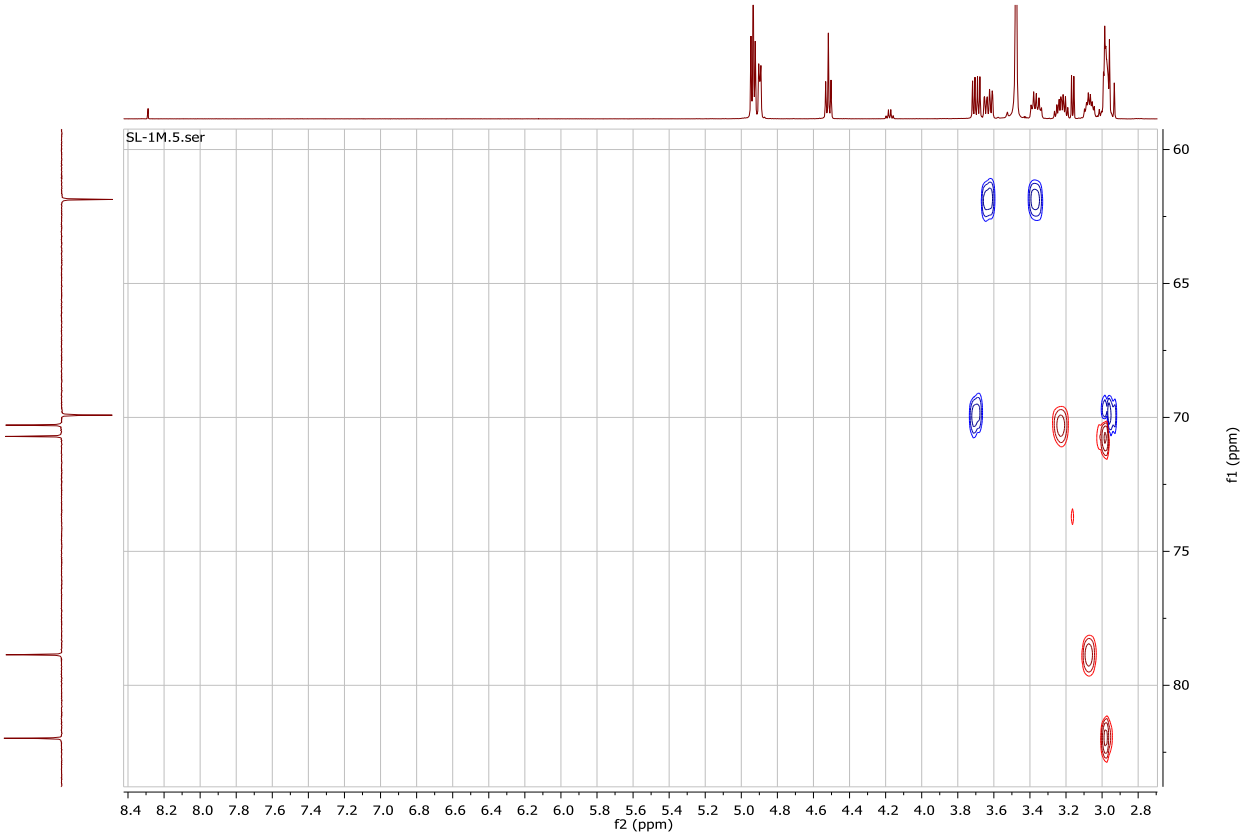
SLM-1

COSY



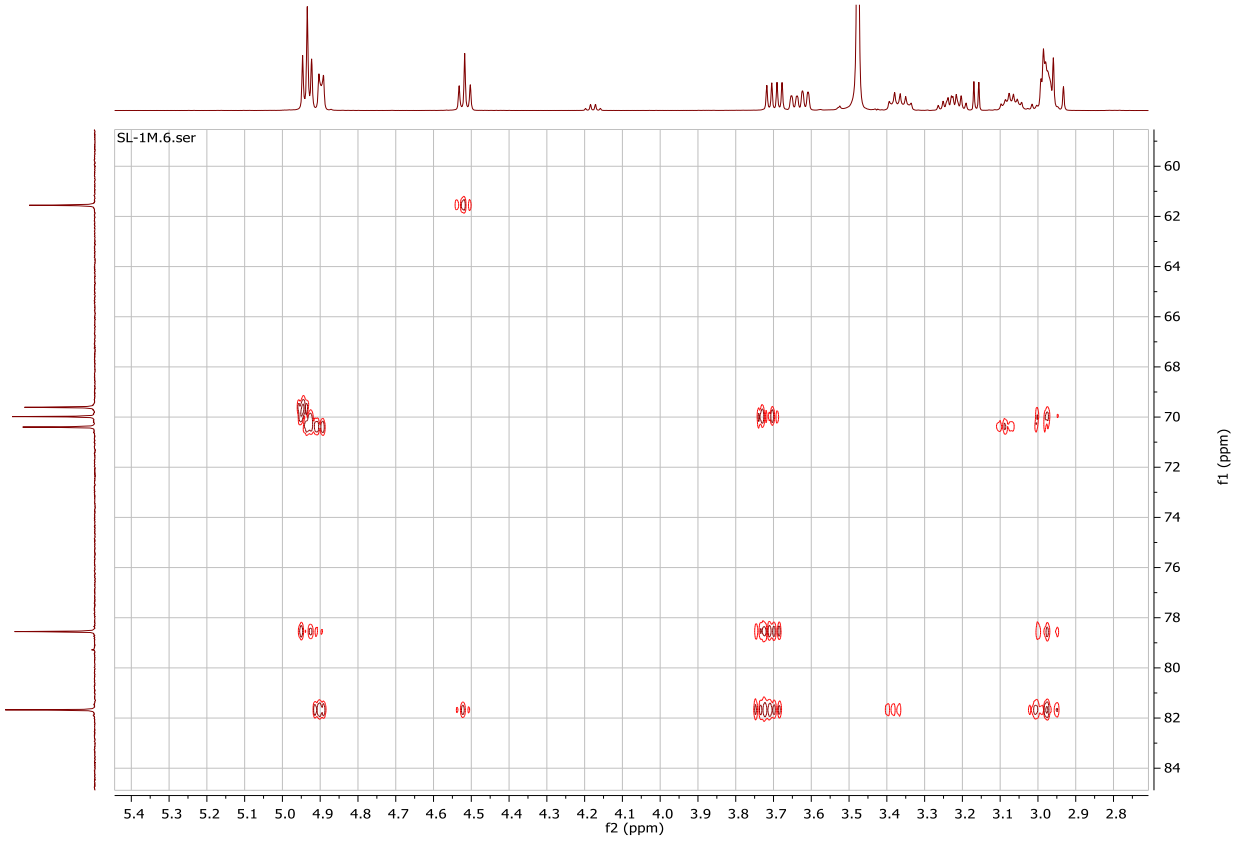
## Appendices -5

SLM-1 HSQC



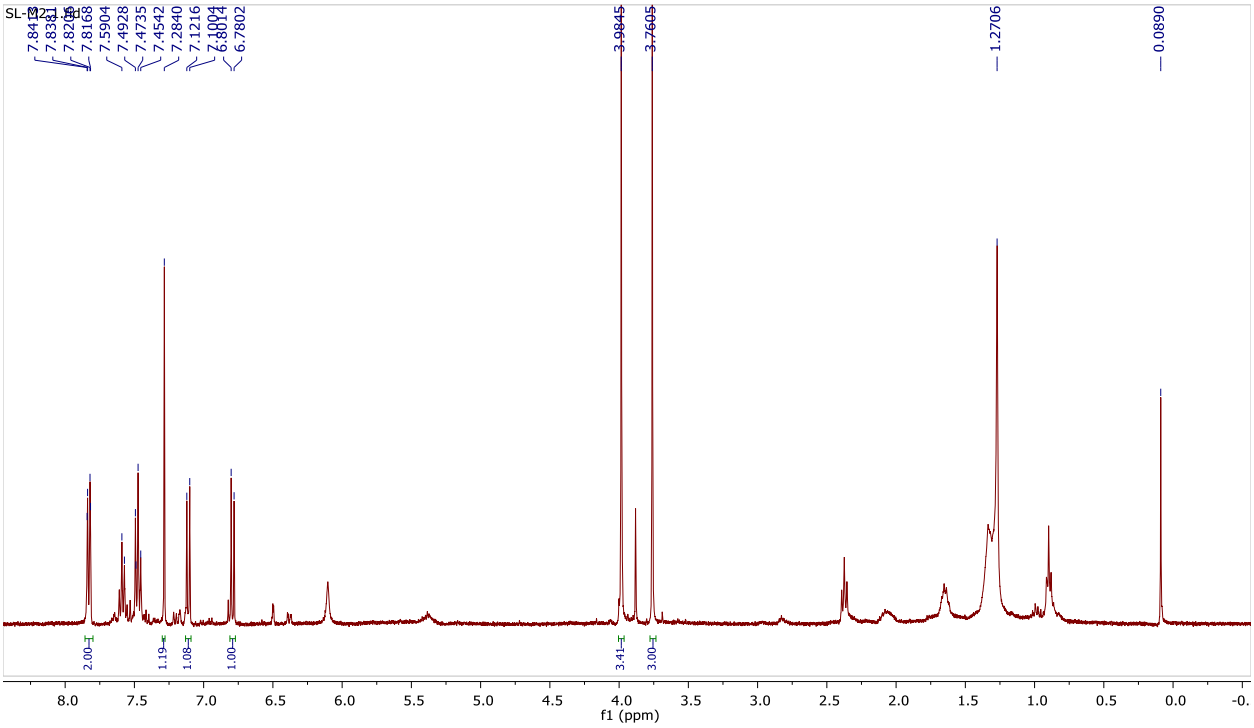
## Appendices -6

SLM-1 HMBC



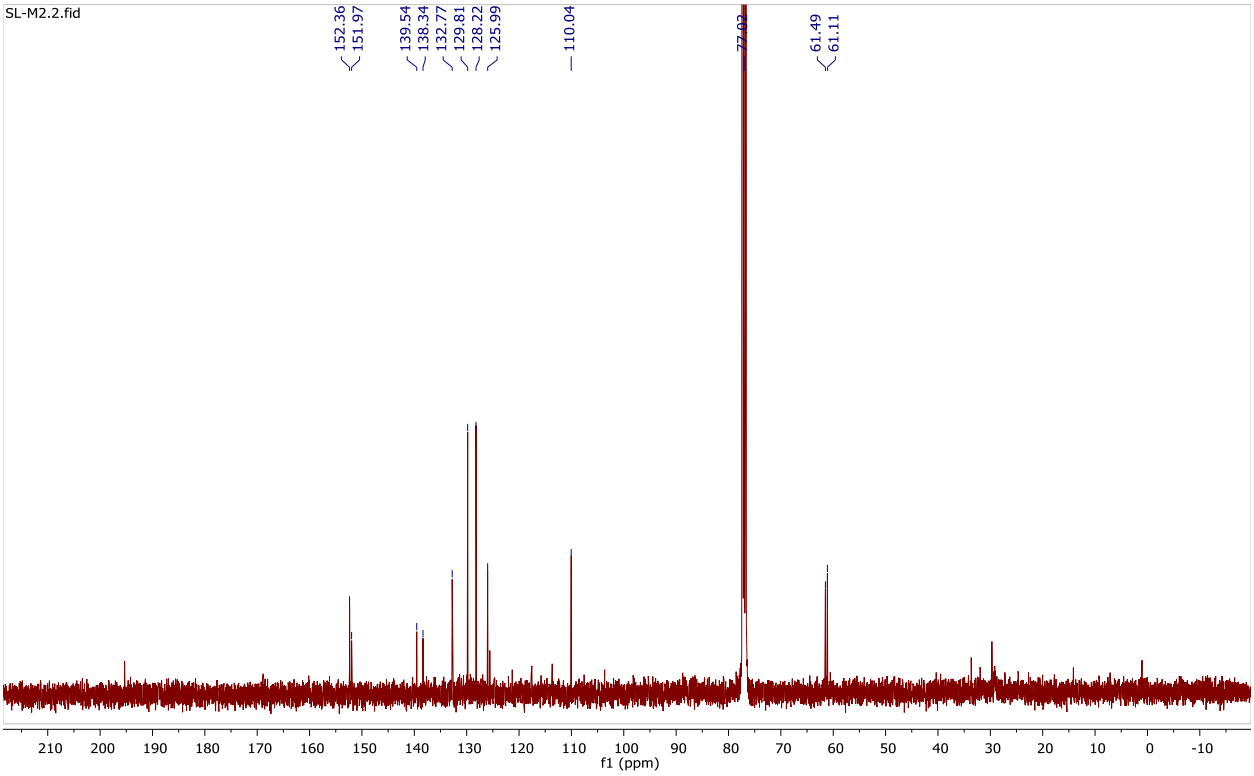
## Appendices 7

SLM-2 $^{1}H$ N M R



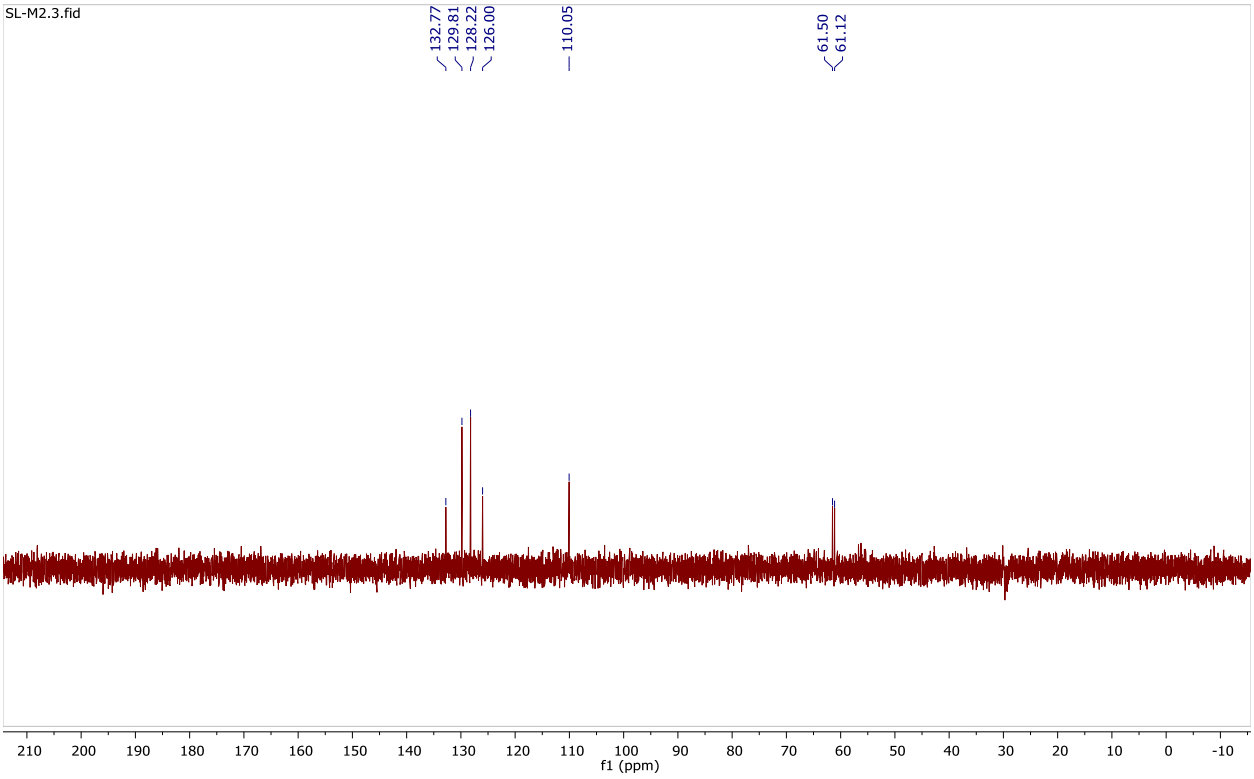
## Appendices 8

SLM-2 $^{13}C$ N M R



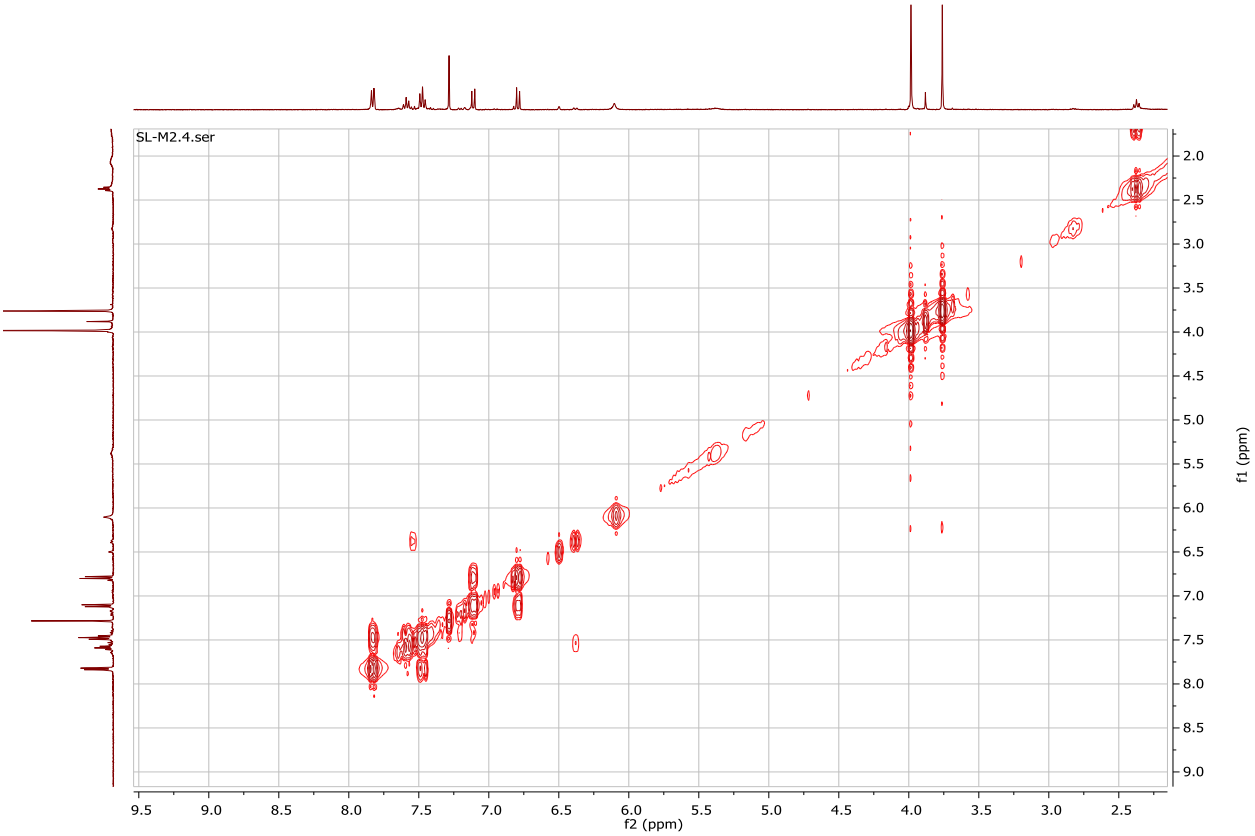
## Appendices 9

SLM-2 DEPT-135



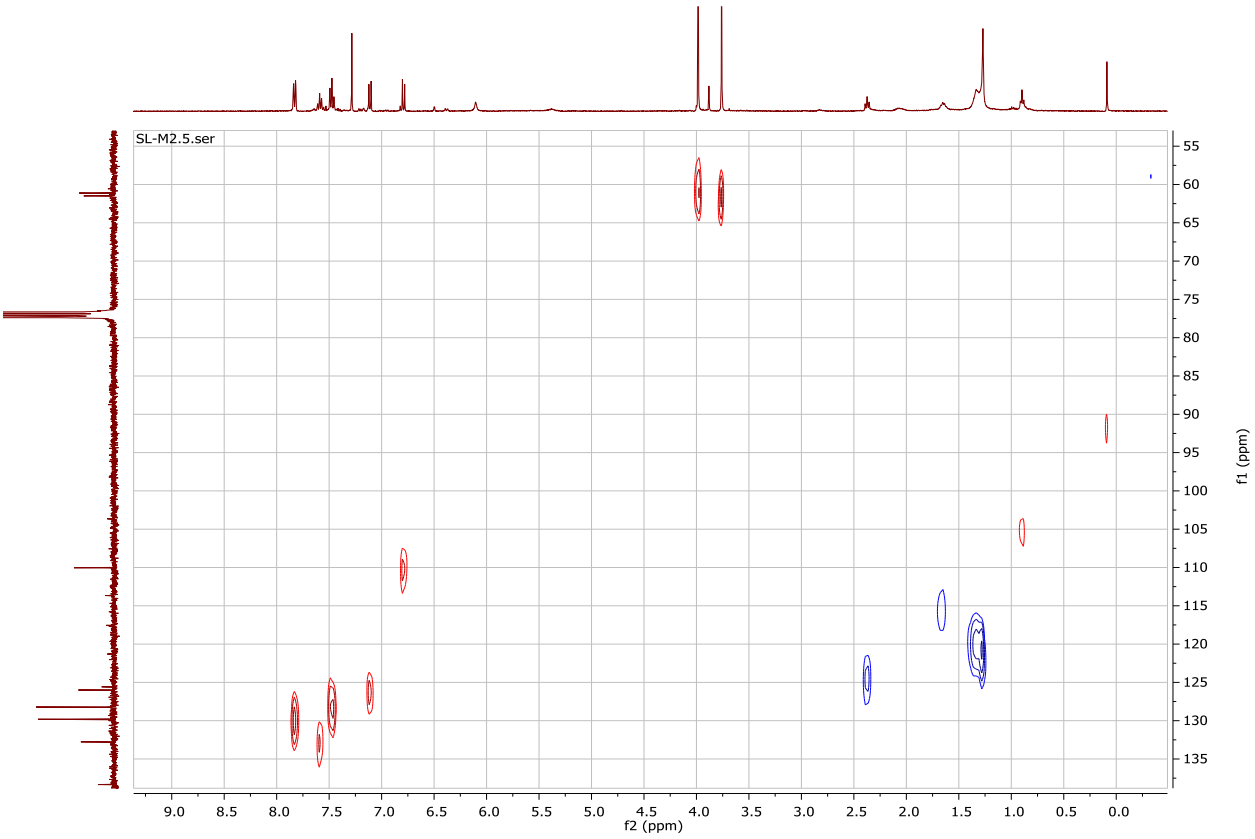
## Appendices 10

SLM-2 COSY



## Appendices 11

SLM-2 HSQC



## Appendices 12

SLM-2 HMBC