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Organic Chemistry Stream

Phytochemical Investigation of extract of *Andrachne aspera Roots*

Thesis submitted for the fulfillment of Master of Science in Chemistry

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SYMBOLS AND ABBREVIATIONS

NMR …… Nuclear Magnetic Resonance

IR …… Infrared

UV ……. Ultra Violat

PTLC …… Preparative thin layer Chromatography

TLC …….. Thin layer Chromatography

DMSO …….. Di-methyl sulfoxide

ppm ……... Parts Per Million

δ (delta) ……. Chemical shift value

I …… Iodine

KI ……. Potasium Iodide

d……Doublet

s…….Singlet

m……Multiplet

t…….Triplet

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Abstract

Andrachne aspera, is a small perennial under shrub, traditional medicine plant used for treating various ailments. In this work aerial and roots part of the plant is collected from grassy land of mountain in Boset Woreda, East Shoa Zone, Oromia region particular place called Usade Mountain. The plant locally is known as "Koricha-hadha" (Oromifa), where its root is used for wound healing.

The crude methanol extract was partitioned with solvents and subjected to column chromatography to yield two compounds.

Phytochemical screening test was done for crude extracts and the result indicated the presence of alkaloids, tannins, terpenoids and saponins in the methanol extract but absent in ethyl acetate and n-hexane extract. The structures of compounds were determined as sucrose and cellobiose using NMR and IR spectroscopic techniques.

Key words:

- *Andrachne aspera, Euphorbiaceae, Sucrose, Cellobiose.*

1. Introduction

1.1Background of the study

The use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries like Africa [1]. The World Health Organization (WHO) reported that 80% of the world's populations depend on traditional medicine and a major part of traditional therapies involve the use of medicinal plant extracts or their active constituents [2].

Scientific studies of Ethiopian plants have shown remarkably effective medicinal values for many ailments and health care needs throughout the country [3]. Most of the Ethiopian population depended on traditional medicine for their health-care delivery and most of this comes from plants [4-5].

1.2 Botanical Description

Genus *Andrachne* belongs to the family Euphorbiaceae. About 15 species of this genus are known. The plants of this genus usually, herbs under shrubs that potentially novel as medicinal plant in some part of the world. The species *Aspera sprengi*s realized to have two subspecies including var.*aspera* and var. *glandulosa.*

Var. *aspera* is open deciduous woodland or bush-land, often growing in rock crevices and stretching from drier areas to 2250 m in Gamo Gofa, Gojam, Tigray regions in Ethiopia; and too in Eritrea as far west as Morocco. Var. *glandulosa* is an open deciduous woodland or bush-land, often growing in rock crevices at an altitude of 1000-2400m. [6]

Andrachne aspera spreng is known in Boset woreda/E/shoa zone, by the vernacular name "Koricha-hadha" and belongs to the family Euphorbiaceae. It grows at an altitude of 1000-2400m in mountain Usade. The plant *Andrachne aspera* is also found in India, Iran, Saudi Arabia and tropical Africa [7].

Figure 1 *Andrachne aspera* plant (photo taken by the author)

1.3 Significance of the study

Several medicinal plants are traditionally used in Ethiopia for the relief of different kinds of ailment. This work relates to isolation and structural studies on the chemical constituents of *Andrachne aspera* roots. Therefore, the result of this study may be used:

- To know the major constituents of roots of *Andrachne aspera* plant.

1.4 Back-ground and Justification of the study

Wound care is constantly evolving with the advanced in medicine. Search for the ideal dressing material still continues as would care professionals are faced with several challenges. Due to the emergence of multi-resistant organisms and decrease in newer antibiotics, would care professionals have revisited the ancient healing methods by using traditional and alternative medicine in would management. People's perception towards traditional medicine has also changed and is very encouraging. The concept of wound healing has been well accepted and traditional medicine has also incorporated this method to fasten the healing process. Several studies using herbal and traditional medicine from different continents have been documents in wound are management [8].

The importance and efficiency of traditional and complementary medicine have risen. Almost four billion people in world-wide use plants as medicines as nothing else in affordable or available.

1.5 Statement of the problem

Analysis of all approved therapeutic agents between 1981 and 2010 indicated that about 405 of drugs are either natural products (NPs) or natural product-derivates (NPDs). Natural products tend to present more structurally diverse 'drug-like' and 'biologically friendly' molecular qualities than pure synthetic compounds. In addition, they are important source of structures for the synthetic and combinational chemistry.

Plants have a long history of use as adjuvant (Ho and Cheong, 2014), prevention (Desa, et *al*… 2008; singh et *at*., 2012, Ji et al., 2014) and treatment (cragg and Newman, 2003). Their use led to the discovery to many novel chemotherapeutic types showing a range of cyclotoxic activities (Qurishi at al, 2011). In this regard cultural medicinal plant 'Korcha-Hedha' or *Andrachne aspera* plant root that served to treat would and it has it own contribution to used interchangeable with modern medicine for healing wound in a place where a health-care system is not accessible. Hence, tangible and effective consistent investigation should be carried-out on this plant widely for further benefit of human being.

1.6. Objective

1.6.1 Main Objective

- To investigate the chemical constituents present in the root of *Andrachne aspera.*

1.6.2 Specific objectives

- To extract the root of *Andrachne aspera* using different solvents and compare their yields.
- To isolate compounds from the crude extracts using column chromatography and PTLC.
- To elucidate structures of isolated compounds using different spectroscopic technique.

2. Review of Related Literature

2.1Medicinal value of *Andrachne aspera*

Before the advent of modern medicine, people of all continents have used medicines from plant origins since pre–historic times.

Ancient Egyptian medicines of 1000 BC are known to have used garlic, opium and other herbs for medicine. Likely in India, ayurvedic medicine has used many herbs such as turmeric possibly as early as 1900 BC. [9]

The use of herbal medicine in wound care has been very encouraging and several researchers around the globe have been strived to exposed their results for the benefit of human being.
Medicinal plants belonging to the genus *Andrachne* are very often used in traditional medicine. For example, medicinally, this plant is used to improve eyesight and treat eye sores in Pakistan. In related manner herbal and traditional medicine from different continents have been documented in wound care management [10-11].
Generally, it's clear that traditional medicine from different parts of plant extract can be used for wound management in certain conditions; in case of the escalating cost of health care particularly in wound management. It's advisable economically to use traditional medicine like *Andrachne aspera* plant root to treat wound. However; large randomized clinical trials are necessary to give more concrete evidence supporting the use of *Andrachne aspera* plant root in wound healing management [12-13].

2.2 Phytochemicals in *Andrachne species*.

a) Alkaloids

Alkaloids isolated from *Andrachne aspera* areal part used to enhance the eye sight and to treat eye sores in Pakistan. As known alkaloids are basic substances that contain one or more nitrogen atoms, usually in combination or as part of a cyclic system [14].

Table 1: Some alkaloids and their pharmacological activities

S.No	Alkaloid	Source	Pharmacological Activity
1	Morphine	*Opium*	Analgesic narcotic
2	Codine	*Opium*	Analgesic strongly addictive
3	Heroine	*Opium*	Analgesic nonaddictive
4	Quinidine	*Cinchona*	Antiarrythmic of ventrical
5	Ajmaline	*Rauwolfia*	Antiarrythmic of auricles
6	Nicotine	*Nicotana*	Respiratory stimulant

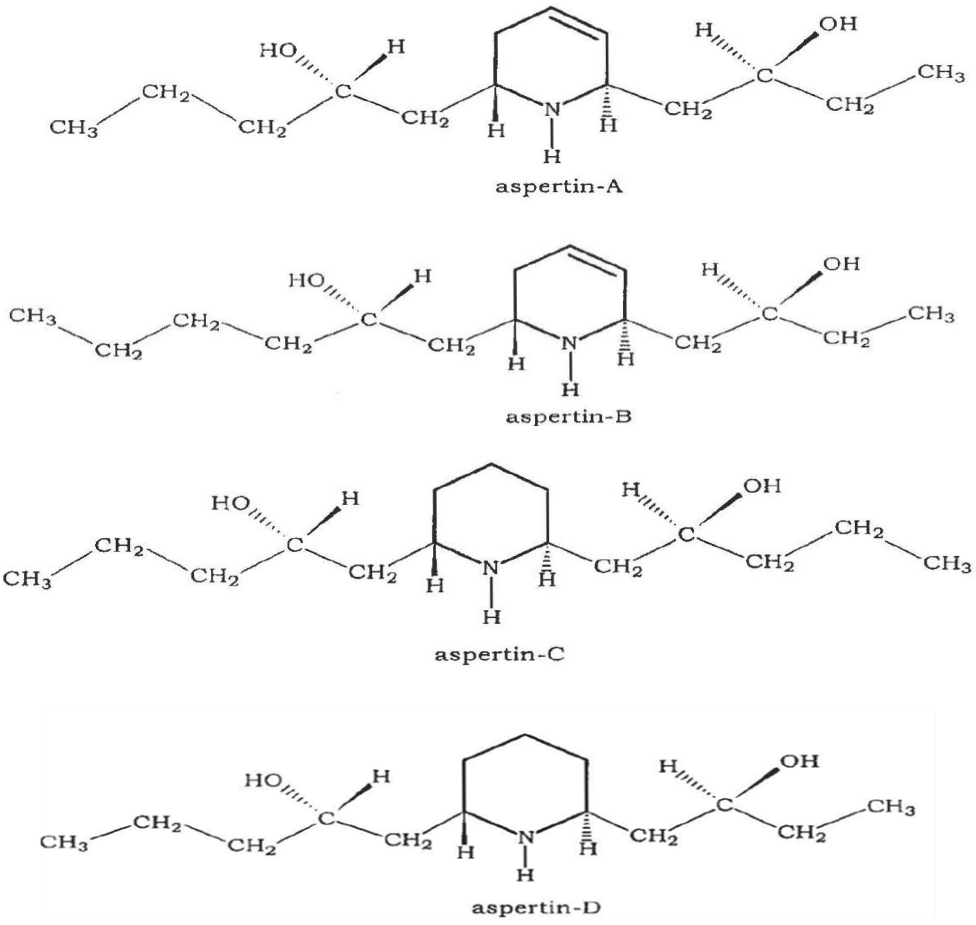


Figure 2 Structures of some alkaloid reported from *Andrachne aspera*

b) Terpenes

Previously one of the known would healing component terpene was isolated from the areal part of *Andrachne aspera* plant (by Kemal A, PhD thesis, University of Karach: Pakistan, 2001).

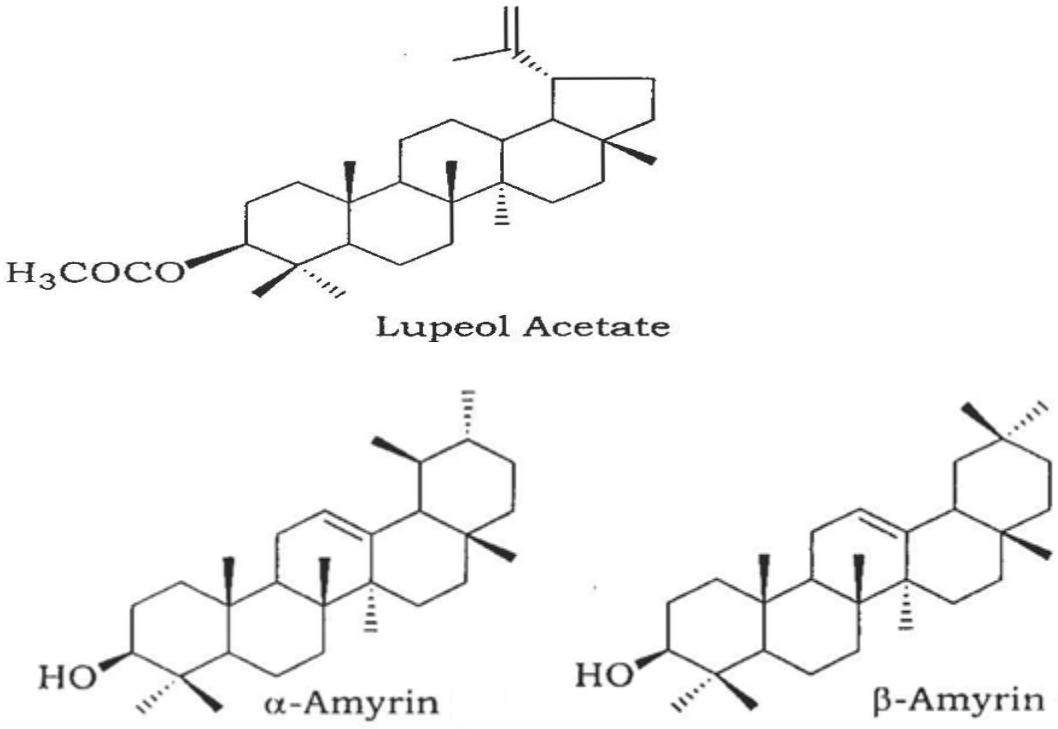


Figure 3 Structures of some terpenes

3. Materials and Method

3.1Materials and chemicals

Materials: The materials used in the experiment were: Electric grinder, Electronic balance, Rotary evaporator, Iodine chamber, PTLC-jar, TLC-chamber, Oven, toung, spatula, Glass rod, IR, NMR (^{1}H, ^{13}C) and Dept-135 spectra were recorded on a Bruker Avace 400 MHz spectrometer with DMSO-d as a solvent, UV, small and large column.

Chemicals: The chemicals applied in the experiment were: Methanol, chloroform, n-Hexane, distilled H_2O, Ethyl acetate, Iodine, DMSO, Acetone, diluted H_2SO_4, KI, $HgCl_2$, NaOH, EtOH, $FeCl_3$, concentrated H_2SO_4.

3.2. Collection and Identification

Roots of *Andrachne aspera* was collected from Oromia regional state, E/shoa zone, Boset woreda, Ethiopia, in particular place called Usade Mountain, in May 2015. The specimen has been deposited at the National Herbarium of Addis Ababa University.

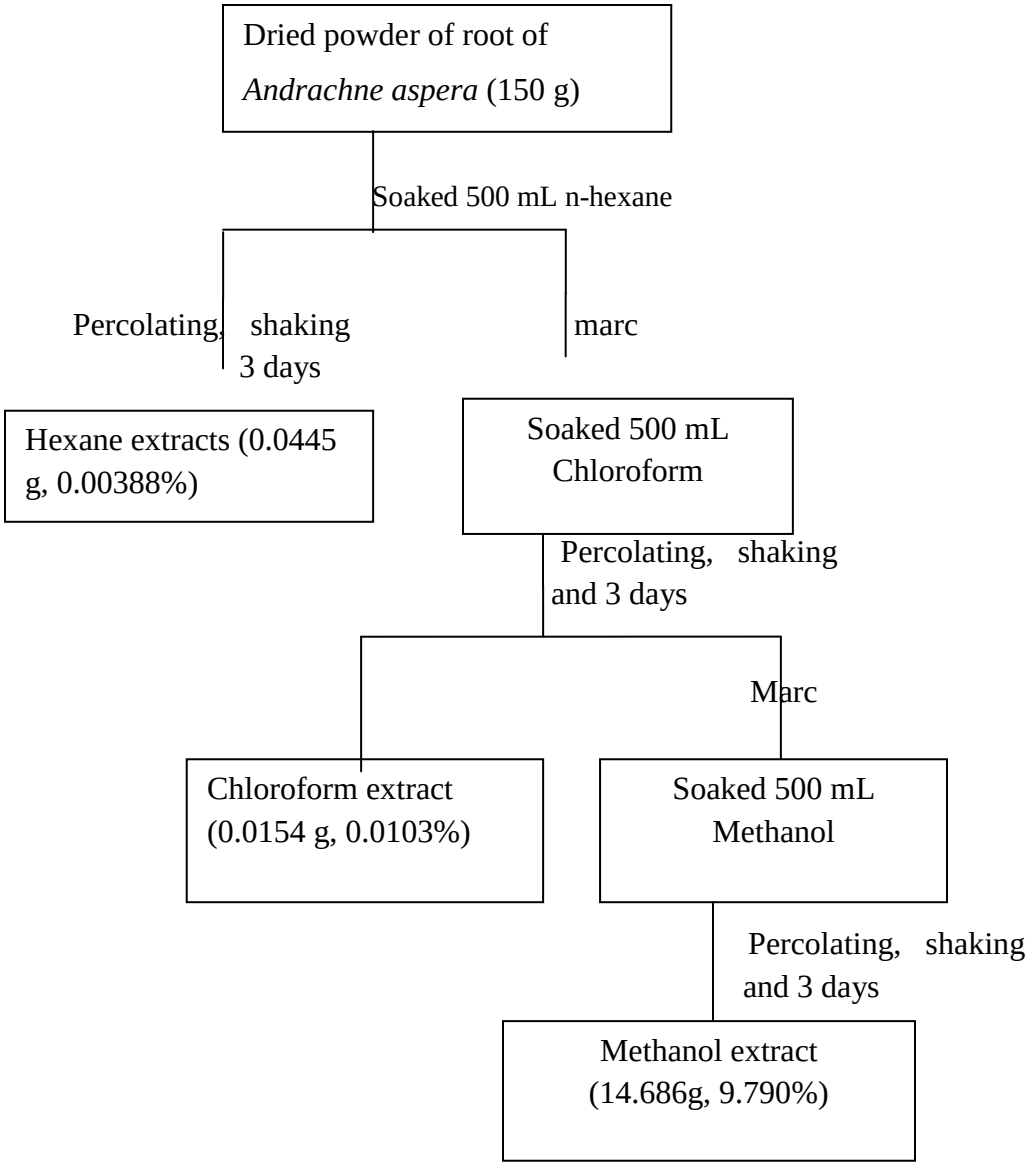
3.3. Extraction of the plant

Andrachne aspera roots were collected, washed and placed in a neatly place. Then, the root left to dry at room temperatures under shade region where an excessive air circulation available.

Dried *Andrachne aspera* roots were milled and homogenized using an electrical grinder after crushed in a mortar and pestle. 2 kg of *Andrachne aspera* root was subjected for this purpose and 703.27g of the powdered form was taken for extraction work.

3.3.1. Extraction

The powdered root of *Andrachne aspera* (150 g) extracted initially by percolation of sample in 500 mL n-hexane for three consecutive days by occasional shaking. The filtrate collected after filtration using Whatman No.1 filter paper and dried using rotary evaporator and yielded 0.004%. The mare left after n-hexane was successively extracted with methanol, chloroform and gave 9.8%, 0.1% and yields. The methanol extract is the highest which means around more than ten folds than the other solvents. Hence the most constituents of the extract indicates polar.[15]



Scheme 1 Extraction of the roots of *Andrachne aspera*

3.4. Preliminary Phytochemical Screening

Screening of the *Andrachne aspera* methanol extract was done to detect the presence of alkaloids, terpenes, tannins, sterols and saponins [13] as follows

3.4.1Alkaloids tests were performed

Mayer reagent

A white to yellowish precipitate was revealed on combining the solutions of $HgCl_2$ and KI in addition of a few drops acidified extract solution.

Wagner reagent

After dissolved 1.27g I_2 and 2.9 g KI in 20mL water, abrown precipitate in acidic solution was suggested the presence of alkaloids.

3.4.2 Terpenoid test

Baljet reagent

The reaction of picric acid and NaOH solutions indicated deep red color on adding 2-3mg of sample.

3.4.3Tannins test (polyphenols)

(Ferric chloride): by adding $FeCl_3$solution to an extract sample, blue-green color produced in the presence of polyphenols.

3.4.4 Sterols test

Salkowski reaction: This screening was carried-out dissolving 1-2mg of the sample in 1mL $CHCl_3$ and 1 mL of concentrated H_2SO_4, forming two phases (layers). Hence; a red color indicating the occurrence of sterols.

3.4.5 Saponins:-several foam was observed when an aqueous solution of a sample shaken well indicating the presence of saponins.

3.5 Thin layer chromatography (TLC) analysis

The crude methanol extract of *Andrachne aspera* was applied on TLC plates coated with silica gel .These plates were developed in chromatography chamber containing hexane, ethyl acetate mixtures (6:4). The developed plates were air dried and visualized under UV light at 254 and 365 nm. [16-18]

3.6. Isolation

According to the TLC analysis using n-hexane/ethylacetate (6:4) and methanol to chloroforn (8:2) have displayed relatively good separation. Depending on this ratio of solvent selected column chromatography was packed with n-hexane after adsorbing the methanol extract on silica gel. The extract was applied on column and eluted with increasing polarity of solvent mixtures.

4. Results and Discussion

Elucidation of the obtained compounds have been done by using UV-visible, infrared (IR), ^{1}H and ^{13}C NMR spectroscopy [19]. From these IR and NMR spectra revealed effectiveness, whereas UV was not active. In former Isolation and characterization of areal part of *Andrachne aspera* used for treating eye sore and to improve eye sight [20].

4.1.Preliminary Phytochemical Analysis

Analysis of the plant extracts revealed the presence of phytochemicals, such as alkaloids, saponins, tannins, sterols, terpenoids in methanol extract.

Table 2. Phytochemical Analysis for methanol extract of *Andrachne aspera*

Extract	Alkaloid test		Sterols test	Flavonoids	Tannins	Saponins	Terpenoids
	Mayer's	Wagner					
Methanol	++++	++++	+++++	-----	++++	++++	++++
Ethylacetate	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-

4.2 TLC Analysis of extracts

4.2.1. TLC analysis of Methanol Extract

Methanol extracts were analyzed by TLC using n-hexane: EtOAc (6:4) and Methanol: Cl (8:2) as eluent spots were detected using vanillin reagent.

4.2.2. Isolation of compounds

4g of methanol extract was dissolved in 200 mL of ethyl acetate using a magnetic stirrer. Then filtered and concentrated using rotary vapor.

Wet method purification mechanism has been applied in 19/26 column size, 60-120 mesh silica gel. 1.24 g of ethyl acetate extract was adsorbed on 1g of silica gel and charged on to column packed with 100 g silica gel using hexane. The column was eluted using the following solvent systems: n-hexane 100% first followed by increasing polarity with addition of Chlorofrom, 10.00, 20.00, 30.00, 40.00, 50.00, 60.00, 70.00, 80.00, 90.00 and 100.00 mL to give 26 fractions. But a single spot was obtained on the solvent system ratio of MeOH:$CHCl_3$ (20:1) in fraction24 which yielded 18.6 mg pure compound.

Fig. 4 Column chromatography of methanol crude extract

4.3. Characterization of isolated compounds

4.3.1. Characterization of compound 1

A white crystalline compound was isolated using CC from ethyl acetate extract. TLC analysis using methanol /chloroform (20:1) showed a single spot with Rf = 0.7 staining red in vanillin reagent.

Its IR spectrum showed an absorption band at 3436 cm^{-1} due to the presence of –OH stretching. The strong absorption band at 2980 and 2990 cm^{-1} were suggestive of aliphatic C-H asymmetrical and symmetrical stretching vibrational frequencies respectively. The absorption band at 1025 cm^{-1} were due to C-O stretching in the molecule (Appendix D).

In the ^{1}H NMR of compound revealed anomeric proton of the glucopyranosyl ring is observed at δ= 5.21 ppm (d) and oxygenated methylene at 4.25 (2H,s) as a result of coupling with H-2g proton and hydroxyl proton), since this proton carbon bonded to two electronegative oxygen it is the one which assigned further downfield in the spectrum. The other proton signals assigned for glucose is at δ = 3.45 which is 4g proton, a triplet due to coupling with protons at 3g and 5g. Likewise signal at δ = 4.1 is assigned for 4f fructose proton which is also a doublet as a result of coupling to proton 3f and 5f. A doublet at 4.2ppm is for 3f proton of fructose which is found on the same carbon of the hydroxyl oxygen. A multiplet for 5f and 5g protons (most probably a quartet at δ=3.82 and 3.9 for 5g and 5f protons due to coupling with 6g and 6f CH_2 protons and 4f and 4g protons. The fructose quaternary carbon which is adjacent to hydrogen 1f have no proton NMR spectrum. The assignments of the 1H signals of sucrose which was obtained from the literature (Timmermans, Waard, Tournois & Leeflang, 1993) is identical to the value of the spectrum under investigation.

The ^{13} C NMR and DEPT-135 signals of the compound are in a good agreement with those sucrose reported in the literature.

This compound is a disaccharide sugar and ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data of the compound is given below.

Table-3 ^{1}H**,** ^{13}C NMR andDEPT-135 of compound **1** (sucrose)

C. No	^{1}H δ value)	Type of C	^{13}C (δ value)	DEPT (δ value)
C-1	-	C	104.43	-
C-7	5.21 (d)	CH	92.17	92.18
C-5	3.82 (m)	CH	82.95	82.96
C-3	4.25 (d)	CH	77.41	77.46
C-4	4.1 (d)	CH	74.67	74.70
C-9	3.75 (d)	CH	73.26	73.29
C-11	3.9 (m)	CH	73.20	73.23
C-8	3.58 (d)	CH	72.04	72.06
C-10	3.45 (t)	CH	70.21	70.24
C-6	3.82 (s)	CH_2	72.58	62.58
C-2	3.65 (s)	CH_2	62.46	62.48
C-12	3.81(d)	CH_2	60.85	60.87

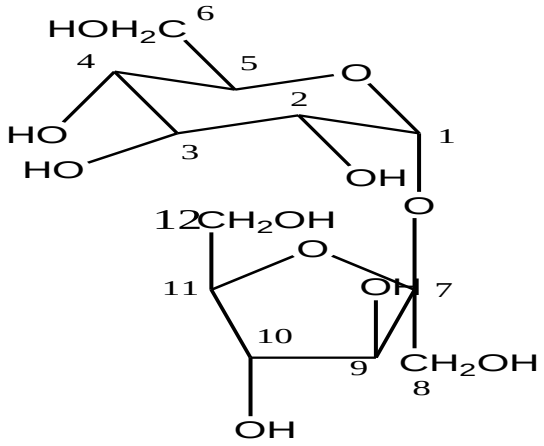


Fig 5: Proposed structure of compound **1** (sucrose)

4.3.2. **Characterization of compound 2**

Compound **2** (amorphous) was obtained from repeated CC of Methanol extract eluting the column with chloroform: water (1:1). Its IR spectrum showed an absorption band at 3434 cm^{-1} due to the presence of –OH stretching. The strong absorption band at 2880 and 2921 cm^{-1} were

suggestive of aliphatic C-H asymmetrical and symmetrical stretching vibrational frequencies respectively (Appendix H). Its ^{1}H NMR showed presence of oxygenated proton in the molecule (Table 4). Its ^{13}C NMR and DEPT-135 showed presence of 12 carbons in the molecule (Table 4). Based on the above spectral data the compound was identified as a disaccharide called cellobiose. Cellobiose sugar contain two glucose units that the glycosidic linkage carried out on β-1,4-linkage. Furthermore cellobiose could be produced by decomposition of cellulose, regarding its biological behavior both the human body and yeast lack the enzyme cellobiose that needed to break the glucose-glucoseβ-1,4-linkage. Therefore, cellobiose cannot be digested by humans or fermented by yeast.

Table: 4 ^{1}H NMR, ^{13}C NMR and DEPT-135 of compound **2** (cellobiose)

C No	^{1}H(δ value)	Type of carbon	^{13}C (δ value)	DEPT(δ value)
C-1	-	CH	104.47	-
C-7	5.02 (d)	CH	92.20	92.19
C-10	3.73(d)	CH	82.98	82.96
C-5	3.97 (m)	CH	77.51	77.45
C-3	4.06 (d)	CH	74.73	74.70
C-2	4.06 (d)	CH	73.20	73.28
C-8	3.82 (d)	CH	73.25	73.23
C-9	3.91 (d)	CH	72.08	72.06
C-11	3.97 (m)	CH	70.28	70.24
C-4	3.93 (t)	CH	62.60	62.58
C-6	3.81(d)	CH_2	62.52	62.48
C-12	-	CH_2	60.91	60.87

The ^{1}H NMR of compound cellobiose displayed anomeric proton of the glucopyranosyl ring is looked at δ= 5.21 (d) and oxygenated CH_2 proton at 3.89 (2H, s).

^{13}C NMR spectral data of the compound was also compared with the reported ^{13}C NMR spectral data of cellobiose in the literature. Based on the above spectral data and comparison with those reported in the literature the following structure was proposed for compound **2.**

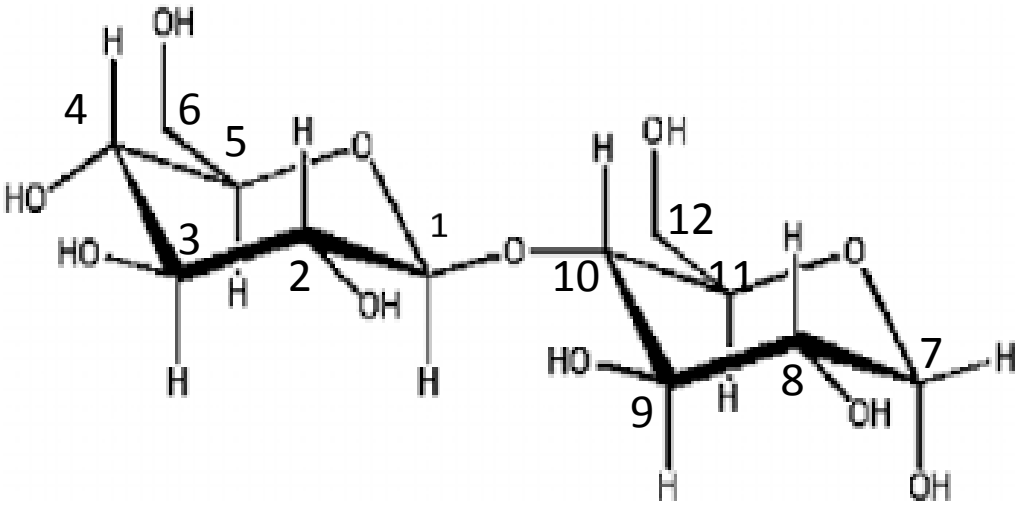


Figure: 6 Proposed structure of compound **2** (cellobiose)

5. Conclusions and Recommendation

5.1 Conclusions

Andrachne aspera, is a small perennial under shrub, traditional medicine plant used for treating various ailments. The roots part of the plant collected from East Shoa Zone, Oromia region in a particular place called Usade Mountain is traditionally use for treatment of wound. The roots of the plants was successively extracted with different solvents.The crude methanol extract was and subjected to column chromatography eluted with different solvents of increasing polarites to yield two compounds.

Phytochemical screening test was done for crude extracts and the result indicated the presence of alkaloids, tannins, terpenoids and saponins in the methanol extract but absent in ethyl acetate and n-hexane extracts. The structures of compounds were determined as sucrose and cellobiose using NMR and IR spectroscopic techniques. Evaluation of antimicrobial activities of crude extracts, fractions and pure compounds of the roots of plant was not conducted due to luck of antimicrobial strains.

5.2 Recommendation

From the previous study, the extract *of Anderachne aspera* possessed a medical functional value as toothache treating, urinary tract infection, respiratory tract infection, to upgrade eye sight and so on. Various chemical compounds from different phytochemical categories involving phenolic compounds, essential oils, terpenoids, fatty acids, sterols and alkaloids were reported to be responsible for wound healing activities. In this regard phytochemical screenings tests conducted revealed that the extract of *Anderachne aspera* consists of the most wound healing components like alkaloids, terpenoids etc. the following recommendations were made.

- Other chromatographic techniques such as PTLC and HPLC should be used to isolate more compounds from the plant.
- More spectroscopic techniques such as 2D NMR (HMBC, HSQC, H-H COSY,) and MS should be used to isolate and characterize more compounds from the plant roots.
- Further phytochemical and biological activity studies should also be conducted on this plant to isolate its specific antimicrobial principles.
- Further bioassay guided isolation and characterization work should be done to fully characterize bioactive constituents of the plant root extracts to identify all active compounds against a variety of bacteria and fungi.
- It is recommended that further similar studies should be conducted on other parts of the plant such as leaves, stem, bark, etc.
- Toxicity studies of the plants should also be done to determine the safety indices of the extracts.

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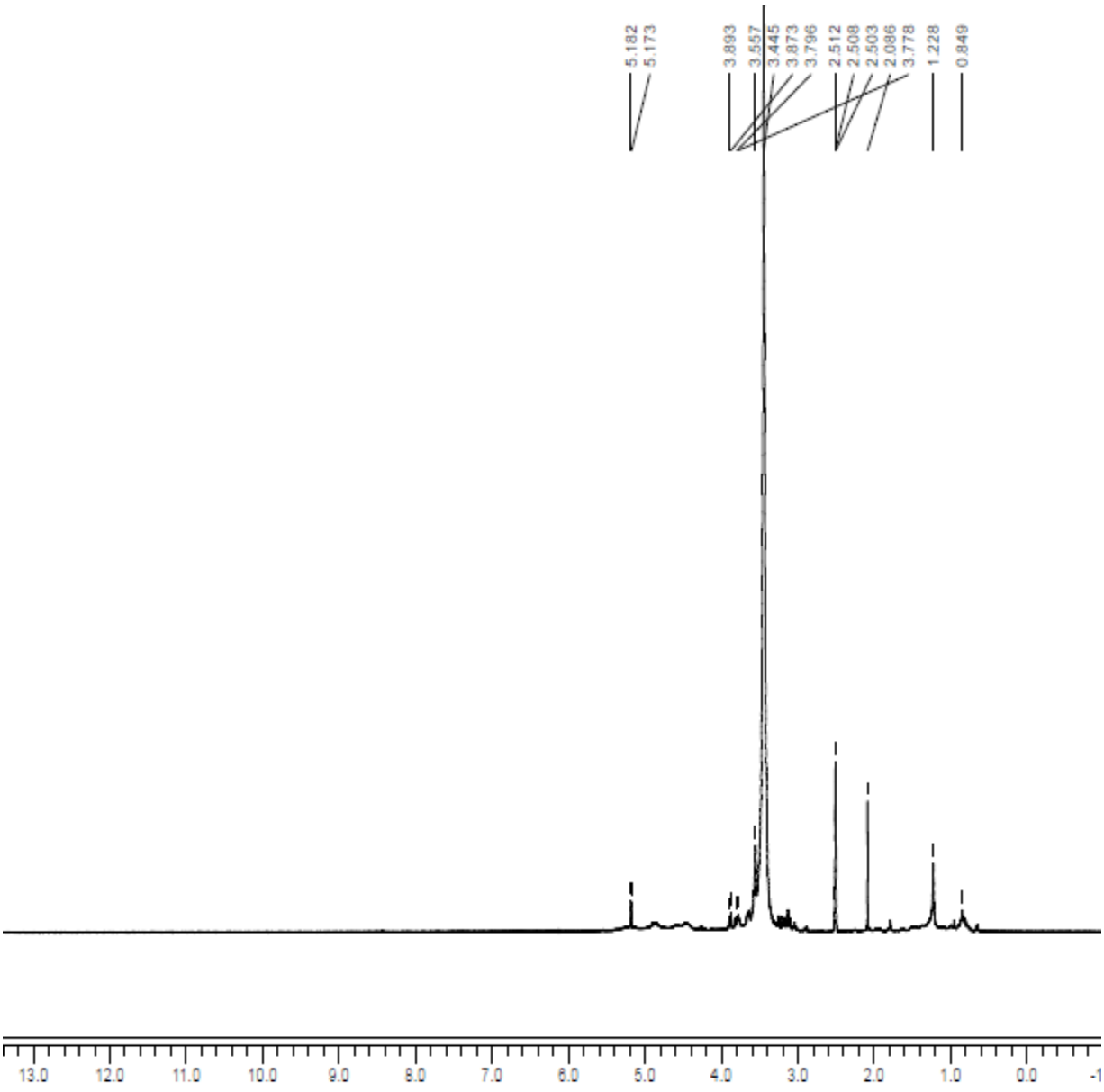
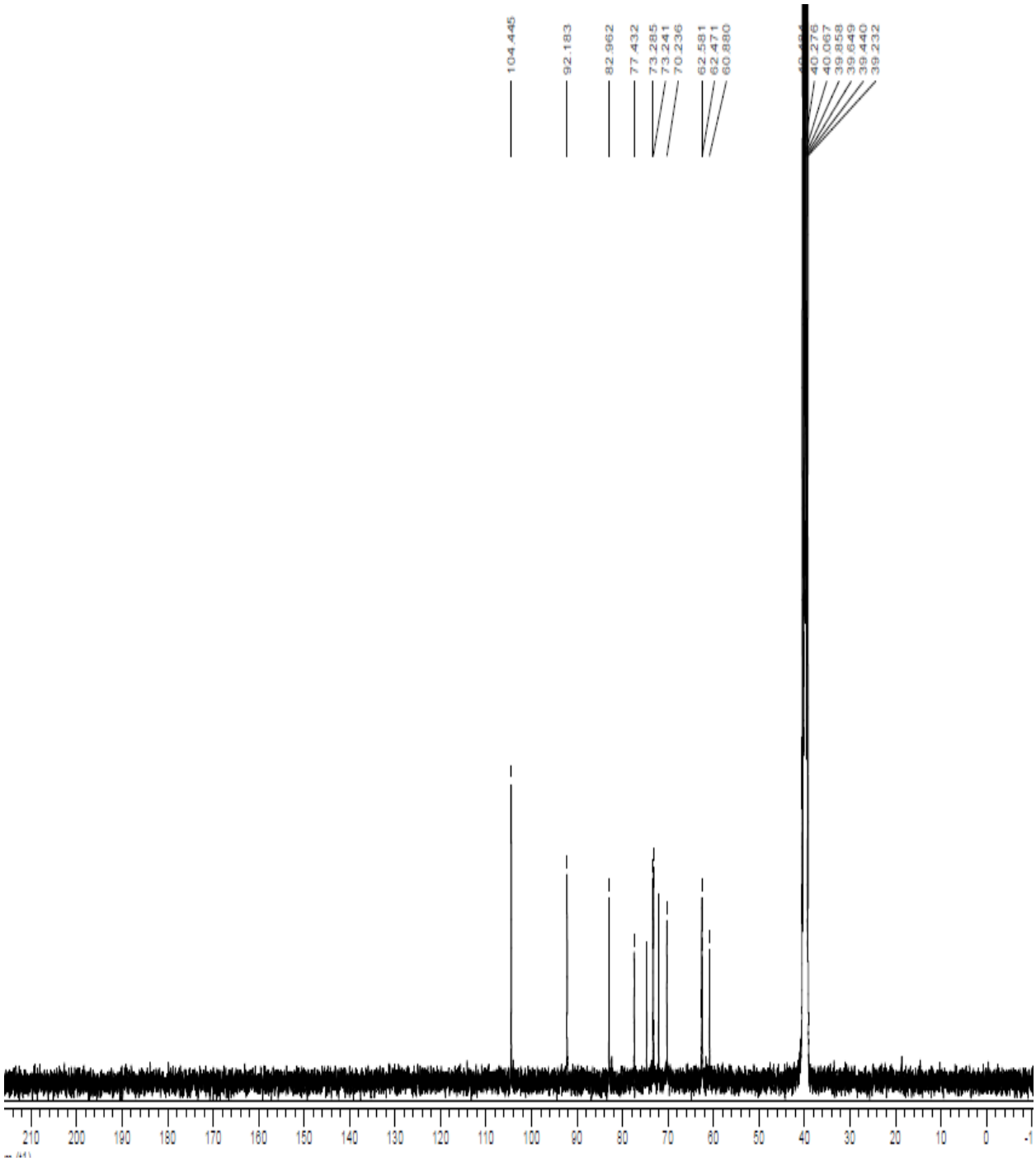
Appendix

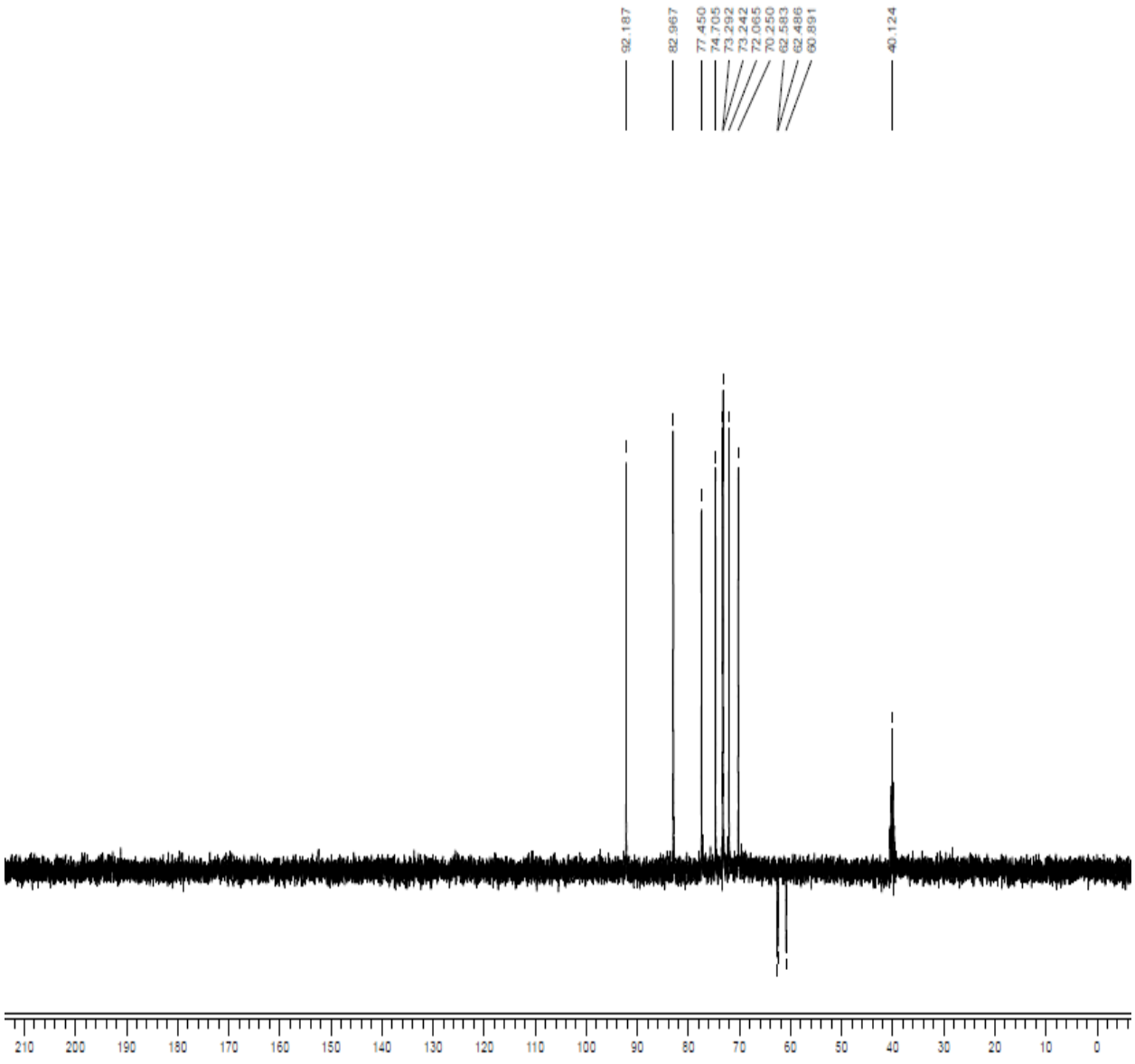
Appendix A. ^{1}H NMR Spectrum of compound **1** (sucrose) in DMSO-d^{6}



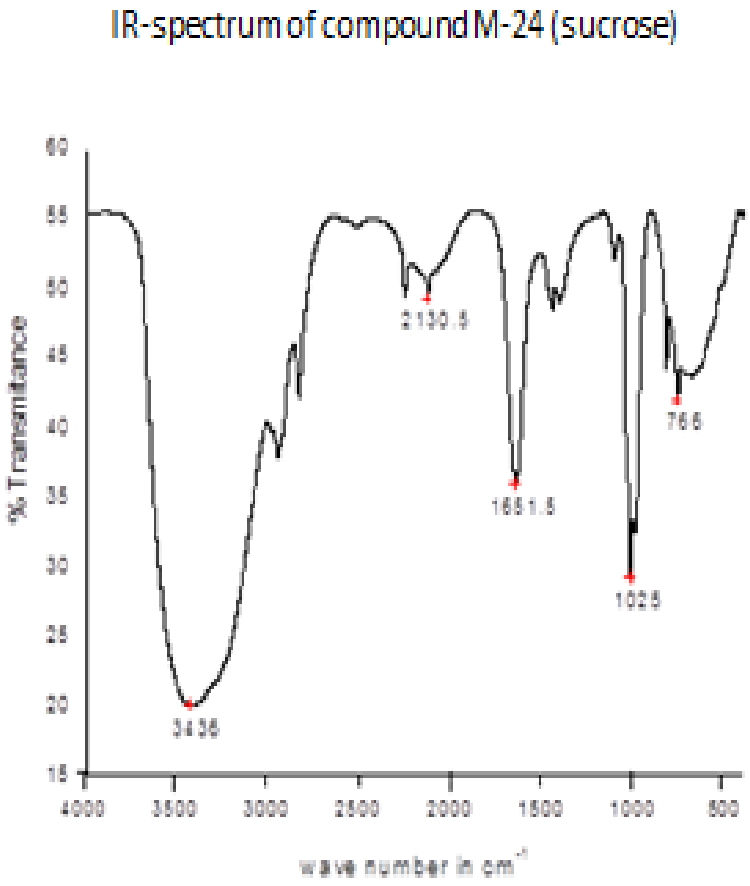
Appendix B. ^{13}C NMR Spectrum of compound **1** (sucrose) in DMSO-d^6



Appendix C. DEPT-135 spectrum of compound **1**(sucrose) in DMSO-d^6

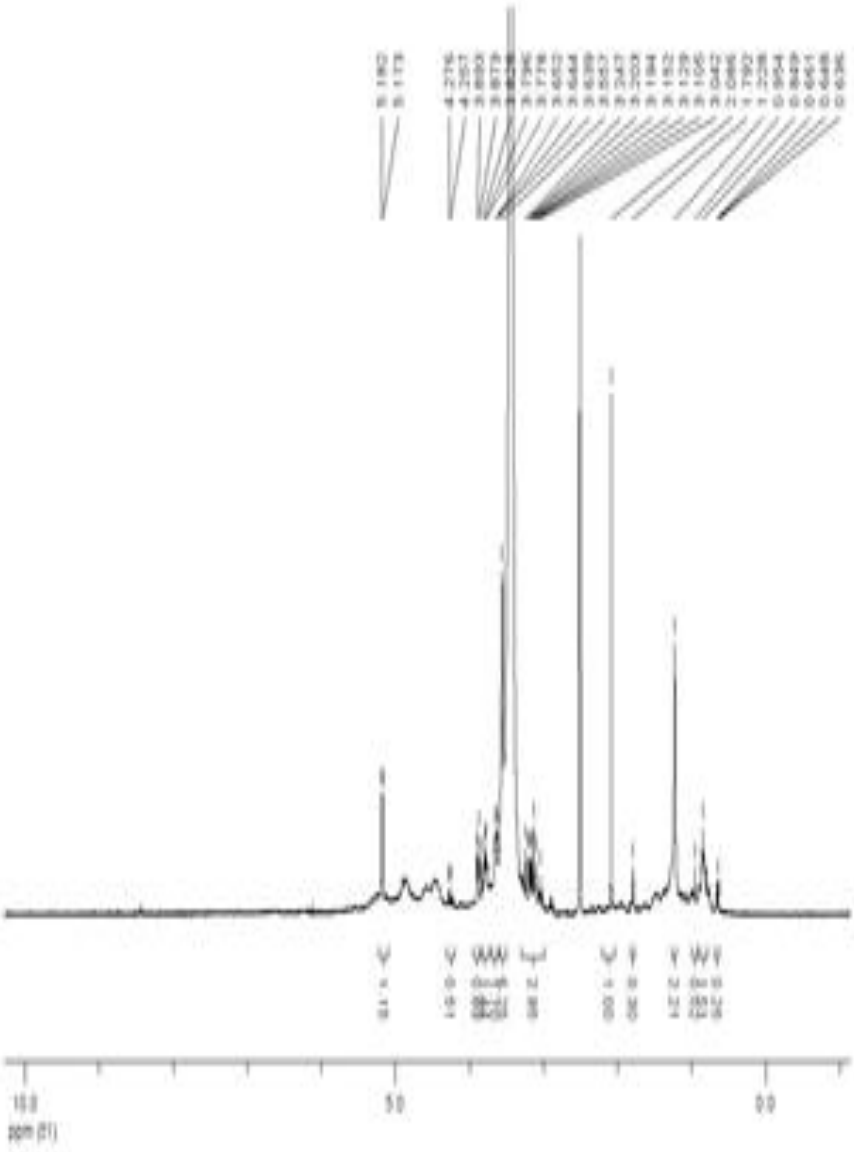


Appendix D


IR-spectrum of compound M-24 (sucrose)
% Transmittance
wave number in cm-1
3436
2130.9
1651.5
1025
766
60
55
50
45
40
35
30
25
20
15
4000
3500
3000
2500
2000
1500
1000
500

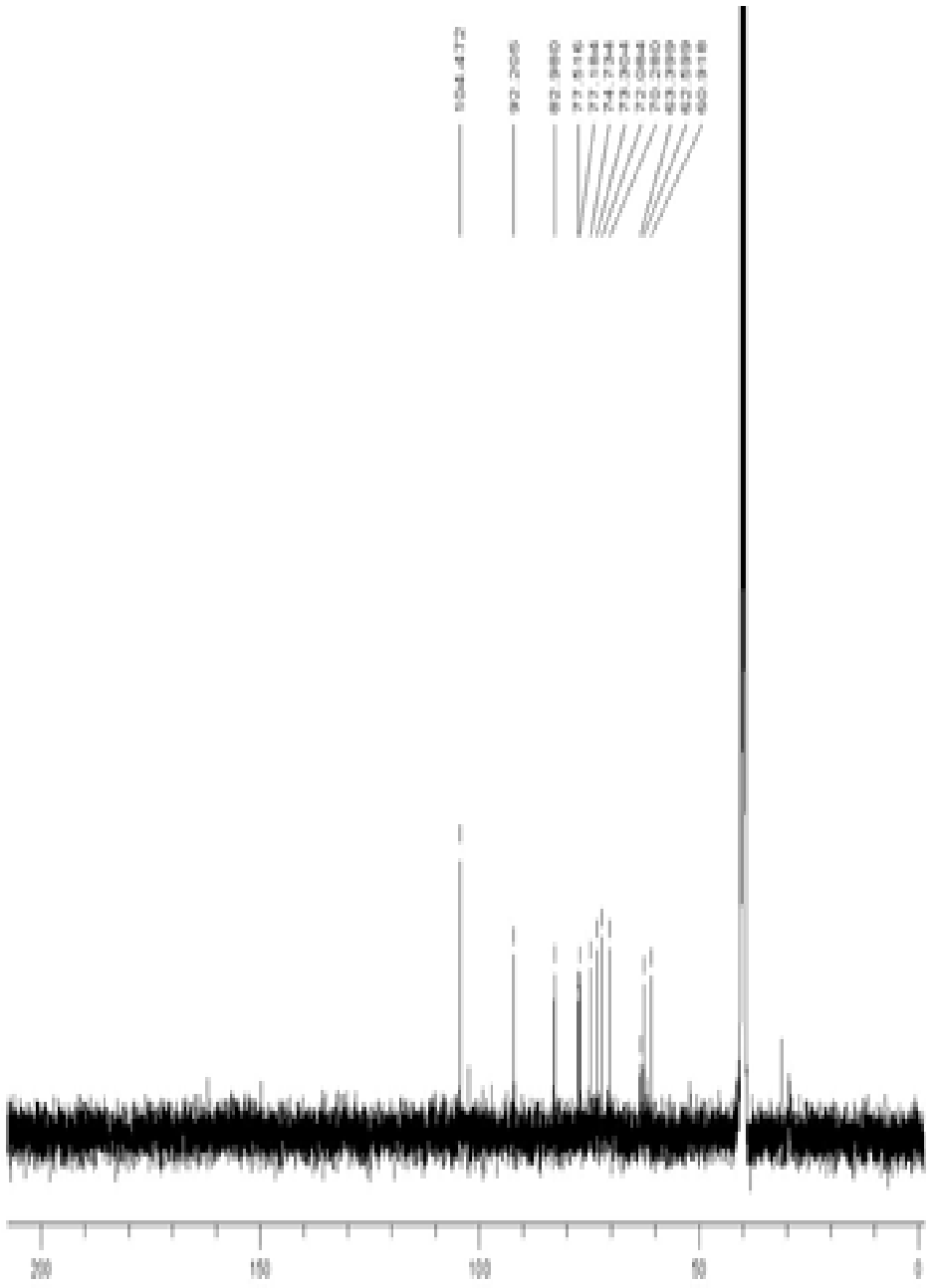
Appendix E

^{1}H NMR Spectrum of compound cellobiose



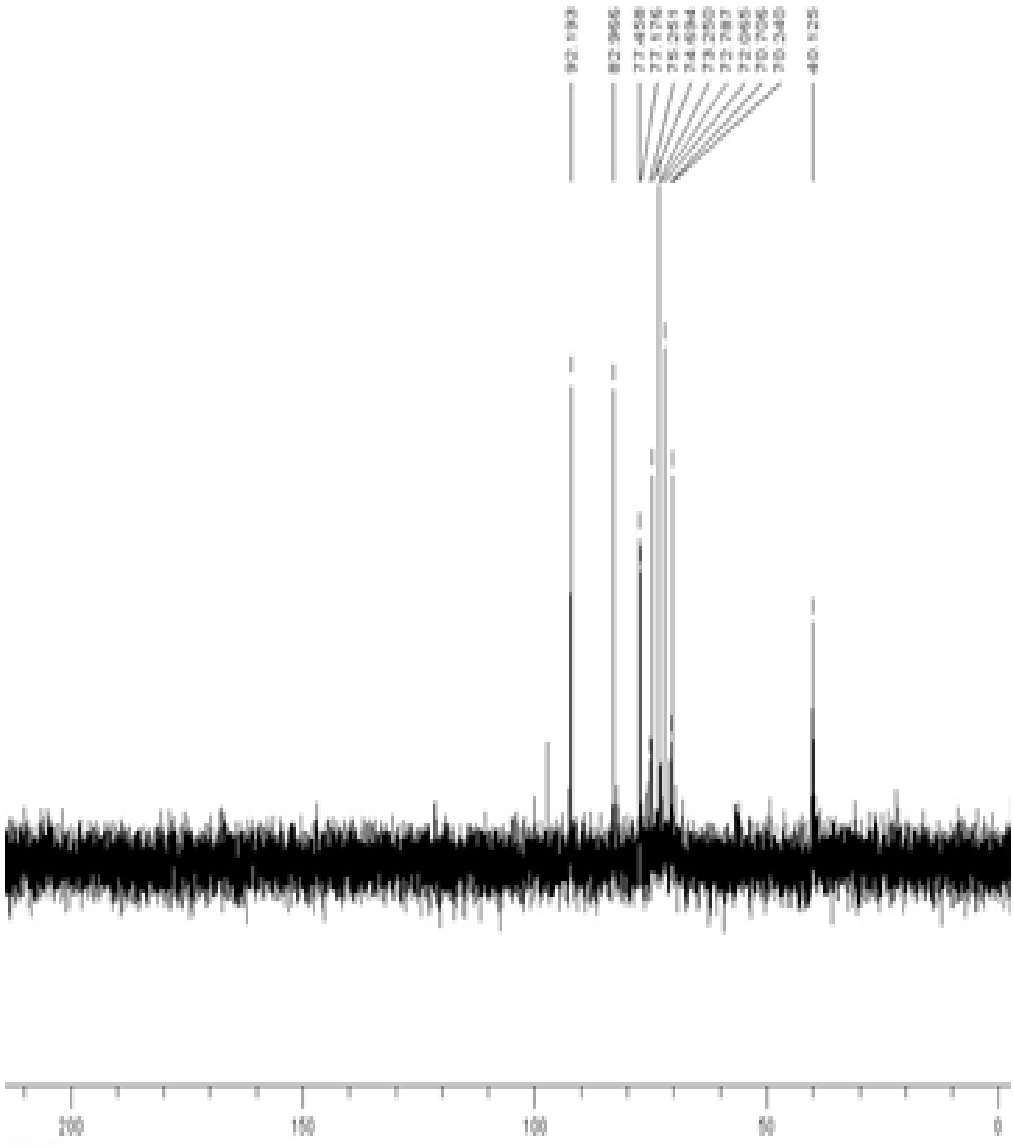
Appendix F

^{13}C-NMR spectrum of compound M-45 (cellobiose)

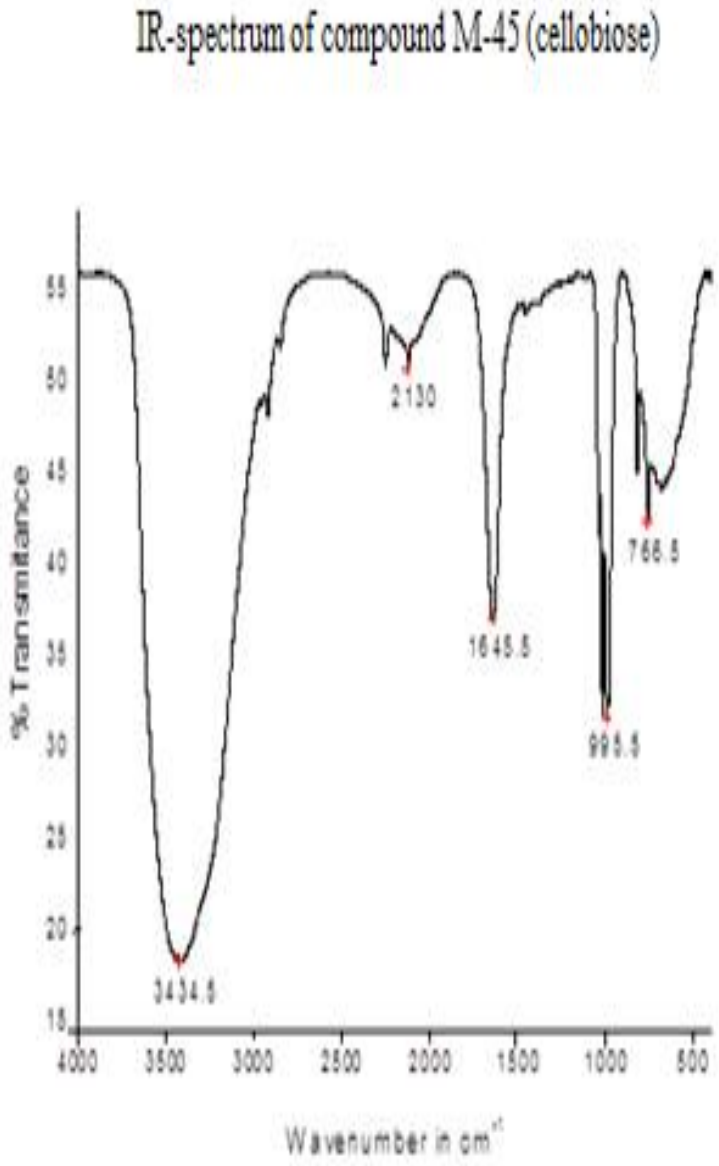


Appendix G

DEPT-135 spectrum of compound M-45 (cellobiose)



Appendix H


IR-spectrum of compound M-45 (cellobiose)
% Transmittance
Wavenumber in cm-1
3434.5
2130
1645.5
995.5
766.5
55
50
45
40
35
30
25
20
15
4000
3500
3000
2500
2000
1500
1000
500