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Phytochemical investigation and structural elucidation on seed extracts of *Datura Stramonium*

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Abstract

Datura Stramonium is found in the family Solanaceaeand and it is available throughout the world. It grows like a weed on loam soil in an Ethiopian context. It is used as a traditional medicine for toothache, skin diseases, and asthma in southern Ethiopia, especially in the Halaba zone. However, there was no research conducted in the study area in this regard. This study aims to isolate and purify alkaloids from Datura Stramonium seeds. Alkaloids are the biologically active substances in this species. Hence, in this study, alkaloid extraction methods were used. Substances besides alkaloids were removed by exhaustive liquid-liquid extraction with diethyl and petroleum ether. The final chloroform extract was tested for alkaloid by Dragendorff's spray reagent. It gave a positive result for alkaloids. The fractionation was done using column chromatography. Chloroform, ethyl acetate, ethanol and methanol were used as eluents. It was done by increasing the polarity of solvents. A total of 26 fractions were obtained. The purity of each sample fraction was checked by thin-layer chromatography (TLC). The fractions that showed the same color and the same Rf value were mixed. The pure fractions with sufficient amounts were studied by 1H NMR, 13 C NMR and DEPT-135 for structural elucidation. In this study, the structures of two compounds, DSA-15 and DSA-21, were identified using IR 1HNMR, 13C NMR, and DEPT-135. Both of them are alkaloids. From this study, it was found out that both DSA-15 and DSA-21 are new compounds.

Keywords: *Alkaloid, Datura stramonium, Ethiopia*

Introduction

Datura stramonium is a plant's botanical name, more commonly known as jimsonweed, angel's trumpet, devil's weed, thorn apple, tolguacha, Jamestown weed, stinkweed, Datura and

moonflower (Kuete 2014; Oseni 2011). It belongs to the genus Datura, which consists of fifteen species, distributed throughout the warmer portion of the world. It is quite common in the British Isles (Government, 2022). Nearly all of them are used locally in medicine and are characterized by similar properties to *Datura Stramonium* (Grieves, 2022).

Datura Stramonium (commonly known as thorn apple) is an annual weed of gardens, roadsides, and other waste or cultivated land. It belongs to the Solanaceae family, which includes the potato and tobacco, and many members of this family contain toxic substances (Aqib, 2014). The *Datura Stramonium* is a large and coarse herb that grows in an annual breaching some what freely, giving a bush to a plant. Its spreading branches cover almost as much rich soil. It may attain a height of six feet. It has long, thick, whitish roots erected and leafy, smooth, peeled yellowish green stems with forked branches and large, angular leaves. The plant flowers are nearly all summer (Karimmojeni et al., 2021). The flowers are large and handsome, about 3 inches in length, trumpet-shaped, and either white or purple (Priyanka, 2012).

Datura Stramonium is frequently used as an anti-asthmatic treatment and is also known for its hallucinogenic and euphoric effects (Aqib, 2014). *Datura Stramonium* possesses anticholinergic properties, and anticholinergics have proved to be of particular value not only in the treatment of asthma but also for chronic obstructive pulmonary disease (COPD); here, vagal cholinergic tone appears to be the only reversible component of airway narrowing, opposite to what happens in asthma (Soni et al., 2012; Maibam, 2011; Pretorius, 2006), and it affects the spermiogramic parameters of aqueous extract of *Datura Stramonium* (Daramola et al., 2009).

Datura Stramonium was used internally to treat madness, epilepsy, and depression. Externally, it formed the basis of ointments for burns and rheumatism. The use of Datura species in phytomedicine for the treatment of cough burns and the healing of wounds is supported in recent studies (Muhammed and Sisay, 2021; Reema and Pankaj, 2020; Khaton, 2012; Donatus and Ephraim, 2009). More recently, preparations from the plant have been used as ingredients in some asthma medicines. With this exception, however, the plant is generally considered too toxic for medical applications nowadays (Sever, 2007). However, there is no study in Ethiopia due to these applications and the benefits of *Datura Stramonium*. Hence, this study aimed to extract and isolate the chemical constituents from the seed of *Datura Stramonium* of Ethiopia.

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Material and methods

Plant material collection and preparation

Datura Stramonium seeds were collected in 2018 from the selected villages of southern Ethiopia State, Halaba zone which is 365 km from Addis Ababa and 90 km from Hawassa (Figure 1). The taxonomic identification of the collected plant was undertaken to identify it as *Datura Stramonium* ("Etsefaris") in the Addis Ababa University Department of Biology National Herbarium. The amount of sample obtained was about 1kg. It was stored in glass flasks to protect it from humidity and light.

a) b)

Figure 1. a) *Datura Stramonium* plant, b) Upon maturity, the plant releases tiny black seeds from spiny capsules [Photo: Alemu Lelago].

Instrumentation

The ultraviolet spectrum has been measured with a GENESYS spectrophotometer methyl trichloride. Infrared spectrum has been recorded as KCl pellets on a Perkin-Elmer BX Infrared Spectrometer ranging from 4000-400 cm_1. 1HNMR and 13 C NMR spectra were recorded on a Bruker advanced 400 MHz spectrometers with Tri-methyl silicate (TMS) as an internal standard. Analytical TLC has been done on a 0.2-mm-thick layer of silica gel on the aluminium card. Dragendorff's spray reagent was used for detecting alkaloids (Sreevidya and Mehrotra, 2019, Raal, Meos et al., 2020). Silica gel 60 (Merck) was used to carry out column chromatography. TLC spots were visualised under UV light (254 and 364 nm), followed by spraying by Dragendorffs' reagents for alkaloid screening (Coskun, 2016; Ngo and Chua, 2019).

Extraction, isolation and purification of compounds

Plant parts collected were crushed into powder by a grinding mill. Powdered plant materials (200g) were wetted with 300 ml of NH4OH (25%m/m), and they were extracted with 2 litres of the mild polar solvent ethyl acetate for 72 hours at room temperature. Then the extract was filtered out and the solvent was evaporated in a rotary evaporator at a reduced pressure of 40 0C. As a result, the residue was dissolved in H2O and acidified with H2SO4 at pH 3.5. It was extracted with petroleum and diethyl ether to remove lipophilic, acidic, and neutral materials. After basifying the aqueous solution to pH 9-10 with NH4OH (25% m/m), it was evaporated again, and its residue was extracted with chloroform. Distilled water was used to wash the extract to neutralise its pH. The extract was dried with Na2SO4 and then concentrated to dryness under reduced pressure to obtain crude extracts (Diego, 2009).

The components of the chloroform extract profile were checked by TLC using 100% chloroform as the mobile phase. The Rf values for each element were calculated with the given solvent system. Depending on the estimated Rf value, the appropriate solvent system was selected to proceed with normal column chromatography.

Fractionation with column chromatography

The column was run using petroleum ether, chloroform, ethyl acetate, ethanol, and methanol by gradient elution technique. The compound fractions were done by developing the solvent gradient system by using a mixture of petroleum ether-chloroform (1:1, 2:3, 1:4, 1:9), ethyl acetate-chloroform (1:9, 1:4, 3:7, 1:1, 4:1), ethyl acetate-ethanol (9: 1, 4:1, 7: 3, 3: 2, 1:1, 2: 3, 3: 7, 1:4, 1:9) and Methanol-ethanol (1:9, 1:4, 2: 3, 1:1). Each fraction was labeled. The fractions with the same color and a single spot with different solvent systems were mixed. The Rf value of each pure compound was carefully determined before proceeding to the instrumental analysis.

Preliminary phytochemical screening

The test of alkaloids was done by Dragendorff's spray reagent, which was prepared in the laboratory as follows (Tiwari, 2011): A) 8-gram potassium iodide was dissolved in 20 ml distilled water; B) 0.85 basic bismuth nitrate + 10ml acetic acid dissolved by 40 ml distilled water. A and B were mixed in a 1: 1 ratio (v/v) and stored at 0 ^{o}C. The spray reagent was made by taking out 5ml from this stoke solution, and 10 ml of acetic acid was added, followed by 90 ml of water. The extract obtained was then measured and the percentage yield of the alkaloid was calculated.

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Structure elucidations

After having the appropriate amount of a pure component, the structures of the selected components were determined using spectroscopic methods, including UV, IR, NMR (both 1H and 13C), and dept -135.

UV experiments were conducted at Hawassa University, Department of Chemistry; 1HNMR, 13C NMR, and dept-135 experiments were conducted at the AAU Department of Chemistry in the NMR Laboratory and at Netherland Vrije University, Department of Chemistry. The IR experiment was carried out in the IR laboratories of an Ethiopian pharmaceutical factory and the Department of Chemistry at the Netherlands Virje University.

Results and discussion

Based on the extraction process and yield, the seeds of *Datura Stramonium* confirmed that the crude extract was alkaloid by Dragendorff's spray reagent test, and moreover, the percentage of the yield was high. This result is in line with the study (Abdelouaheb, 2006); the amount of alkaloids in *Datura Stramonium* seed is only 0.2%. This indicates the high percentage yield of alkaloids in *Datura Stramonium* was investigated in the study, and this shows that it is a potential medicinal plant (Grzegorz and Maria, 2008; Christen, 2000).

The TLC profile was done using 100% chloroform as the mobile phase. The three groups of spots were observed in this solvent system. The Rf values of these spots were calculated as 0.2, 0.23, and 0.89, respectively. These Rf values did not indicate that the crude extract contains only three compounds because each spot contains many compounds with the closest Rf values. To purify such compounds with different Rf values and almost similar Rf values, the solvent system was developed with increasing polarity as described in the experimental part (Anvir et al., 2017).

Pure fractions obtained from column chromatography

The TLC column chromatography fractionation test showed that: among the 26 fractions obtained from column chromatography fractionation, 8 of them had no spots, and 6 of them have been observed as single spots with similar Rf values and the same color by using ethyl acetate-chloroform in a 1:1 ratio as eluent. Then, the fractions with similar spots were mixed and dried in a rotary evaporator, and their Rf values were calculated as 0.62 in a given solvent system. It gave a positive result for alkaloids. However, further identification and structural elucidation of this

compound was not conducted because of its' small amount, the cost of the instruments, and time limitations.

A further spectroscopic technique was studied for both DSA-15 and DSA-21 for structural elucidation. Fractions 15 (coded as DSA-15) and 21-26 (coded as DSA-21) were found to be pure and positive for the alkaloid test (Alabri et al., 2014). Fraction-15 Rf value was calculated in an ethyl acetate:ethanol 1:1 ratio as eluent to be 0.74 and weight to 80mg. At the same time, fractions 21-26 showed a single spot with the same color and Rf value using different solvents (eluent). Then its Rf value was calculated by using methanol-ethanol in a 1:1 ratio as a solvent system and found to be 0.85. This indicated that they were all the same compound and measured at 38 mg.

Fractions 16–20 were pure and positive for alkaloid tests. But for fractions 16, 18, and 20, further study of these compounds was not conducted due to the cost of the instruments and time limitations. However, fractions 17 and 19 had pure and positive results for alkaloid tests; hence, further investigation by spectroscopic technique was conducted. Unfortunately, the spectra of DSA-17 and DSA-19 were not interpreted because their proton NMR was not good and required further running, even though their carbon NMR showed the same skeleton as that of compound DSA-15.

Characterization and identification of pure compounds by spectroscopic techniques

Characterization of DSA-15

Based on the IR spectrum 8, the absorption band of 2922.25cm^{-1} and 2852.81 cm^{-1} revealed the existence of C-H asymmetric stretch for CH_3 and CH_2, respectively (Fig. 2). At the same time, a strong absorption band of 1685.84 cm^{-1} revealed the presence of the ester C=O functional group. A weak band at 1658.84 cm^{-1} revealed the existence of alkenes C=C stretch. The fact of the absorption band of 1587.47 $cm^{-1}$and 1512.24 cm^{-1} revealed the presence of aromatic ring stretch at C=C absorption. The existence of absorption bands from 1300 to1000cm^{-1} showed C-O bits of the ester functional groups. Absorption bands of 1452.45 cm^{-1} and 1386.86 cm^{-1} revealed the existence of CH_2 and CH_3 bending absorption, respectively. The presence of an absorption band from 1350 cm^{-1} to 1000 cm^{-1} indicated C-N stretch absorption.

The UV spectrum absorption band (Klotz, 1945) at λ_{max} (in $CHCl_3$) 241 nm indicated the presence of a conjugated system (Tong, et al., 2020). The ^{1}HNMR spectrum of the compound DSA-15 (Figure 2) shows the peaks at δ 0.8 ppm, 1.06 ppm and δ 1.2 ppm Showing the presence of methyl

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protons. Also, singlet peaks of δ 2.0ppm and δ 3.81ppm indicated the methyl protons that are substituted on the benzene ring and on the carbon next to nitrogen of the compound respectively. The singlet peak at δ 3.81 ppm showed the presence of methylene hydrogen which is on the carbon that directly attached to the nitrogen of the compound. The multiplet peak at δ2.11ppm, the triplet peak at 2.01 ppm and other triplet peak at 2.6ppm showed the presence of methine hydrogens on the cyclic ring carbon atom. The singlet at δ5.4 ppm, doublet at δ 6.2 ppm and 6.9ppm, and triplet at 6.72ppm represented for hydrogen on olefinic carbon. The triplet at δ 6.72ppm showed the presence of hydrogen on the olefinic carbon. The singlet at δ7.1 ppm showed the presence of hydrogen on the aromatic ring.

The ^{13}C NMR and DEPT-135 indicated that DSA-15 has 30 non-equivalent carbons. The spectra showed eight methyl carbons at δ 17.137ppm, 25.517ppm, 27.063ppm, 29,106ppm, 29.218ppm, 29.419ppm, 29.604ppm, and 34.523ppm. The downfield chemical shifts for methyl carbons indicated that the shielded carbon was due to nitrogen atoms' conjugation and electro-negativity. Two methyl carbons are overlapped at δ 29.106ppm. The peaks at δ 31.802ppm indicated the presence of aliphatic quaternary carbon because, on dept-135, there is no peak for this carbon. The peak at δ 40.975ppm, 48.513, and 48.734 showed the presence of cyclic methine carbons. One oxymethylene peak was observed at δ 55.698ppm since it is an inverted peak on dept -135. Peaks at δ 48.310 ppm, 48.734 ppm and 49.594 ppm are represented for cyclic quaternary carbons because there is no peak on dept-135 for these carbons. The peaks at δ 110.044ppm, 115.019ppm, 115.310ppm, 117.578 ppm, 122.028 ppm and 129.803ppm showed the existence of olefinic methine carbons. Peaks of δ 147.776ppm and 155.238 ppm showed the olefinic quaternary carbons since there is no peak on dept-135 for these carbons. The peaks at δ129.677 ppm and 141.063 ppm showed the presence of unsubstituted aromatic carbons. The peaks at δ126.946ppm and 147.287ppm indicated the quaternary aromatic carbon. The peak revealed the existence of the ester functional group at 167.128 ppm. Finally, the peak at δ 188.695 represented the carbonyl carbon of conjugated ketone. Three peaks at δ 77.107 ppm, 77.308 ppm and 426 ppm are due to the solvent $CDCl_3$ (Table 1).

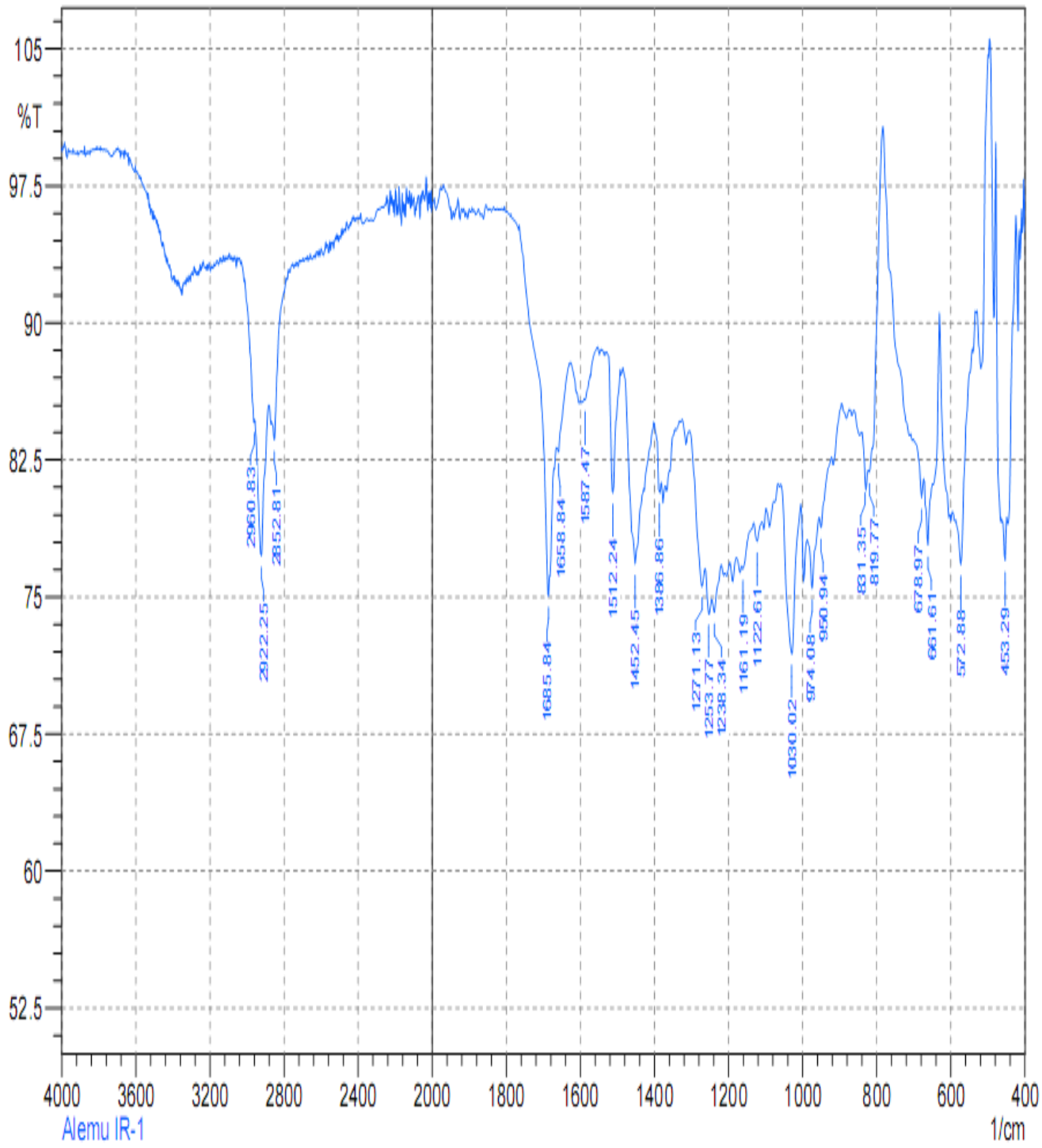

Figure 2. IR spectrum of DSA-15

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Table 1. ^{1}H, ^{13}C, and dept- 135 spectra data for DSA- 15

Position	δ ^{13}C	δ ^{1}H	DEPT-135	Remark
1	129.803	6.2d	CH	
2	117.578	6.2d	CH	
3	155.238	-	-	Quaternary carbon
4	110.044	5.4s	CH	
5	48.734	-	-	Quaternary carbon
6	115.310	6.20d	CH	
7	115.019	6.72t	CH	
8	48. 948	2.01m	CH	
9	48.513	2.34t	CH	
10	48.301	-	-	Quaternary
11	115.019	6.72t	CH	
12	115.310	6.20d	CH	
13	49.164	-	-	Quaternary
14	40.971	2.60t	CH	
15	122.028	6.90d	CH	
16	147.776	-	-	Quaternary carbon
17	188.695	-	-	Quaternary carbon
18	17.137	1.2s	CH_3	
19	25.517	0.8s	CH_3	
20	167.128	-	-	Quaternary carbon
21	27.063	2.0s	CH_3	
22	31.802	-	-	Quaternary carbon
23	29.604	1.06S	CH_3	
24	29.419	1.06s	CH_3	
25	29.218	1.06s	CH_3	
N-CH_3	34.523	3.3s	CH_3	
1'	55.698	3.81s	CH_2	
2'	126.946	-	-	Quaternary carbon
3', 7	129.677	7.1s	CH	
4', 6'	147.287	-	-	Quaternary carbon
5'	141.063	7.1s	CH	
8',9'	29.106	2.0s	CH_3	

From UV, IR, ^{1}H, ^{13}C NMR NMR and DEPT-135 spectrums the following structure is expected for DSA-15 (Figure 2).

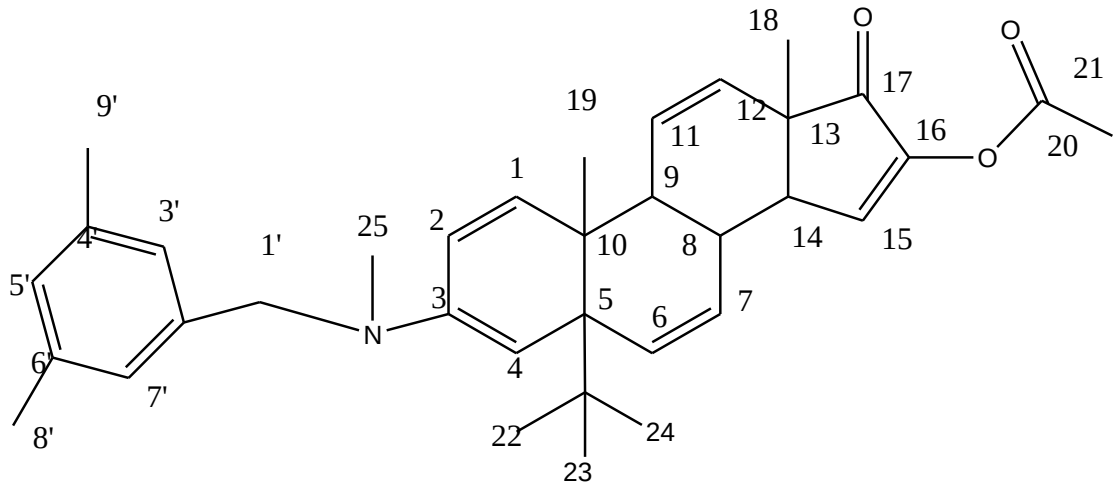

Figure 2. The expected structure of DSA-15

Characterization of DSA-21

IR spectrum of DSA-21, the O-H stretch brought the absorption band of 3435.34 cm^{-1}. The absorption bands of 2955.04cm^{-1} and 2852.81cm^{-1} revealed the presence of C-H asymmetric stretch for CH_3 and CH_2 stretches, respectively. A strong absorption band of 1660.05cm^{-1} revealed the existence of the ester C=O functional group. The weak band of 1600.84cm^{-1} indicated the existence of alkenes of C=C stretch. The existence of absorption bands from 1300-1000cm^{-1} explained C-O stretching of ester and ether functional groups. Absorption bands of 1456.30 cm^{-1} and 1386.86 cm^{-1} revealed the existence of CH_2 and CH_3 bending absorption, respectively (Figure 3). The presence of absorption bands from 1350 cm^{-1} to 1000 cm^{-1} indicated C-N stretch absorption. The UV spectrum absorption band at λ_{max} (in $CHCl_3$) 271 nm indicated the presence of a conjugated system in compound DSA-21. From UV, IR, 13C NMR, 1H NMR, and DEPT-135 spectrums, the following structure is expected for DSA-21 (Figure 2). Giday et al. (2015), reported another structure of tropine alkaloid.

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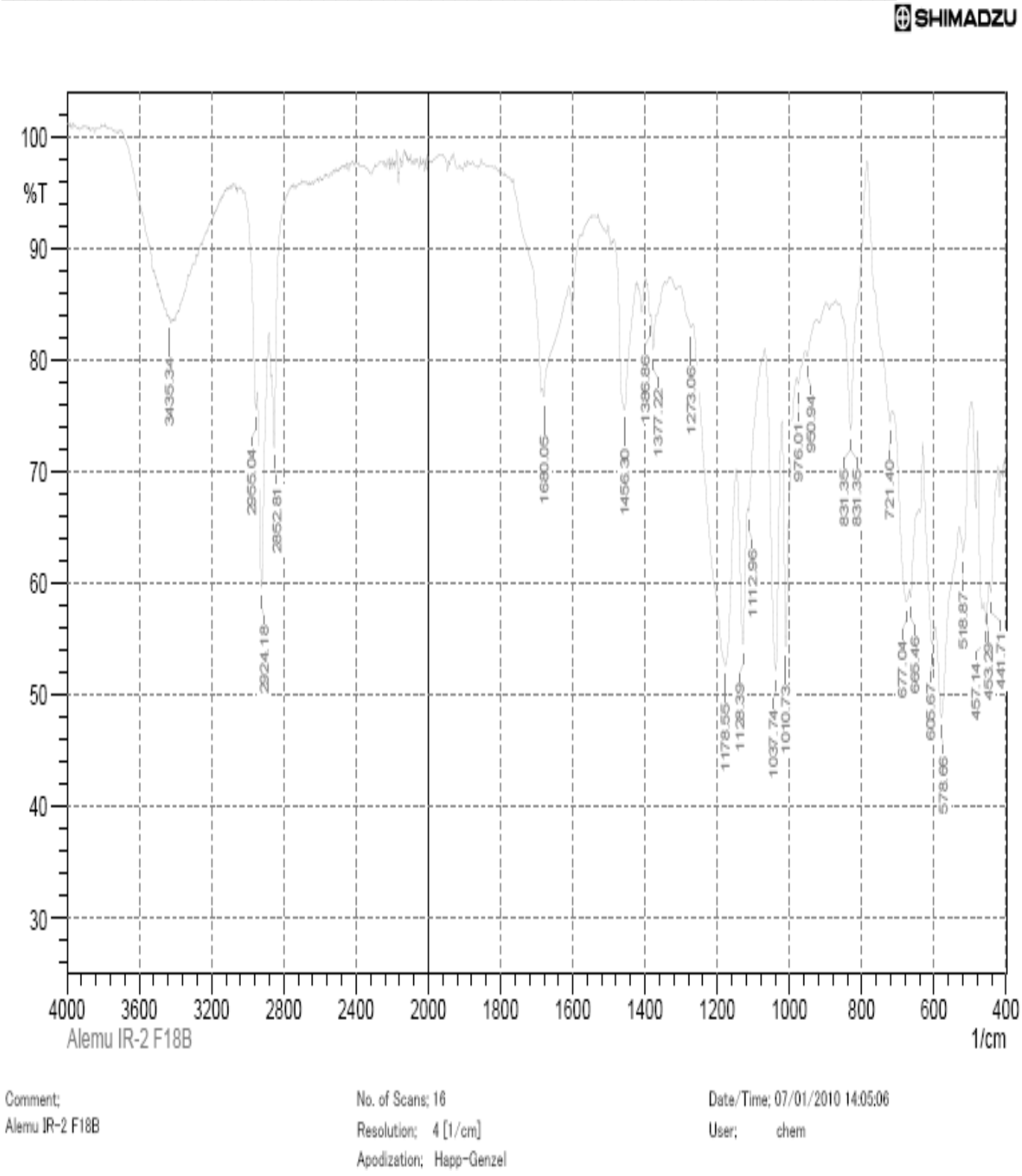

Figure 3. IR spectrum of DSA-15

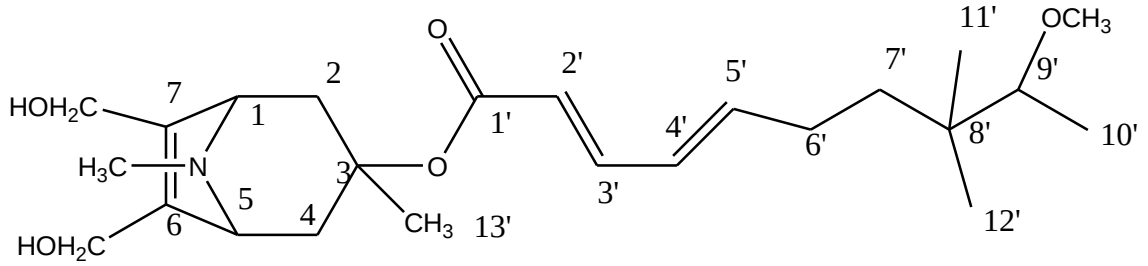

Figure 4. The expected structure of DSA-21

^{1}HNMR spectrum showed for compound DSA-21, the singlet peak at δ 1.2 ppm indicated hydrogen of methyl attached to the quaternary carbon. The doublet at δ 1.8 ppm and multiplet at δ 2.0 ppm represented methylene hydrogens respectively. The singlet peak at δ 2.5 ppm represented three hydrogens of N-$CH_{3.}$ The triplet at δ 2.6 ppm represented the methine hydrogens on the carbon attached to the nitrogen of the compound. The singlet at δ 3.2 ppm represented hydrogen of the O-H functional group. The tall singlet at δ 3.24 ppm showed the presence of methoxy hydrogen, and the other tall singlet at δ3.8ppm showed the presence of hydrogen of methylene attached to O-H. The quartet peaks at δ 5.5 ppm, doublet peaks at 6.2 ppm, triplet at 6.4 ppm and triplet at 7.5 ppm indicated the presence of olefinic carbon.

The ^{13}CNMR and DEPT-135 indicated that DSA-21 has 22 none quivalent carbons. The spectra showed three methyl carbons at δ 17.643 ppm, 26.471 ppm and 26.587 ppm. Four methylene signals appeared at δ32.928 ppm, 32.095 ppm, 33.167 ppm and 33.479 ppm. Oxymethyl signal appeared at δ 70.104 ppm. The peak at δ 35.745 ppm represented aliphatic quaternary carbon. The peak at 43.699 ppm was defined for the methyl group deshileded by nitrogen. The peaks at δ 58.483 ppm, 59.322 ppm and 59.591 ppm showed methine carbon. The peaks at δ 61.217 ppm and 61.333 ppm represented two oxymethylene carbons, respectively. The peak at δ 67.116 ppm represented quaternary carbon. The signals indicate four olefinic methine carbons at δ 131.702 ppm, 131.831 ppm, 132.139 ppm, and 132.734 ppm. An olefinic quaternary carbon appeared at 139.723 ppm. Finally, the ester functional group carbonyl carbon was revealed by the peak of δ 175.636 ppm. The seven peaks near δ 51 ppm and three peaks near δ 81 ppm are due to the solvents $MeOD_{4}$ and $CDCl_{3,}$ respectively (Table 3). Similar to this study by Giday et al. (2015)

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reported 3-(3'-methoxytropoyloxy)-6-tigloyloxy -7-Hydroxy tropane from crude extract of Datura Stramonium seed by using IR, 1H- NMR, 13C-NMR, DEPT-135 and GC-MS.

Table 2. ^{1}H ^{13}C and dept 135 spectra data DSA- 21

Position	Delta carbon	Delta carbon	Dept	Remark
1	59.591	2.6t	CH	
2	33.475	1.8d	CH_2	
3	67.116	-	-	Quaternary carbon
4	33.167	1.8d	CH_2	
5	59.322	2.6t	CH	
6,7	139.723	-	-	Quaternary carbon
1'	175.36	-	-	Quaternary carbon
2’	131.702	6.4t	CH	
3'	132.734	7.5 t	CH	
4’	132.139	6.2d	CH	
5’	131.831	5.6q	CH	
6'	32.928	1.9m	CH_2	
7’	33.095	1.25t	CH_2	
8'	35.745	-	-	Quaternary carbon
9’	70.104	2.8m	CH	
10’	17.643	1.1d	CH_3	
11’, 12’	26.471	1.2s	CH_3	
13’	26.587	1.2s	CH_3	
OCH_3	58.483	3.8s	CH_3	
$N\text{-}CH_3$	43.669	2.5s	CH_3	
OCH_2	61.217	3.8s	CH_2	
OCH_2	61.333	3.8s	CH_2	
OH	-	3.2s	-	

Conclusions

In this study, two alkaloids, DSA-15 and DSA-21, were identified. To investigate the structures of the noble compounds, DSA-15, and DSA-21, the 2D_NMR technique should be used to know the correct structure of the identified compounds, DSA-15 and DSA-21. MS experiment should be conducted to see the fragmentation patterns of the compounds. Finally, the bioassay-guided fractionation of the chloroform extract should be done to understand the biological activity of the compounds.

Conflict of interest

The authors declared that they have no conflict of interest

Data availability statement

The data used to support the findings of this are available from the corresponding author upon request

Funding statements

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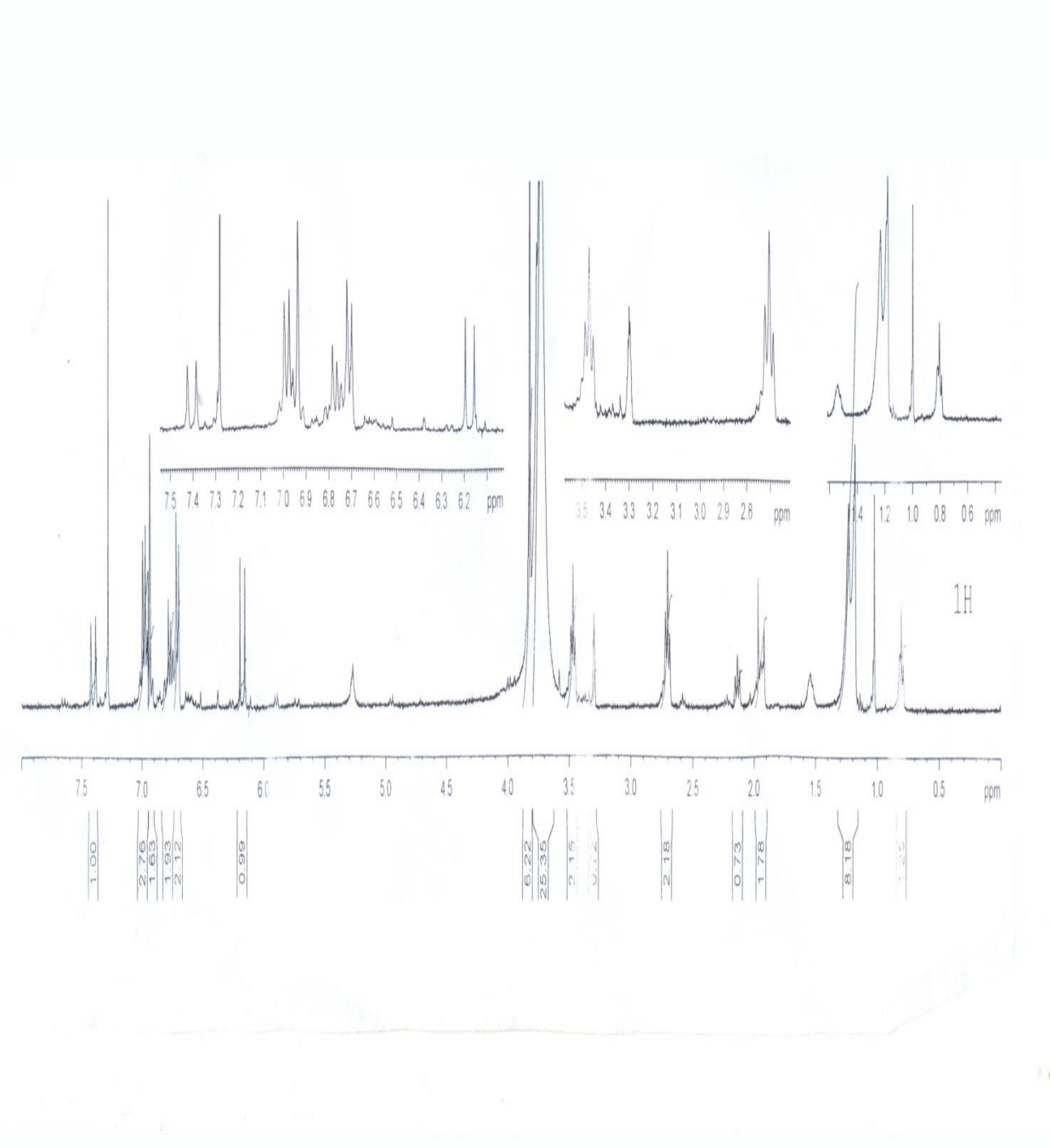
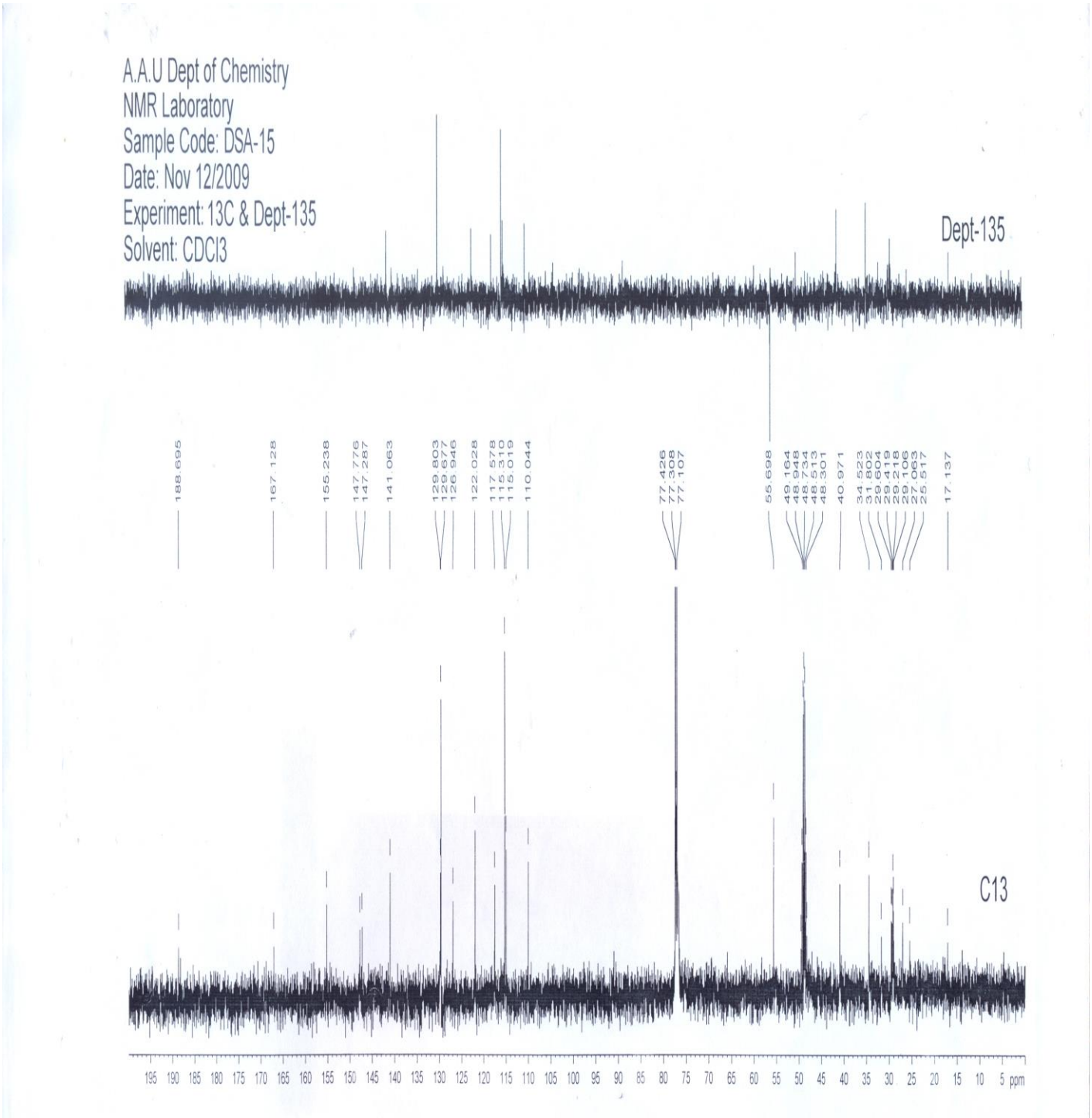
Appendices

Appendix-1 ^{1}H NMR spectrum of DSA-15



Appendix 2. ^{13}CNMR and dept-135 of DSA-15



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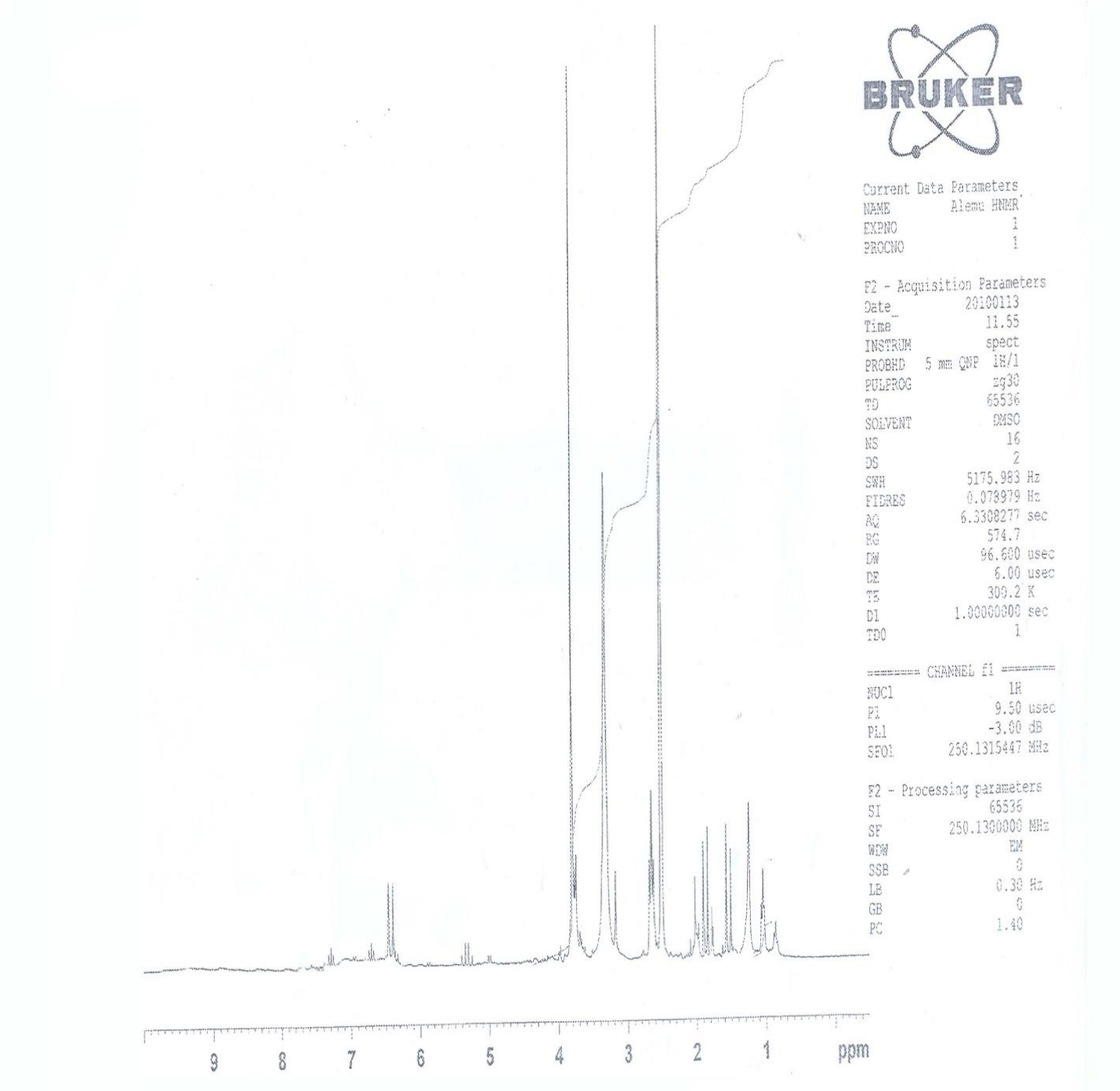
Appendix 3. ^{1}H NMR spectrum of DSA-21



Appendix 4.^{13}CNMR and dept-135 of DSA-21