**ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY**

**SCHOOL OF APPLIED NATURAL SCIENCES**

**CHEMISTRY PROGRAM**

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# PHYTOCHEICAL INVESTIGATION ON THE FRUITS OF *SOLANUM XANTHOCARPUM*

**RESEARCH WORK FOR THE PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ORGANIC CHEMISTRY FROM THE SCHOOL OF APPLIED SCIENCES IN ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY**

**BY:**

**HENOK BREHANU**

**ADVISOR Dr. H/MICHAEL TESSO**

**AUGUST, 2015, ADAMA, ETHIOPIA**

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**APPROVAL SHEET**

**ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY**

**SCHOOL OF APPLIED SCIENCES**

**DEPARTMENT OF APPLIED CHEMISTRY**

As thesis advisor, hereby I certify that I have read and evaluate this thesis prepared under my guidance by Henok Brehanu entitled phytocheical investigation on the fruits of *Solanum xanthocarpum.* I recommended that it shall be submitted as fulfilling thesis requirement.

**Dr. Hailemichael Tesso (Ph.D)** _____ _____

Advisor signature date

As member of the examining board of the M.Sc. thesis defense examination, we certify that we have read and evaluated the thesis prepared by Henok Brehanu and examined by the candidate. We recommended that the thesis shall be accepted as fulfilling the thesis requirement for the degree of Master of Science in organic chemistry.

_____ _____ _____

Name of the chairman signature date

_____ _____ _____

Name of internal examiner signature date

_____ _____ _____

Name of external examiner signature date

**Statement of the author**

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

Name: Henok Brehanu

Signature _____

Date _____

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**Abstract**

The plant used for this study was *Solanum xanthocarpum*, a prickly diffuse, bright green perennial herb, locally known as enbuay. The aqueous extract of the fruit of the plant *S. xanthocarpum* is administered by some traditional healers for the treatment of tuberculosis, locally known as samba nekersa and other diseases. The phytochemical tests of *S. zanthocarpum* fruits revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, tannins and saponins in chloroform, ethyl acetate and methanol extracts. Then isolation and purification of phytoconstituents from chloroform and methanol extracts by column chromatography led to the isolation of two new compounds, one from each extract. Chloroform extract gave a simple 1, 2 di-substituted phenyl ester, which has not been reported before from this plant. And methanol extract gave a steroidal glycoside of furostane parent aglycone skeleton. The structures of both compounds have been elucidated by interpretation of their UV-Vs, IR, and NMR spectral data and comparison of these spectral data with literatures.

Key words: *Solanum xanthocarpum,* furostane, spirostane, steroidal glycosides, saponin.

**Abbreviations**

AAU: Addis Ababa University

ASTU: Adama Science and Technology University

DEPT: Distortionless enhancement by polarization transfer

EtOAc: Ethyl acetate

EtOH: Ethanol

IR: Infrared

MeOH: Methanol

MS: Mass spectrometry

NMR: Nuclear magnetic resonance

2DNMR: Two dimensional nuclear magnetic resonance

ppm: Parts per million

PTLC: Preparative thin layer chromatography

RF: Retardation factor

TLC: Thin layer chromatography

TMS: Tetra methyl silane

UV-VIS: Ultra violet-visible

# 1. Introduction

## 1.1. General

Plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock since ancient times. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians [1].

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. [2].

Plants constitute a rich source of bioactive compounds and these compounds are biodegradable to nontoxic products and potentially suitable for use in integrated pest management programs, for example they could lead to the development of new classes of safer insect control agents. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals and the emphasis is inevitably on chromatographic techniques. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom. The continuous improvement in chemical technology allowed the isolation, structural characterization and further synthesis of the pharmacologically active plant constituents. The

enormous variety of organic substances that are elaborated by plants with different chemical structure, biosynthesis, turnover and metabolism, natural distribution and biological function, separation, purification and identification of the many different constituents present in the plant are secondary metabolites [3].

Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy and chemical constituents of some of the herbs used in the treatment of some illness would be worthwhile. This research work, therefore, deals with extraction, isolation and structural elucidation of some of the chemical constituents of the fruits of *S. xanthocarpum,* locally known as enbuay*,* a plant that is used in treatment of various illnesses in different countries' traditional medicinal practices.

### 1.2. *Solanum xanthocarpum* Schrad and Wendl

The genus *Solanum* belongs to the family Solanaceae and comprises more than 1700 species, and, is found in the tropical and temperate regions [2, 4]. *S. xanthocarpum* is a very prickly diffuse, bright green perennial herb, 2-3 m high. Stems are zigzag; prickles compressed, straight, yellow and shining; leaves 5-10 by 2.5-5.7 cm ovate or elliptic, sinuate or sub pinnatified, hairy on both sides, Petiole prickly. Flowers are small, in extra-axillary few flowered cymes. Corolla is purple, lobes deltoid, hairy outside. Fruits are of 1.3 cm diameter berry, yellow or white with green veins, surrounded by enlarged calyx. It grows on all kind of soil but does well on dry and hot temperate region [5].

**Fig. 1:** Fruits of *S. xanthocarpum*

### 1.3. Statement of the problem

The genus *Solanum* belongs to the family Solanaceae and comprises more than 1700 species, and, is found in the tropical and temperate regions [2, 4]. *S. xanthocarpum* one of the ten plants in the Ayurveda (the science of life prevention and longevity – the oldest traditional system of medicine in India) [6].

In Ethiopia, despite the availability of the plant in different parts of the country, no research work is reported up to now, to the best of our knowledge except that we found some traditional medicinal scripture related to the Orthodox "Debteras" reporting the use of the fruit of *S. xanthocarpum* for the treatment of tuberculosis, locally known as samba nekersa. Hence, this research emphasized on the investigation of the chemical constituents of fruits of *S. xanthocarpum.*

## 2. Objective of the study

### 2.1. General objective

The aim of this study is to isolate and elucidate the structures of the chemical constituents of the fruit of *S. xanthocarpum*.

### 2.2. Specific objectives

- To extract the fruits of the plant *S. xanthocarpum* successively with petroleum eater, chloroform, ethyl acetate and methanol.
- To isolate compound(s) from the fruits of *S. xanthocarpum* using various chromatographic techniques such as column chromatography and preparative thin layer chromatography.
- To chemically test the presence and/or absence of phytochemicals in the different extract(s) of the fruit of *S. xanthocarpum.*
- To elucidate the structure(s) of isolated compounds by means of spectroscopic techniques.

## 3. Literature review

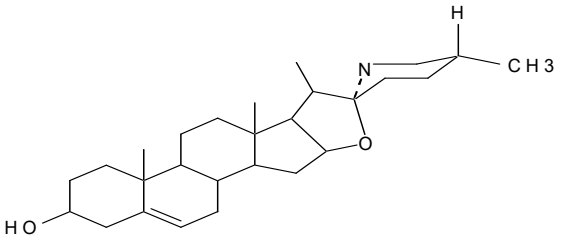
### 3.1. Medicinal uses of *S. xanthocarpum*

The plant has been reported to possess antispasmodic, cardiotonic, hypotensive, antitumor, antianaphylactic and cytotoxic activities [4 – 6]. It is also useful in the treatment of asthma, cough, fever, enlargement of liver, spleen, controlling stones in bladder and pain in chest [7]. Various studies indicated that *S. xanthocarpum* possesses antiasthmatic, hypoglycemic, hepatoprotective, antibacterial and insect repellent properties. Various traditional claims like immunomodulation, anti-inflammatory, antiallergic, antianaphylactic and antitumor effects of the plant are still remains to be validated scientifically [13]. Clinical trials for the reported preclinical studies should be performed to further validate the claims of local people [14].

### 3.2. Previous phytochemical investigation on the plant *S. xanthocarpum*

The fruits are reported to contain several steroidal alkaloids like solasodine **(1)** and

solamargine **(2)**. Other constituents like caffeic acid **(3)**, coumarins like esculetin **(4)**, steroids like carpesterol **(5)**, diosgenin **(6)**, campesterol **(7)**, β-sitosterol **(8)** and triterpenes like cycloartenol **(9)** and cycloartanol **(10)** were reported from the fruits [13]. Structures of some isolated compounds from the fruits of *S. xanthocarpum* are shown in Figure 2.



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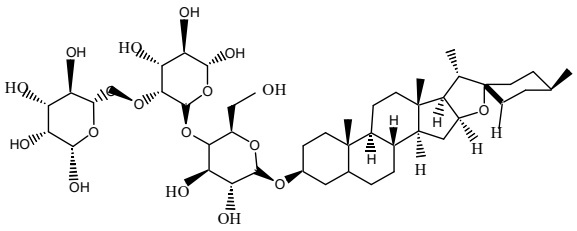

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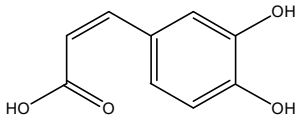

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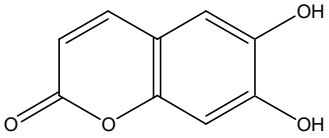

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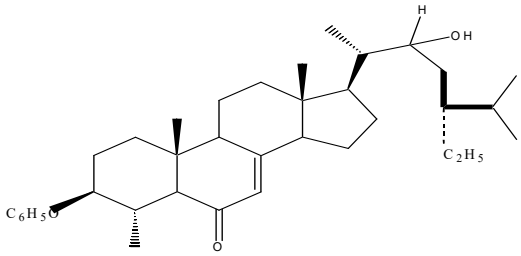

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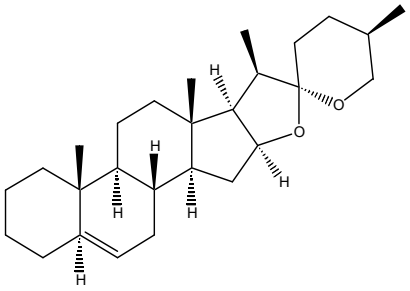

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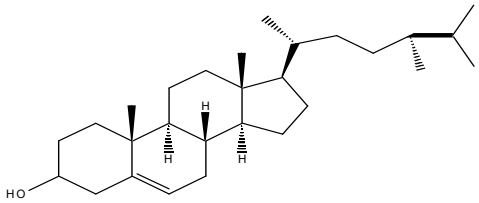

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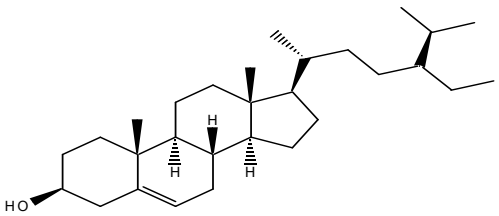

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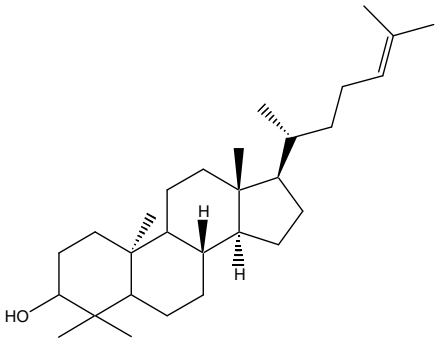


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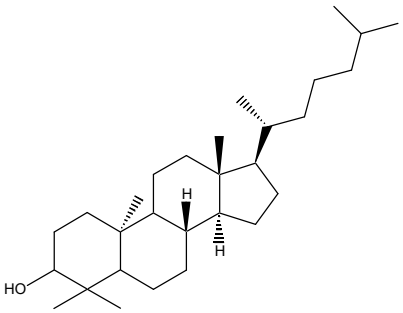


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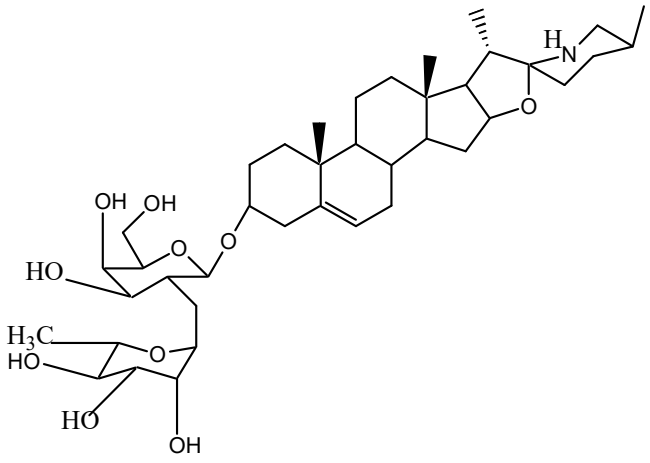
**Fig. 2:** Previously reported compounds from fruits of *S. xanthocarpum*

Recently a new steroidal glycoalkaloid *O*-(3){α-Lrhamnopyranosyl-(1—>2gal)-β-D-galactopyranosyl}-solasodine is reported from berries of *S. xanthocarpum*. This new compound is renamed solaxanine (11)[16].

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# 4. Materials and Methods

## 4.1. Sample Collection and Preparation

Plant material**:** Fruits of the plant, *S. xanthocarpum* was collected from Adama Science and Technology University campus. The sample was shade dried and powdered into similar mesh size.

## 4.2. Chemicals and Apparatus

### 4.2.1. Chemicals and solvents

Some of the chemicals and solvents that were used in this study include: methanol, distilled water, petroleum ether, silica gel, tetra methyl silane, $H_2SO_4$, HCl, NaOH, acetone, ethyl acetate, n-hexane, $CHCl_3$ (chloroform), $CH_2Cl_2$ (dichloromethane), $FeCl_3$ (ferric chloride), $Hg(NO_3)_2$ (mercuric nitrate), mercuric chloride ($HgCl_2$), Iodine ($I_2$), potassium iodide (KI), ammonia ($NH_3$), benzene ($C_6H_6$), vanillin, zinc ribbon.

### 4.2.2. Apparatus and Instruments

Some of the apparatus used in this study were: electronic balance, rota evaporator, glass Column, filter paper, measuring cylinders, pippets, test tubes, reagent bottles, analytical TLC, preparative thin layer lhromatography (PTLC), UV-light cabinet, and UV-Vis spectrophotometer.

## 4.3. Methods

### 4.3.1. Extraction, TLC analysis and fractionation

170 gm. of powdered fruit of *S. xanthocarpum* was extracted successively with petroleum ether (1.5L), chloroform (1.5L), ethyl acetate (1.5L), and methanol (1.5L) for 72 hours each. And the solutions were filtered with filter papers and evaporated under reduced pressure using rotary evaporator and crude extracts were obtained for each solvent used. The yields from each extract were measured with analytical balance and recorded. TLC was used for the analysis of components in each extract and for the determination of solvent system to run the column chromatography. Column chromatography and preparative tin layer chromatography were used for the fractionation and purification, respectively, of chloroform and methanol extracts. NMR, IR and UV-Vis spectroscopy were used for the structure determination of isolated compounds. But the dry crude extracts were first stored in a refrigerator for the upcoming phytochemical analysis, isolation and purification of natural products.

### 4.3.2. Phytochemical screening tests

#### 4.3.2.1. Test for carbohydrates

**Molisch's test**

The extracts are combined with drops of molisch's reagent (α-naphtol dissolved in ethanol) in a test tube. And the mixtures were shaken well and a small amount of concentrated sulfuric acid is slowly added down the side of the sloping test tubes without mixing to form a layer. A positive result is indicated by appearance of a purple ring at the interface between the acid and test layer. The results for each extracts are as follows.

Carbohydrates were not detected in petroleum ether extract but were detected in ethyl acetate and methanol extract.

#### 4.3.2.2. Test for proteins and amino acids

**Millon's test**

To 2ml of each extract 2 ml of millon's reagent (mericuric nitrate in nitric acid) was added. A positive result that indicates the presence of proteins and amino acids is the appearance of white precipitate which turns red up on gentle heating. The results for each extracts are as follows.

Proteins and amino acids were not detected in petroleum ether extract but were detected in ethyl acetate and methanol extract.

#### 4.3.2.3. Test for oils and fats

**Stain test**

Small amounts of each extract were spotted on the same filter paper and left to dry. A positive result indicating the presence of fixed oils is that the spotted place remains stained. Hence, only the petroleum ether extract gave a positive result for the stain test. For confirmation a saponification test was done as follows for petroleum extract. To 1 ml of petroleum ether extract 3 drops of sodium hydroxide solution and 1 drops of ethanol was added and heated in a water bath. Froth started to form after 30 minute.

Fats and oils were detected in petroleum ether extract but were not detected in ethyl acetate and methanol extract.

#### 4.3.2.4. Test for alkaloids

**Wagner's test**

Wagner's reagent was added to a petroleum ether and methanol extract and gave a brown precipitate for the methanol extract which is a positive test for alkaloids.

Alkaloids were not detected in petroleum ether extract but were detected in methanol extract.

#### 4.3.2.5. Test for flavonoids

**Zinc hydrochloride reduction test**

To petroleum ether and methanol extract, 1 ml each in a test tube, zinc metal in HCl was added. A positive result is the turning of the test solution to red color after few minute. Neither of the two extract gave a positive result.

**Alkaline reagent test**

To 1 ml petroleum ether and 2 ml methanol extract 3 drops of sodium hydroxide solution was added. The formation of yellow color would indicate the presence of flavonoid. Neither of the two extract gave a positive result.

#### 4.3.2.6. Test for saponins

To each 2ml extract in 3 different test tubes 5 ml distilled water was added and shaken. A positive result indicating the presence of saponins is the formation of froth which persists for few minutes. The results are as follows.

Saponins were not detected in petroleum ether extract but were detected in ethyl acetate and methanol extract.

#### 4.3.2.7. Test for phenolics and tanins

**Ferric chloride test**

Ferric chloride solution was added to petroleum ether and methanol extract and no color change for the petroleum ether extract but a greenish black color for methanol extract which is a positive test for phenolics and tannins.

Phenolics and tanins were not detected in petroleum ether extract but were detected in methanol extract.

| Phytochemicals | | Petroleum ether exract | Ethyl acetate extract | Methanol extract |
|---|---|---|---|---|
| Carbohydrates | Molisch's test | - | + | + |
| Proteins and amino acids | Millon's test | - | + | + |
| Fats and oils | Stain test | + | - | - |
| | Saponification test | + | | |
| Alkaloids | Wagner's test | - | | + |
| Flavonoids | Zinc hydrochloride reduction test | - | | - |
| | Alkaline reagent test | - | | - |
| Saponins | | - | + | + |
| Phenolics and tanins | | - | | + |

## 5. Results and discussion

### 5.1. TLC analysis of the extracts

Petroleum ether extract gave five clear spots on TLC with methanol and petroleum ether (1:6) solvent system. The chloroform extract gave six spots with 100% chloroform. The ethyl acetate extract gave many spots with methanol and ethyl acetate (1:9) solvent system. The methanol extract was unable to move with 100% methanol using a normal phase TLC plate but gave many spots using a reversed phase TLC plate with 100% methanol.

### 5.2. Isolation of compounds from methanol extract

Isolation of compounds from methanol extract was achieved first using column chromatography over silica gel followed by purification of fraction 13 by preparative thin layer chromatography (PTLC) by using methanol : glacial acetic acid (10 : 1). First slurry of 120 gm. silica gel was prepared in petroleum ether and poured in to the column. Then the methanol extract (17 gm.) was loaded by dissolving in 50 ml methanol. 23 fractions were collected from the column by eluting with methanol : glacial acetic acid (10 : 1). Fraction 13 showed a single spot with some impurity and taken for further purification by preparative TLC (PTLC) with the same solvent system (i.e. 10:1 methanol and glacial acetic acid). The isolated compound was analyzed by NMR, IR and UV spectroscopy at AAU 4 Kilo campus.

TLC chromatogram for methanol extract. TLC chromatogram for SX-13. TLC chromatogram for SX-13 after PTLC

**Fig. 3:** TLC for methanol crude extract and fraction 13

### 5.3. Isolation of compounds from chloroform extract

Isolation of compounds from chloroform extract was achieved first using column chromatography of the partitions over silica gel followed by purification of some fractions by preparative thin layer chromatography (PTLC). First a slurry of 70 gm. silica gel was prepared in chloroform and poured in to the column. Then the chloroform extract (1.1 gm.) was loaded by dissolving in 15 ml chloroform. The column was eluted with increasing polarity of $CHCl_3$/methanol solvent mixtures. 46 fractions were collected from the column. Fraction 21-29 were taken and mixed for further purification by preparative TLC (PTLC). After concentrating it gave 3 different spots on TLC having a very close RF value with acetone and petroleum ether (5:3) solvent system. The three compounds were isolated using PTLC with the specified solvent system as a mobile phase and analyzed by NMR and IR spectroscopy at AAU 4 Kilo campus. Two of them are phthalate esters which we thought are impurities most probably from the solvent container. But the 3$^{rd}$ compound is partially characterized by NMR and IR spectruml data.

**Fig. 4:** TLC for chloroform extract.

### 5.4. Partial Characterization of Isolated compounds

#### 5.4.1. Characterization of compound $SX_{-13}$

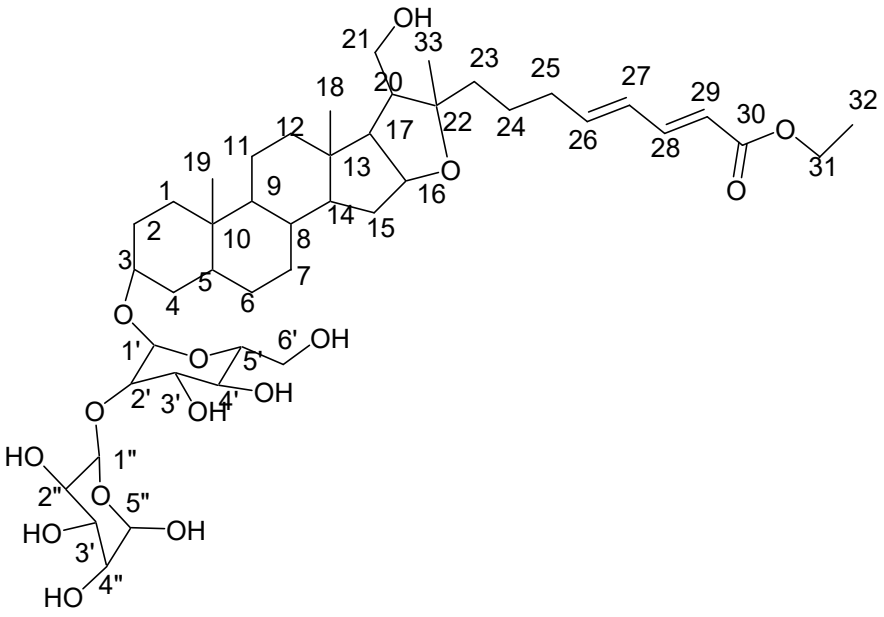
$SX_{-13}$ was obtained as a red solid compound. The UV-Vis (Appendix-1) spectrum showed absorption maxima at 277. This suggests that the compound is composed of a conjugated diene system that would absorb around 200 nm and this increase to 277 nm is possibly due to the extending conjugation of the conjugated double bonds being next to a carbonyl group. Extensive analysis of the $^{1}H$ and $^{13}C$ NMR spectrum of the compound and comparison with literature data led to a frostane type skeleton for the aglycone of the compound. The $^{1}H$-NMR spectrum provided key information about the skeleton of the compound. The $H_{-16}$ resonates between 4.1 and 4.5 ppm as a quartate, doublet of doublet, having $^{3}J_{16,15a} = {}^{3}J_{16,15b} = {}^{3}J_{16,17} = 6.2$ -8.2 in the case of both spirostanes and furostanes [20]. The $^{1}H$-NMRspectrum (Table 2, δH and δC in ppm) showed resonance for $H_{-16}$ at 4.112 ppm as doublet of doublet with $^{3}J_{16,15a} = {}^{3}J_{16,15b} = {}^{3}J_{16,17} = 7$. The $^{1}H$-NMRspectrum displayed signals at 0.83(s), 0.911(s) and at 1.20 (s) ppm indicating singlet methyl protons located at the junction of the rings A-E. If there is any substituent on a furostane ring at the junction carbon, then it will result in the non-protonation of these carbons. The presence of multiple down field peaks in the aliphatic protons region in the range from 3-4.9

ppm (almost nine peaks) indicates the protons are connected to an electron withdrawing group most likely oxygen as in cyclic sugars connected by a glycosidic bond or otherwise to other deshielding groups. The presence of sugar moieties is also supported by its $^{13}C$ NMR spectrum. There are also resonances for olifinic protons at around 5.3 ppm. For $^{1}H$-NMR a well-known difficulty with the assignment of proton resonances of oligosaccharides is the overlap of constituent proton resonances in a narrow spectral width (3.2-4.5 ppm). An exception is the anomeric resonances of the sugar moieties which occur largely in a well-defined region (4.4-6.4) [20]. So, $^{1}H$-NMR spectrum shows anomeric proton resonances at 4.900 and 4.995 ppm. $^{13}C$-NMR spectrum showed signals for methyl carbons 11.21, 14.35, 18.17 and 19.26 δC in ppm and 15 methylene carbons from 23.69 to 71.71 ppm. There are four double bond carbons and DEPT-135 suggests they all are methines (CH) and the UV-Vis spectrum shows absorption maxima at 274 nm. This tells the two double bonds are not only conjugated with each other but also with another group like C=O. Therefore, there is no chance for the double bonds to be inside the cholestane ring since a double bond at any position or substituents at junctions cause non-protonated nature of these carbons. The $^{13}C$ anomeric region (92-108) is straight forward and hence the number of C-signals in this region usually reflects the number of sugar units, considering $C_{-22}$ can appear to confuse in this region which usually can be identified by its none protonated nature [20]. Its $^{13}C$ NMR spectrum shows multiple methine resonances in region between 68 and 80 ppm and their anomeric carbons at 92 .60 and 97.19 ppm. Although the complete assignments of the $^{1}H$ and $^{13}C$ chemical shifts, coupling constants and configuration in order to establish the nature and types of the sugar moiety and their connectivity with each other as well as with the furostane ring requires extensive 2D NMR spectral analysis [20-23], we proposed the structure of compound $SX_{-13}$ based on the above spectral data.

Table 2: $^{1}H$, $^{13}C$ and DEPT spectral data of compound $SX_{-13}$

| Position | $\delta^{1}H$ (400MHz, DMSO-d6) | $\delta^{13}C$(400MHz, DMSO-d6) | DEPT-135 implications |
|---|---|---|---|
| 1 | 1.45 (t, J=15) | 30.20 | $CH_2$ |
| 2 | 1.07 (t, J=15) | 24.89 | $CH_2$ |
| 3 | 4.66 (m) | 72.70 | CH |
| 4 | 0.89 | 28.91 | $CH_2$ |
| 5 | 1.96 (m) | 34.13 | $CH_2$ |
| 6 | 1.30(q) | 29.08 | $CH_2$ |
| 7 | 1.30 | 29.36 | $CH_2$ |
| 8 | 2.14 (t, J=18) | 38.51 | CH |
| 9 | 2.14 (t, J=18) | 39.42 | CH |
| 10 | - | 35.51 | C |
| 11 | 1.20 | 23.69 | $CH_2$ |
| 12 | 1.20 | 26.94 | $CH_2$ |
| 13 | - | 37.33 | C |
| 14 | - | 53.75 | CH |
| 15 | - | 31.67 | $CH_2$ |
| 16 | 4.11(dd, $^{3}JH_a$= $^{3}JH_b$ =$^{3}JH_c$ =7) | 77.06 | CH |
| 17 | 1.95 | 68.10 | CH |
| 18 | 0.82 (s) | 14.36 | $CH_3$ |
| 19 | 0.91 (s) | 14.37 | $CH_3$ |
| 20 | 2.87 (t, J=20) | 70.67 | CH |
| 21 | 4.26 (d, J=18 ) | 63.12 | $CH_2$ |
| 22 | - | 97.19 | C |
| 23 | 1.45 (t, J=16) | 29.27 | $CH_2$ |
| 24 | 1.2 (m) | 28.79 | $CH_2$ |

| | | | |
|---|---|---|---|
| 25 | 1.30 (q) | 29.46 | $CH_2$ |
| 26 | 5.29 | 128.12 | CH |
| 27 | 5.30 | 129.13 | CH |
| 28 | 5.33 | 130.12 | CH |
| 29 | 5.31 | 132.08 | CH |
| 30 | - | 167.60 | C |
| 31 | 3.5 | 67.97 | $CH_2$ |
| 32 | 0.84 (t, J=15) | 18.26 | $CH_3$ |
| 33 | 1.07 (t, J=15) | 19.28 | $CH_3$ |
| 1’ | 4.90 (d, J=7) | 92.60 | CH |
| 2’ | 4.26m | 77.01 | CH |
| 3’ | .3.98 m | 76.99 | CH |
| 4’ | 4.60 (m) | 75.23 | CH |
| 5’ | 4.11 (m) | 73.46 | CH |
| 6’ | 4.26 m | 63.12 | $CH_2$ |
| 1” | 4.99m | 97.19 | CH |
| 2” | 3.25m | 73.46 | CH |
| 3” | 3.57 | 72.70 | CH |
| 4” | 3.39 | 70.89 | CH |
| 5” | 3.42 | 70.32 | CH |




Compound SX-13

### 5.4.2. Characterization of compound SX-3

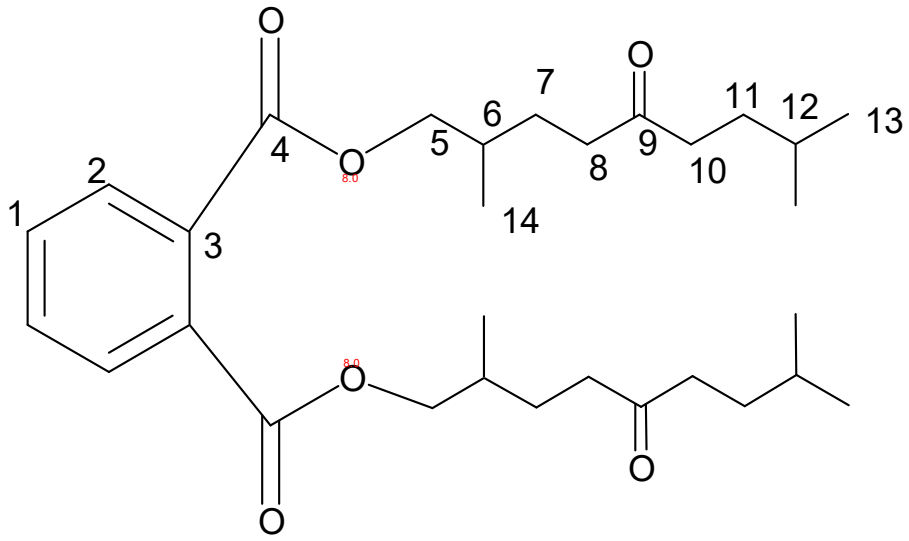
Compound **SX-3** was obtained as a yellow crystalline solid. Its IR (KBr, $cm^{-1}$) spectrum showed absorptions at 2925 $cm^{-1}$ for the aliphatic C-H stretching vibrations, 1720 $cm^{-1}$ for the ester C=O stretching vibration. And it also shows for the aromatic C=C stretching vibration at 1632 $cm^{-1}$, for the aromatic C-H bending vibration at 1465 $cm^{-1}$ and at 743 $cm^{-1}$ signal for the chain bending vibration. The $^{1}$H-NMR spectral data (δH and δC in ppm, Table 1) displayed signals at [δH 0.91 (d, J= 6), 0.94 (s), 1.27 (s), 1.36 (t, J= 13), 1.41 (q, J= 12), 1.68 (t, J= 15), 2.19 (s), 4.23 (t, J= 15), 7.55 (dd, J= 9, 8), 7.71 (dd, J= 9, 9)]. $^{13}$C-NMR showed a total of 15 peaks for the sample, ten peaks in the aliphatic region at δC in ppm [10.95, 14.04, 22.98, 23.74, 28.92, 29.69, 30.36, 30.91, 38.73, and a deshielded at 68.17 which an indicative of a carbon atom connected to an electronegative atom oxygen as displayed by the IR spectrum containing the ester functional group and also consistent with the proton signal at 4.25 which integrate for two protons], three

aromatic carbons at δC in ppm 128.80, 130.89, 132.44], and two carbonyl carbon at 167.79 ppm for the ester C=O and at 207.09 ppm for a ketone C=O (the ketone C=O vibration is not displayed in the IR spectrum probably, it is overlapped with the ester C=O). The DEPT-135 showed a total of eleven peaks: two methyl, five methylene, and four tertiary carbons and three missing peaks indicating the compound has three set of equivalent quaternary carbons.

Table 3: $^{1}H$, $^{13}C$ and DEPT spectral data of compound $SX_{-3}$

| No | δH (400MHz, $CDCl_3$) | δC (400MHz, $CDCl_3$ ) | DEPT-135 implications |
|---|---|---|---|
| 1 | 7.72 (d) | 130.89 | CH |
| 2 | 7.55 (d ) | 128.80 | CH |
| 3 | - | 132.44 | C |
| 4 | - | 167.79 | C |
| 5 | 4.23 (m ) | 68.17 | $CH_2$ |
| 6 | 2.65 | 38.73 | CH |
| 7 | 1.34 | 23.74 | $CH_2$ |
| 8 | 1.70 (m) | 30.36 | $CH_2$ |
| 9 | - | 207.09 | C |
| 10 | 1.45 | 28.92 | $CH_2$ |
| 11 | 1.30 | 22.98 | $CH_2$ |
| 12 | 2.33 (m) | 30.91 | CH |
| 13 | 0.92 (d, J=6 ) | 10.95 | $CH_3$ |
| 14 | 0.99 (d, J=14) | 14.04 | $CH_3$ |

Therefore, based on the above spectral data the following structure was proposed for compound $SX_{-3}$:




Compound $SX_{-3}$

## 6. Summary

170 gm. of powdered fruit of *S. xanthocarpum* when successively extracted with petroleum ether (1.5L), chloroform (1.5L), ethyl acetate (1.5L), and methanol (1.5L) yielded 0.8 gm., 1.1 gm., 0.6 gm. and 17.6 gm. crude extracts respectively. The petroleum ether extract gave clearly separated spots but there was no separation between the starting spot and the first eluted compound for ethyl acetate extract. Methanol extract was not able to move on normal phase TLC plate at even 100% methanol solvent system. Petroleum ether extract gave a positive result for the "fats and oils" test and a negative result for the "carbohydrates, (proteins and amino acids), saponins, (phenolic and tannins), flavonoids and alkaloids" tests by the methods employed in this work. Ethyl acetate and methanol extracts gave a negative result for the "(fats and oils) test and a

positive result for the “carbohydrates, (proteins and amino acids), saponins, (phenolic and tannins), flavonoids and alkaloids by the methods employed in this work. When chloroform and methanol extracts were fractionated by column chromatography over silica gel, three compounds form chloroform extract fractionation and one compound from methanol extract fractionation were isolated and purified by PTLC and further analyzed by IR, NMR, and UV spectrophotometers for their structural information.

In recent years, although technology and medicine have developed extensively due to decrease in natural richness and other drawbacks, some countries have made it obligatory to use natural products for many goals [1], Ethiopia is becoming one amongst them. For this reason we have chosen a medicinal plant *S. xanthocarpum* which is claimed to treat TB, locally known as samba nekersa for the study of its natural constituents. Hence, 170 gm. of powdered fruit of *S. xanthocarpum* when successively extracted with petroleum ether, chloroform, ethyl acetate, and methanol gave *a* maximum yield, 17.6 gm., 10.4%, for the methanol extract and none of the other gave a yield that exceeded 1.1gm (the amount obtained from chloroform extract), is an indicative of the plant having more proportion of polar compounds. The presence of these secondary metabolites detected in different solvent extracts of fruits of *S. xanthocarpum* is consistent with the previous reports. Almost entirely, literature on the isolation of natural products from this plant reports that the plant has steroidal saponins and steroidal glyco-alkaloids. And compound SX-13 of the methanol extract reveals this fact. It is a steroidal saponin with a furostane aglycon skeleton. Compound $SX_3$ that was isolated from chloroform extract is a 1, 2 di-substituted phenyl ester having a ketone function at $C_9$. We don’t find literature reports that this kind of compound, a 1,2 di-substituted phenyl ester or even amide, is isolated from the plant *S. xanthocarpum*.

## 7. Conclusion and Recommendation

The fruits of *S. xanthocarpum* are the source of natural products; carbohydrates, fixed fats and oils (which are detected in only petroleum ether extract), proteins, amino acids, saponins, phenolic, tannins, flavonoids, alkaloids and glycosides whose presence and/or absence are confirmed by the methods employed in this work. Although plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock since ancient times, research works must proof the traditional claims that this plant's , *S. xanthocarpum,* aqueous fruit extract can be used for treating bacillus tuberculosis (locally known as samba nekersa). Although the most common natural steroidal saponin are from spirostane parent skeleton in which case $C_{22}$ connected to two oxygen atoms usually resonates in a narrow spectral width between 108.9 and 110.0 ppm (20, 23), the lack of signals not only even in the vicinity of this narrow band, but also from 103 to 127 ppm in our $^{13}C$ spectrum led us to the conclusion that our compound (SX-13) is of a furostane parent skeleton in which case $C_{22}$ resonates at 97.19 ppm. Because of money and time constraints we only isolated and partially characterized two compounds, one from methanol extract and one from chloroform extract. Thus we hope our study titled "phytochemicals investigation of fruits of *S. xanthocarpum"* will help other researchers who are interested to isolate and characterize more constituents from *S. xanthocarpum* plant.

**References**


1. Endashaw Bekele, Study on Actual Situation of Medicinal Plants in Ethiopia (Japan Association for International Collaboration of Agriculture and Forestry; December 2007

2. Mazen E. and Jehad A.**,** Manual of Phytochemistry**.** Al Azhar University Faculty of Pharmacy Department of Pharmacognosy, 2010. P. 7-8.

3. Farnsworth N., Soejarto D., Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H, editors. The Conservation of Medicinal Plants. *Cambridge University Press*, Cambridge; 1991. p. 25–51.

4. Muralidharan S., Rajan S. and Danaphal S. P., Simultaneous Estimation of Plumbagin and Embelin by Reverse Phase-High Performance Liquid Chromatographic method, 2009*,* 1(1): 135-142

5. Okram Mukherjee S. and Thokchom Prasanta S., Phytochemistry of *Solanum xanthocarpum*: an amazing traditional healer, *Journal of Scientific & Industrial Research,* 2010, pp. 732-740.

6. Sachin P., Amit G., Navin S., *Solanum xanthocarpum* (Yellow Berried Night Shade): A review. *Der Pharmacia Lettre*, 2010, 2(4): 373-383.

7. Smit H., Woerdenbag H., Singh R., Meulenbeld G., Labadie R., Zwaving J., Ayurvedic herbal drugs with possible cytostatic activity. *J. Ethanopharmacol* 1995; 47: 75- 84.

8. Mayer B., Ferrigni N., Putnam J., Jacobsen L., Nichols D., McLaughlin J., Brine S., A Convenient General Bioassay for Active Plant Constituents. *Planta Med* 1982; 45(5): 31-34.

9. Dixit V., Gupta R., Antispermatogenic/antiandrogenic properties of solasodine (C27H43O2N) obtained from *solanum xanthocarpum* berries on the male genital tract of dog (Canis-familiaris). A Histophysiological approach. *Int J Androl* 1982; 5(3): 295 307.

10. Heble M., Narayanaswami S., Chadha M., *Am Assoc Adv Sci Stable* 1967; 161(13): 1145.

11. Sato Y., Latham J., The Isolation of Diosgenin from *Solanum Xanthocarpum. J Am Chem Soc* 1953; 75(23): 6067-6067.

12. Lu Y., Luo J., Kong L., Steroidal alkaloid saponins and steroidal saponins from *Solanum surattense*. *Phytochemistry* 2011; 72(7): 668-673.

13. Sachin P., Amit G. and Navin S., *Solanum xanthocarpum* (Yelow Berried Night Shade): A review. *Der Phrmacia lettere*, 2010, 2(4): 372-383

14. Kusano G., Beisler J., Sato Y., *Phytochemistry* 1973; 12(2): 397-401.

15. Singh O., Subharani K., Singh N., Devi N. and Nevidita L., Isolation of steroidal glycosides from *Solanum xanthocarpum* and studies on their antifungal activities. *Nat Prod Res* 2005; 21(7): 585-590.

16. Divya K., Mayak G., Amit K., Gopal N., Yamini B., Tripathi J., Tiwari S., Comparative Evaluation Phenol and Flavonoid Content of Polyherbal Drugs. *Pharmacologyonline* (2011) 3: 1365-1373.

17. Nasir E, Ali S., Flora of Pakistan, Pakistan Agricultural Research Council Islamabad. 1985; 168:1-3.

18. Parveen F., and Rahat A., Isolation of a New Steroidal Glycoalkaloid from *solanum xanthocarpum*, *Journal of Basic & Applied Sciences,* 2014, *10,* 28-32.

19. Poul R. and Datta K., An Ubdated Overview on *solanum xanthocrarpum* Schrad and wendl, *IJRAP* 2011, 2(3) 730-735.

20. Pawan K., A SYSTEMATIC NMR APPROACH FOR THE DETERMINATION OF THE MOLECULAR STRUCTURES OF STEROIDAL SAPONINS, Central institute of Medicinal and Aromatic Plants, *Lucknow* – 226 015, INDIA.

21. Agrawal P.k., Pathak A.K., NMR spectroscopic approaches for the determination of interglycosidic lincage and sequence in oligosaccharides, *phytochem. Anal.,* (2014) 7: 113-130.

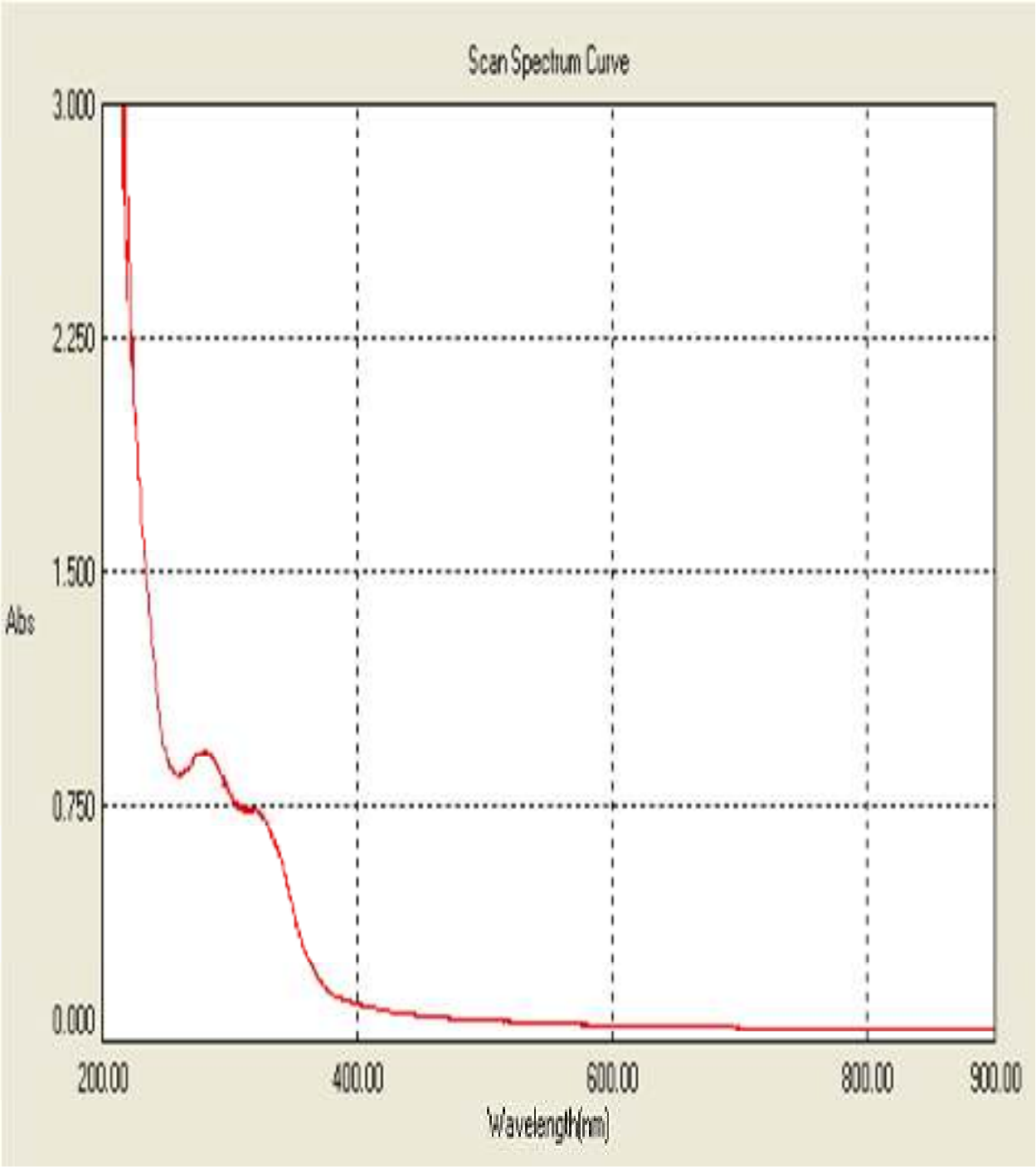
22. Chen S. and Snyder J.k., Diosgenin-bearing molluscidal saponins from *Allium vineale*: an NMR approach for the structural assignment of oligosaccharide units. *J. Org. chem.*, (1988) 54:3679.

23. Jacek W., Morzycki, Katarzyna P., Karolina D., Izabella J., Leszek S., Iwona W., $^{13}C$ NMR study of spirostanes and furostanes in solution and solid state, Institute of Chemistry, University of Bialystok, Pilsudskiego 11/4, Bialystok (2005) 15-443, Poland.

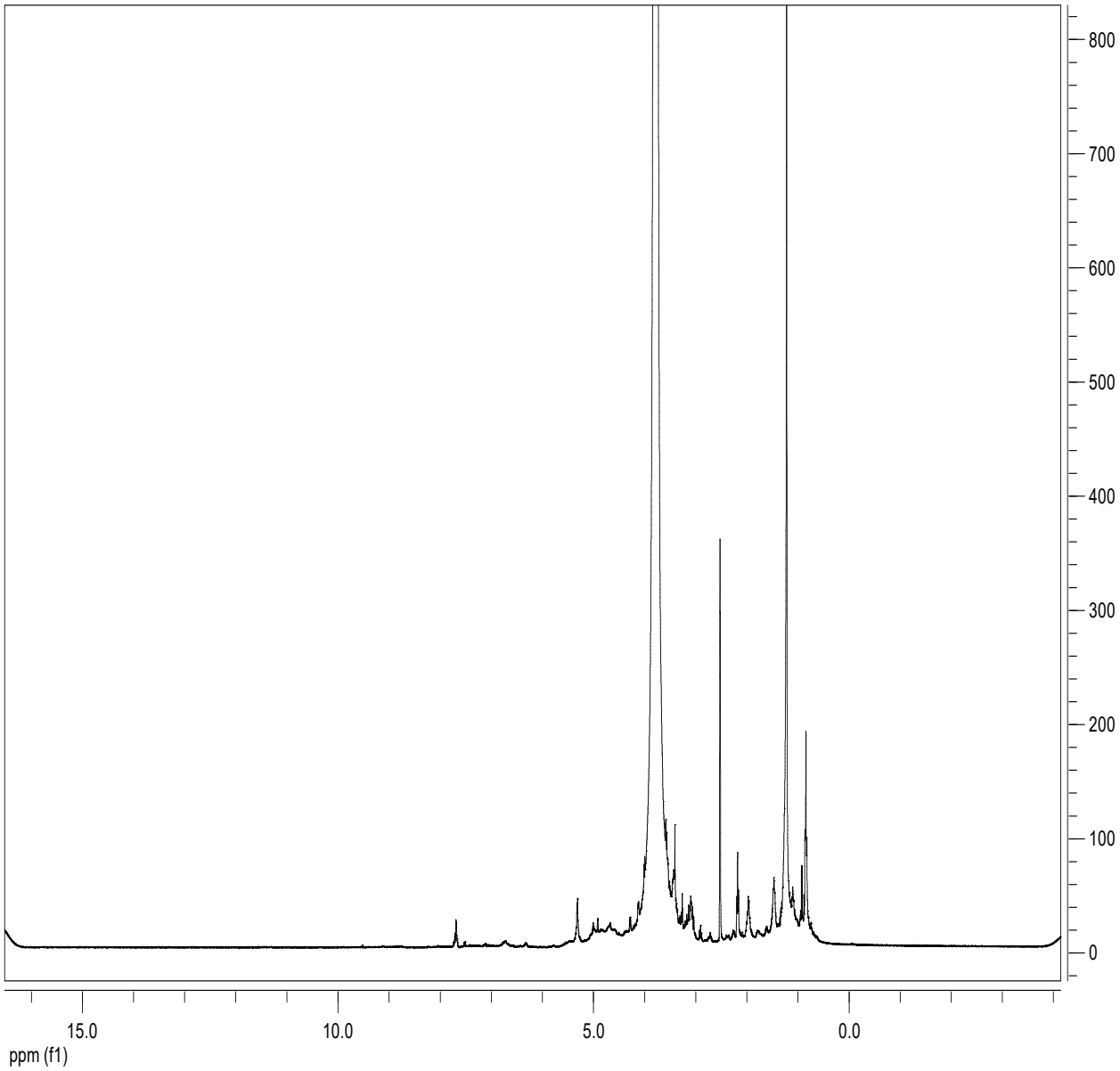
24. Agrawal P., Jain D., Gupta R., Thakur R.., Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins., *Phytochemistry*, (1985) *24*, 2479-2496.

## Appendix

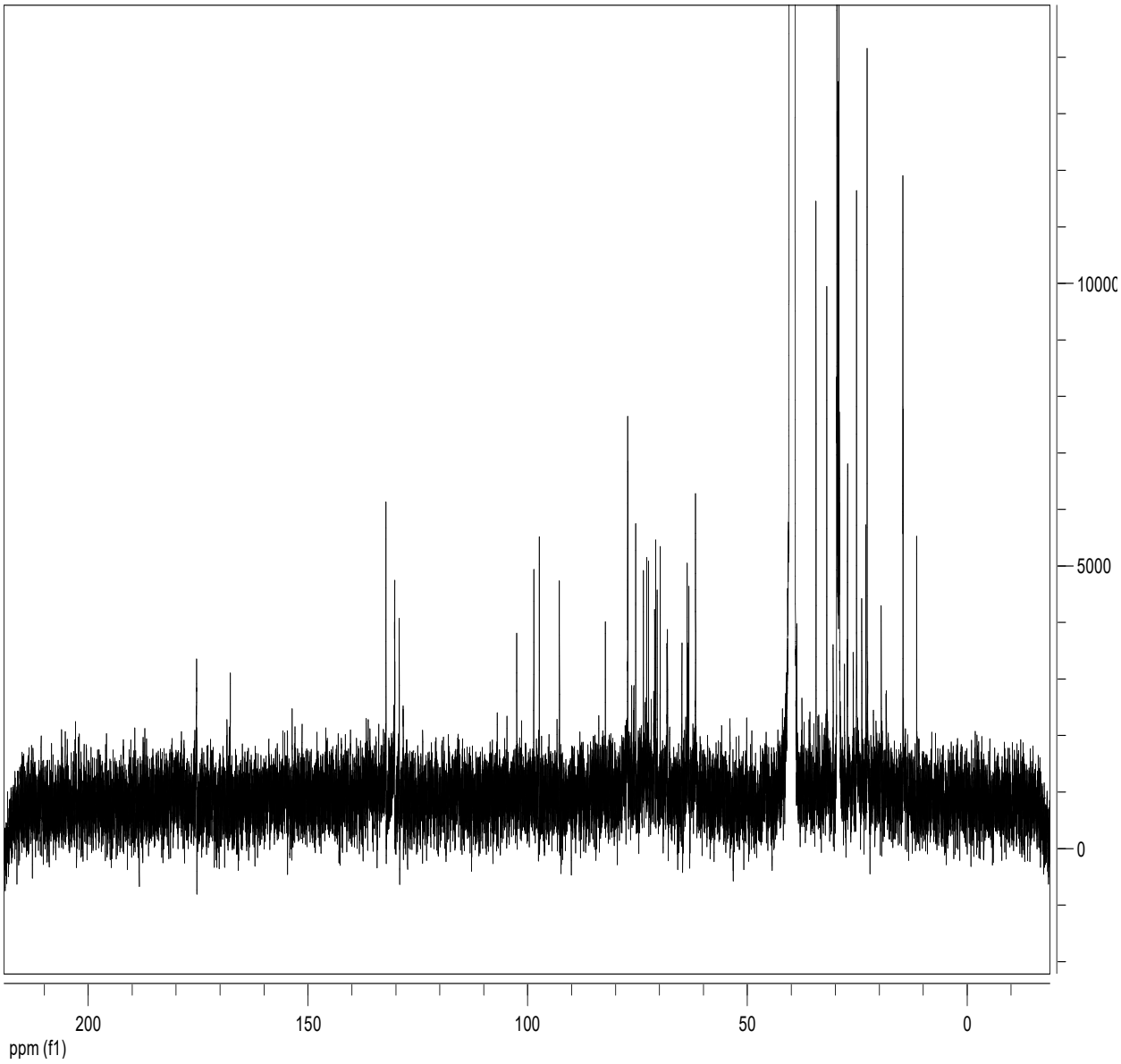
Appendix 1: UV-Vis spectrum of $SX_{-13}$ in methanol



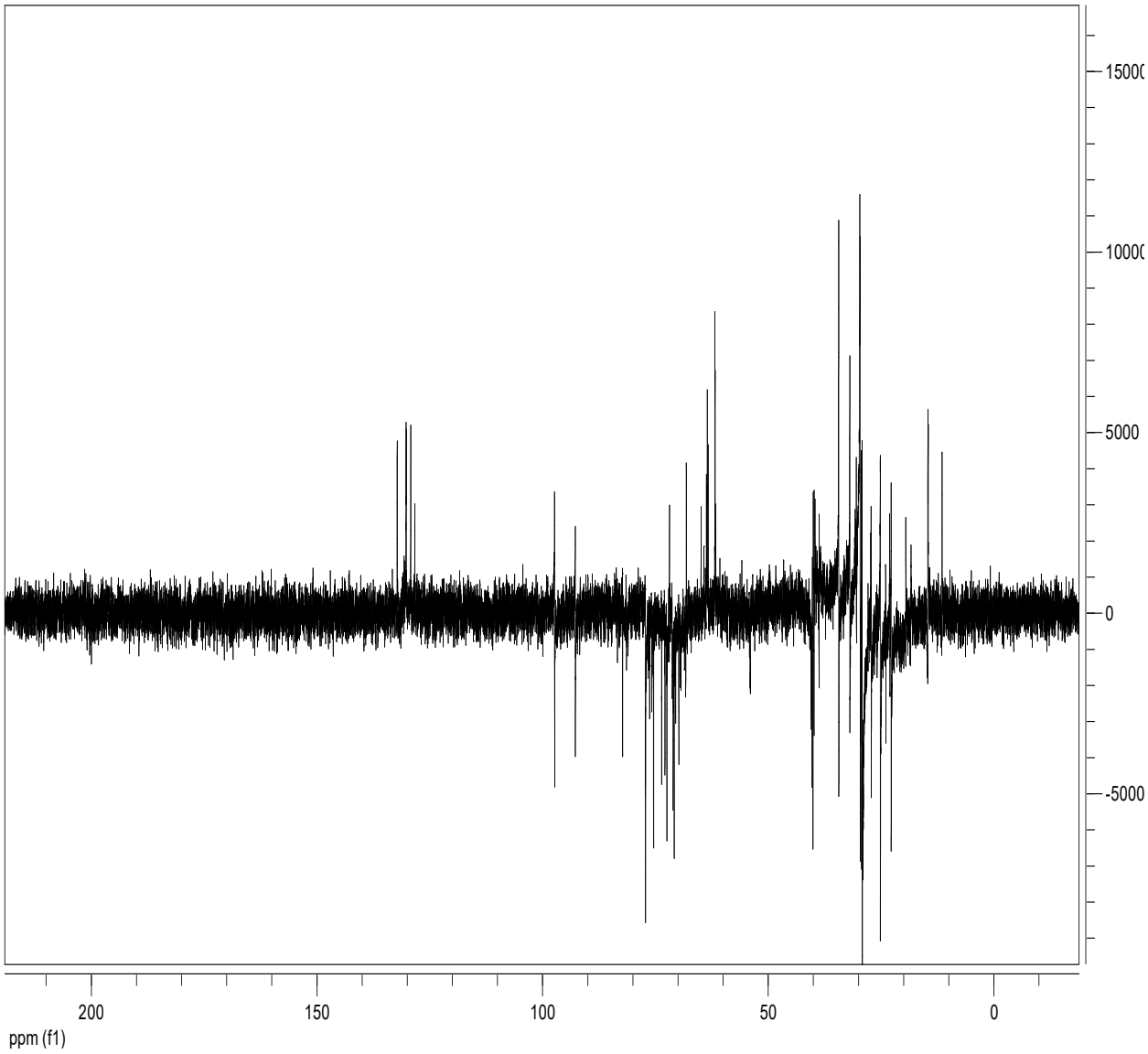
Appendix 2 $^{1}H$ NMR spectrum of $SX_{-13}$ in DMSO-6



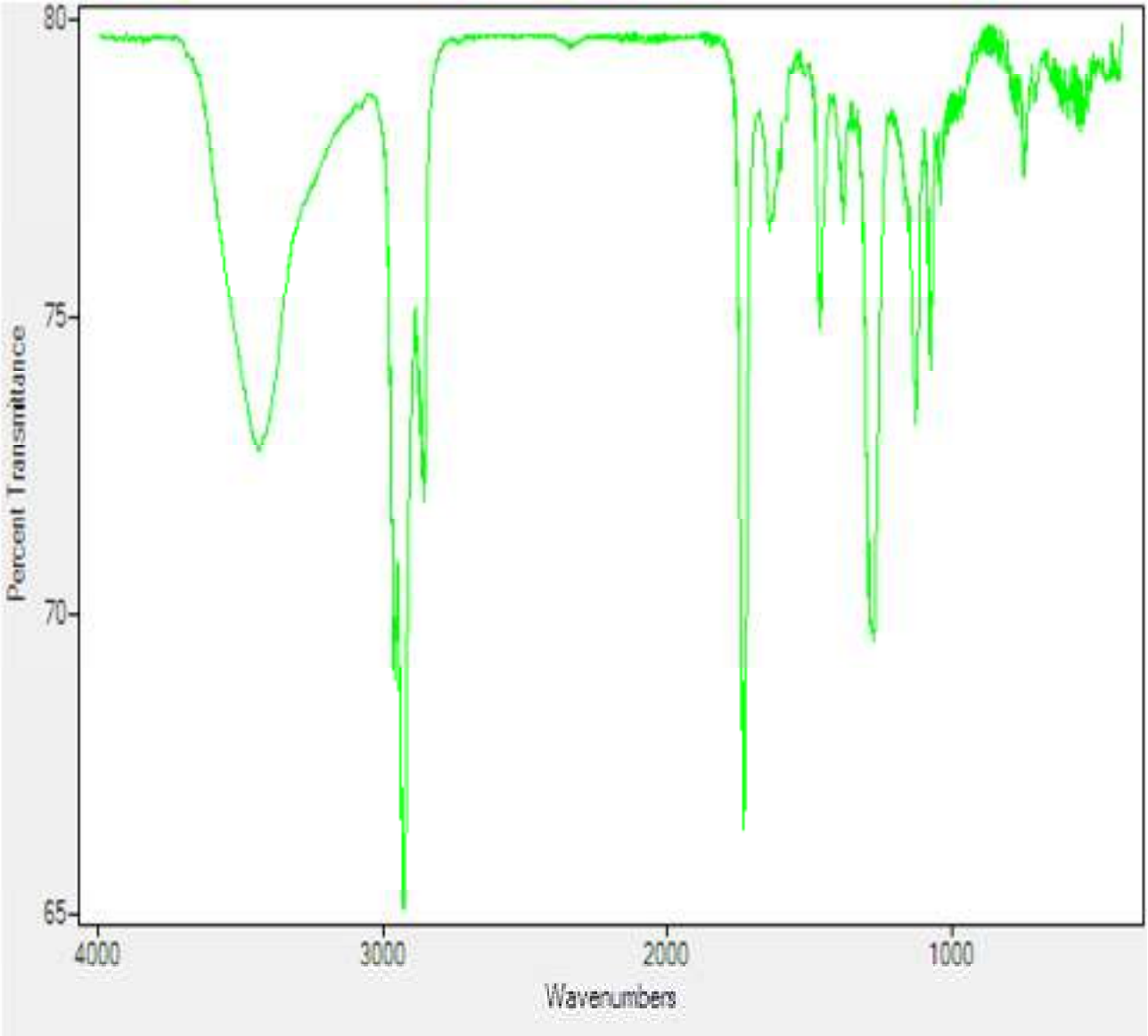
Appendix 3: $^{13}C$ NMR spectrum of $SX_{-13}$ in DMSO-6



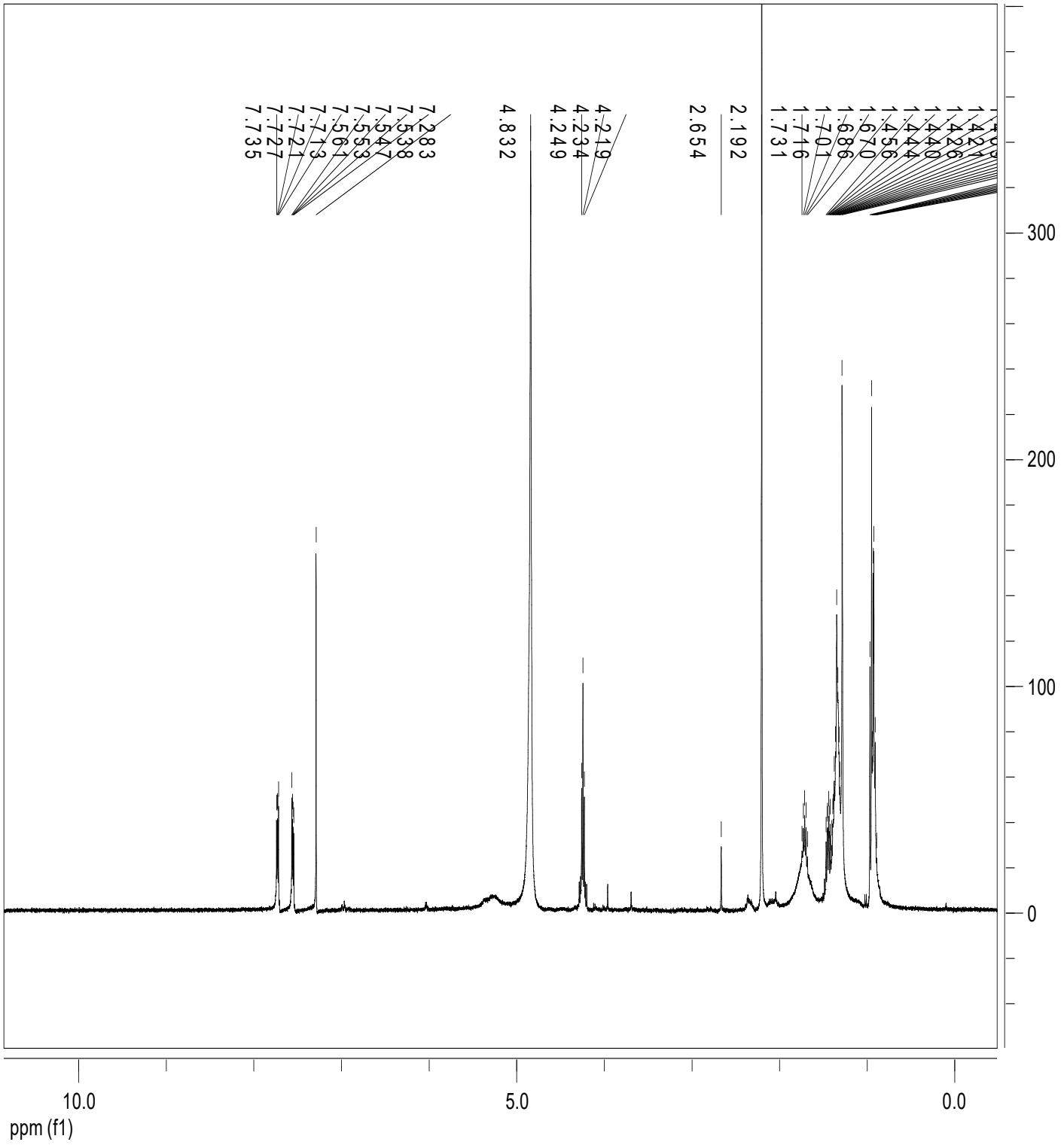
Appendix 4: DEPT-135 spectrum of $SX_{13}$ in DMSO-6



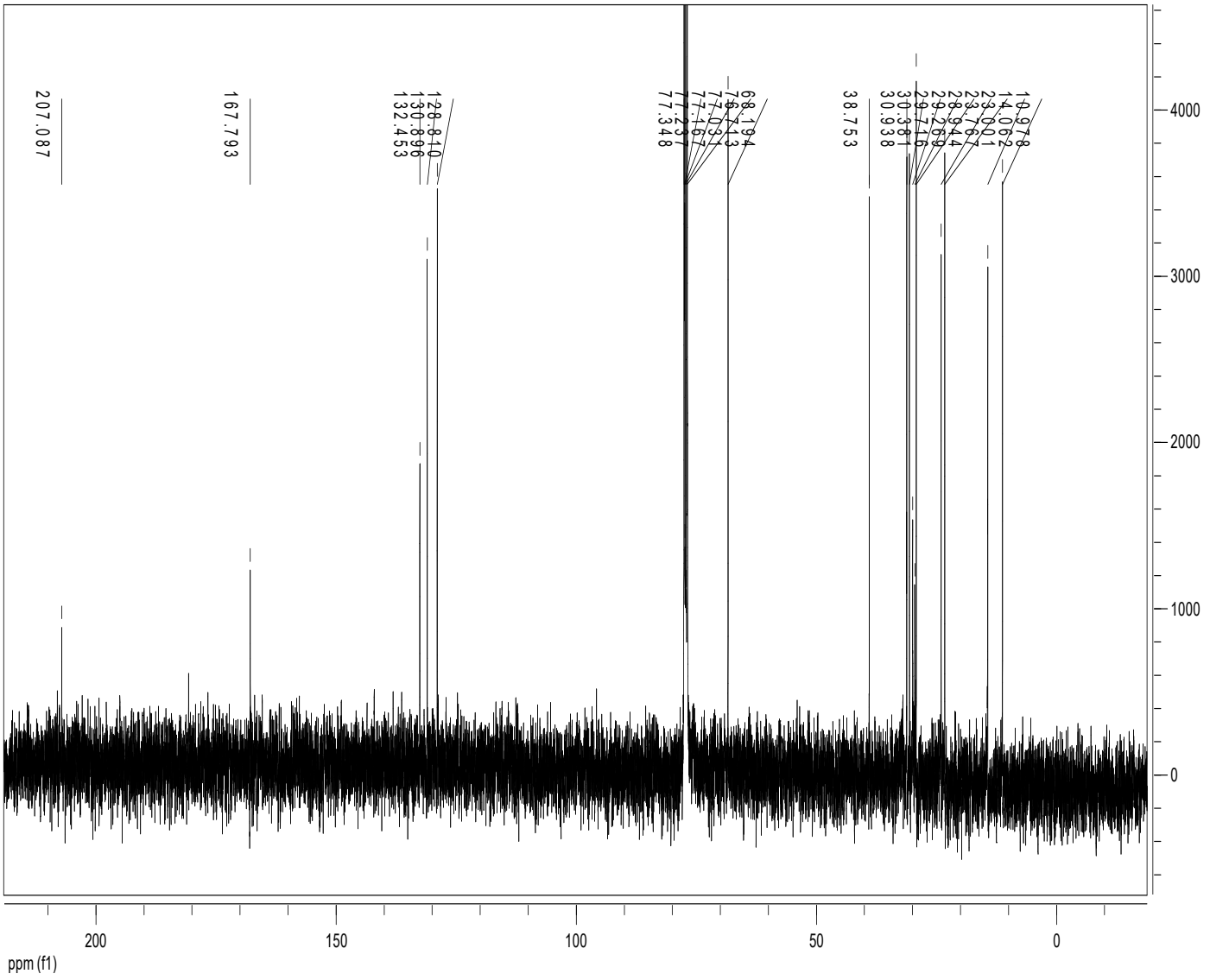
Appendix 5: IR spectrum of $SX_3$



Appendix 6: $^{1}H$ NMR spectrum of $SX_{-3}$ in $CDCl_3$



Appendix 7: $^{13}C$ NMR spectrum of $SX_3$ in $CDCl_3$



Appendix 8: DEPT-135 spectrum of $SX_{-3}$ in $CDCl_3$