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Extraction, Isolation, Characterization and Antioxidant Potential of Pure Compounds from *Adonsonia digitata* Leaf Extract

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Abstract

Introduction: Adonsonia digitata L. (Malvaceae) commonly known as Baobab is a medicinal and nutritional plant. The plant parts are used to treat various ailments such as diarrhoea, malaria and microbial infections. It is reported that it is an excellent antioxidant due to its vitamin C content. Baobab has numerous biological properties including antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities amongst others. Objective: The study involves extraction, Isolation, Characterisation of phytochemicals and evaluation of antioxidant potential of the pure compounds. Methods: The dried leaf powder was subjected to rotary evaporator to obtain crude extract which was subjected to isolation using chromatography analysis and elucidation using NMR and FTIR. Antioxidant (IC50) potential was determined using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH). Result: Phytochemical investigation of the Baobab leaf extract through isolation and characterization of a bioactive compound was observed. The leaf extract yielded three compounds characterised as D-Limonene, Thunbergol and (z)-2-Methyl-7-octadecene. Conclusion: The analysis of the chemical component reported identified compounds from Baobab leaf as; DLimonene; (1) Thunbergol (2) and Cis-2-Methyl-7-octadecene (3) with significant antioxidant potential. The chemical components were identified for the first times.

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Keywords: Adonsonia digitata L, Baobab, Medicinal plant, Phytochemical extraction, Antioxidant potential

INTRODUCTION

Adonsonia digitate L (Malvaceae) popularly known as Baobab plant is a tree belonging to the Malvaceae. It is widespread throughout the hot, drier regions of tropical Africa (De Caluwé et al., 2010; Bremer et al., 2003). It is a deciduous, tree with a height up to 25 m high, which may live for hundreds of years (Gebauer et al., 2002). The trunk is swollen and stout, up to 10 m in diameter, Branches are large and distributed irregularly. The bark is smooth, reddish brown to grey, soft and fibrous (Gebauer et al., 2002).

The Leaves alternate arranged with a size of about diameter of 20 cm. Flowers are pendulous, solitary large with globose bud (Sidibe and Williams, 2002). The fruit ovoid, with brownish seeds, embedded in a white powdered pulp (Nnam and Obiakor,2003).

Baobab leaves and fruit are used as food and for medicinal purposes in many parts of Africa. In the Sahel, for example, baobab leaf is a staple food, the Michika people in north east of Nigeria used to make "miyan kuka" soup. Powdered leaves are used to reduce high cholesterol level in the blood system (Umaru, et al., 2003). The leaves are also used traditionally to treat Guinea worm and dysentery (Dan and Dan, 1986; Sidibe and Williams, 2002).

Baobab leaves are potential protein source among Michika people to complement the amino and minerals demand as reported by Boukari et

al. (2001). Some studies reported that baobab leaves are an important source of iron (Yazzie et al,1994;), and have a higher content of iron and are a rich source of calcium (Barminas et al., 1998). Umaru et al, 2003, also show that baobab leaves possess taurocholate binding capacity properties.

The present study is focused on the isolation and characterization of three bioactive compound from the leaves of Adonsonia digitate L (Baobab) and the antioxidant potential of the isolated compounds.

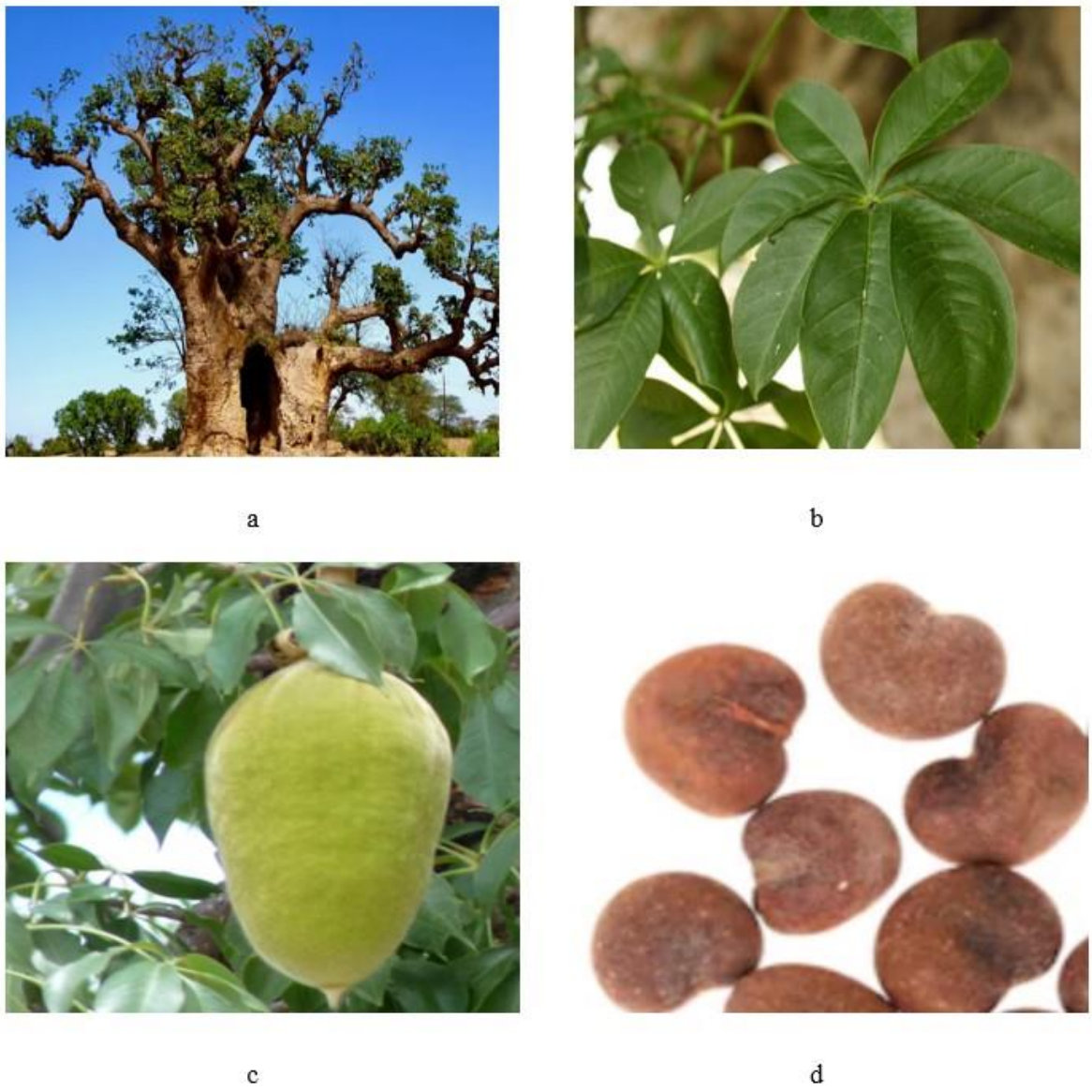


Figure 1: shows different parts of Adonsonia digitata L (Baobab) a, b, c, and d.

MATERIALS AND METHODS

Plant collection and identification

The leaves of baobab (Adansonia digitata) were obtained from Michika Local Government Area of Adamawa state, Nigeria. The plant parts were authenticated in the Botanical laboratory of the Department of Botany, Modibbo Adama University of Technology Yola, Adamawa State. The leaves were washed, air-dried and ground into fine powder using mortar and pestle in the laboratory.

Preparation of Extract of Leaves of Adansonia digitata

Adansonia digitata leaves extract were prepared by cold maceration. The extract of the leaves of Adansonia digitata were prepared by soaking 500 g of finely grounded powder of leaf in 1000 ml of dichloromethane for 72 hours. After the extracts were filtered through Whatman No. 1 filter Paper and the residual matter was again soaked with 500 ml of

Dichloromethane for 24 hours. The extract was filtered through Whatman No. 1 filter paper, pooled together and then combined. The extracts were then concentrated in a rotary evaporator. The concentrated extract was transferred into clean and dried universal bottle and stored in the refrigerator until needed for analysis.

Determination of Percentage Yield

The percentage yield of the extract was calculated using the formula below:

Percentage (%) yield = Weight of the extracted oil (g) x 100

Dried weight of sample (g)

Isolation and Identification of Secondary Metabolites

Isolation and Purification

Isolation and Purification of secondary Metabolites in the crude extract of Adonsonia digitata L (Baobab) was carried out using chromatographic procedure namely column chromatography (CC), with thin layer chromatography (TLC) plate as a medium for visual identification.

Procedure reported by Zeb et al., (2014) was used to remove tannin from the methanol crude extract which was dissolved in a small volume of methanol and added with petroleum ether to remove tannin. This was performed by adding the dissolved methanol extract into a separating funnel and then followed by adding the petroleum ether with the ratio 1:1. The sample in the separating funnel was shaken slowly and allowed to settle for 5 min until two layers of solution were clearly observed. At the end the petroleum ether extract was removed, allowing further reseparation of the methanol sample with petroleum ether. This process was repeated until the petroleum ether layer became colourless. Thus, the sample ready for isolation and purification using thin layer chromatography and column chromatography.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was carried out using aluminium plate of 20 x 20 cm coated with silica gel 60 F254 (Merck 1.05554.0001) and Rf value of each spot was determined (Umaru et al., 2019).

Column Chromatography (CC)

The crude extract was examined on TLC plate in different solvent ratio; hexane, hexane-dichloromethane, dichloromethane, dichloromethanechloroform, chloroform, chloroform - ethyl acetate, ethyl acetate, ethyl acetate–methanol and methanol.

The column was eluted using suitable solvent systems with increasing polarity (Fasihuddin et al., 2010). The column's valve was then opened and about 10-20 mL fraction of the eluate was collected in test tubes (Patra et al., 2012). The procedure was repeated using different solvent systems. Samples collected from the column were examined by using Thin Layer Chromatography (TLC) plates. Fractions with similar Rf values were combined (Patra et al., 2012). Fraction with single component spot (one spot) that appeared on TLC plate was treated as possible pure secondary metabolite.

Chemical Structure Elucidation

The identification of the isolated secondary metabolite was made by spectroscopy method namely Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infra-Red spectrometry (FTIR) as described by Fasihuddin et al. (2010). The elucidation of chemical structure for the extracted secondary metabolite was made based on the data obtained from various spectroscopy methods and also comparison with published information.

Gas Chromatography-Mass Spectrometry (GC-MS)

The single spot obtained in TLC was further analysed by GC-MS (model Clarus 680) to obtain molecular mass of pure compounds according to mass per charge (m/z) ratio as described by Kalaiselvan et al. (2012).

Isolated compound was matched with the retention times with those of authentic compounds information, and identification with obtained mass spectral from library data of the corresponding compounds (Kalaiselvan et al., 2012).

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectrometry was performed by using JEOL JNM-ECA 500 Spectrometer, based on the method as described by Umaru et al., (2019), Efdi et al., (2010) and Danelutte et al., (2003). Identification of the type of each 1H-NMR and 13C-NMR detected was based on the Table of characteristic NMR absorptions published in Organic Chemistry (Janice, 2008) and with the guide of the possible proposed structure given by NIST library.

Fourier Transform Infra-Red Spectrometry (FT-IR)

The functional groups of the compounds were detected by using Fourier Transform-Infra Red spectrometry (FT-IR). Characteristic of the chemical bond was read by spectrum produced through transmittance of wavelength of the light. The chemical bond in a molecule was detected by interpreting the infra-red transmittance spectrum. Identification of functional group in the compound was based on the Table of characteristic IR absorptions published in Organic Chemistry (Janice, 2008).

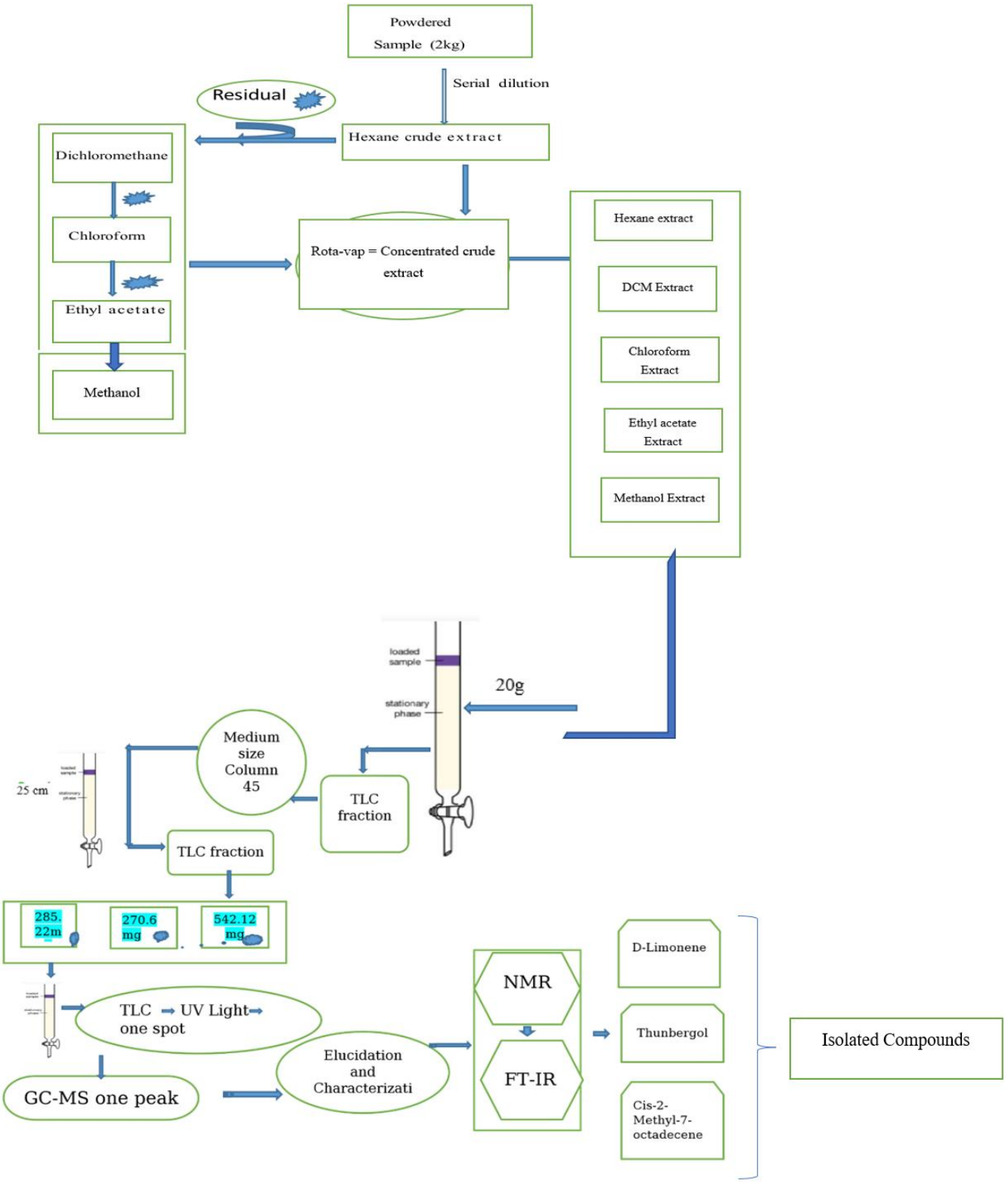


Figure 2: Showing the pure compound Isolation from *Adonsonia digitata* L (Baobab) Leaves

RESULTS AND DISCUSSION

Results: Compound 1, 2, and 3.

The GC-MS analysis of *Adonsonia digitata* L (Baobab) Isolate *indicated* the presence of three constituents. Comparison of the mass spectra of bioactive constituents with internal standards of the National Institute of Standard and Technology and Willey libraries carried out identified three bioactive compounds. The active principles with their respective retention time, molecular formula, molecular weight and concentration (peak area, %) were considered

Isolation and Identification of Secondary Metabolites

Compound 1, 2 and 3 were isolated from ethyl acetate leaf crude extract of *Adonsonia digitata* L (Baobab). About 20 g of the crude extract was loaded using dry pack method into a column with silica gel in hexane 100 %. The crude extract was then eluted from the column with solvent system in sequence as shown in Table 1.

Table 1: Solvent System used for Column Chromatography (300mL each solvent)

Solvent	Volume to volume (v/v)
Hexane	1
Hexane: Dichloromethane	1:1
Hexane: Dichloromethane	1:2
Dichloromethane	1
Dichloromethane: Chloroform	1:1
Dichloromethane: Chloroform	1:2
Chloroform	1
Chloroform: ethyl acetate Chloroform: ethyl acetate	1:1
	1:2
Ethyl acetate	1
Ethyl acetate: Methanol Ethyl acetate: Methanol	1:1
	1:2
Methanol	1

Table 2: All the fractions were labelled as ADLEA (1-10) for *Adonsonia digitate*

Fractions code	Fraction (mg) weight	Fraction colour
ADLEA 1	12.8	Colourless
ADLEA 2	30.4	Dark yellow
ADLEA 3	177.6	Light yellow
ADLEA 4	145.5	Light brown
ADLEA 5	189.8	Light brown
ADLEA 6	270.6	Dark brown
ADLEA 7	330.32	Dark brown
ADLEA 8	285.22	Dark yellow
ADLEA 9	345.18	Dark yellow
ADLEA 10	542.12	Dark yellow

Purification and Structural Elucidation of Compound 1

Isolation and Purification

Compound 1 was obtained from the combined fraction of ADLEA10 with 542.12 mg of Dark yellow colour fraction. TLC analysis fraction was carried out with the following solvent; hexane: chloroform (3:7), hexane: ethyl acetate (1:9) and hexane: ethyl acetate (2:8) as shown in Table 3.

Table 3: TLC and R_f value of ADLEA10 in Different Solvent Ration System under UV Light.

Solvent system (v/v)	Number spots of	R_f value	Stained TLC colour
Hexane: Chloroform (3:7)	2	0.49 0.51	Brown
Hexane: Ethyl acetate (1:9)	2	0.55 0.13	Brown
Hexane: Ethyl acetate (2:8)	3	0.67 0.58 0.63	yellow

Fraction of the same R_f value from ADLEA 10-1 and ADLEA 10-12 were combined and labelled as ADLEA 10-A. The combined fraction (ADLEA 10-A) was introduced to

smaller column for further purification, fraction was observed under UV and spots with similar R_f value were combined and labelled as ADLEA10-A1. ADLEA10-A1 was repeated using a suitable solvent ration hexane: ethyl acetate (1:4) and hexane: ethyl acetate (3:8). The TLC gave a good separation where the targeted spot was combined labelled ADLEA10-A2 the TLC result is as shown in Table 4.

Table 4: TLC and R_f Value of ADLEA10-A2 in Different Solvent Ration System Under UV Light.

Solvent system (v/v)	**Number spots of**	**R_f value**	**Stained TLC colour**
Hexane: Ethyl acetate (2:8)	2	0.56	Light brown
Hexane: Ethyl acetate (3:8)	1	0.62	yellow

The fraction of ADLEA10-A2 was then repeated in a smaller column by changing the solvent ratio hexane: ethyl acetate (1:9) and TLC was observed under UV, a single spot was obtained labelled ADLEA10-A3 as shown in Table 5.

Table 5: TLC and R_f value of ADLEA10-A3 in Different Solvent Ration System Under UV Light.

Solvent system (v/v)	**Number spots of**	**R_f value**	**Stained TLC colour**
Hexane: Ethyl acetate (1:9)	1	0.64	yellow

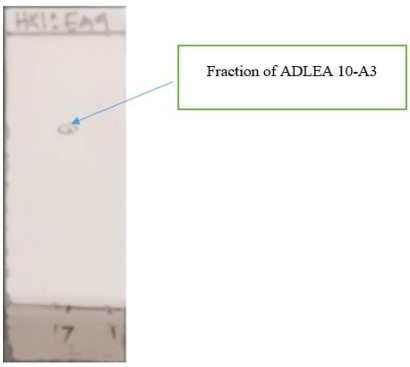


Figure 3: TLC plate showing one spot from the combined fraction of ADLEA10-A3 in hexane and ethyl acetate (1:9).

The GC-MS analysis of the combined fraction of ADLEA 10-A3 was carried out and the result from the GC showed a single peak at the retention time of 12.248 min. this confirmed that ADLEA 10-A3 is a pure compound and its was renamed as Compound 1.

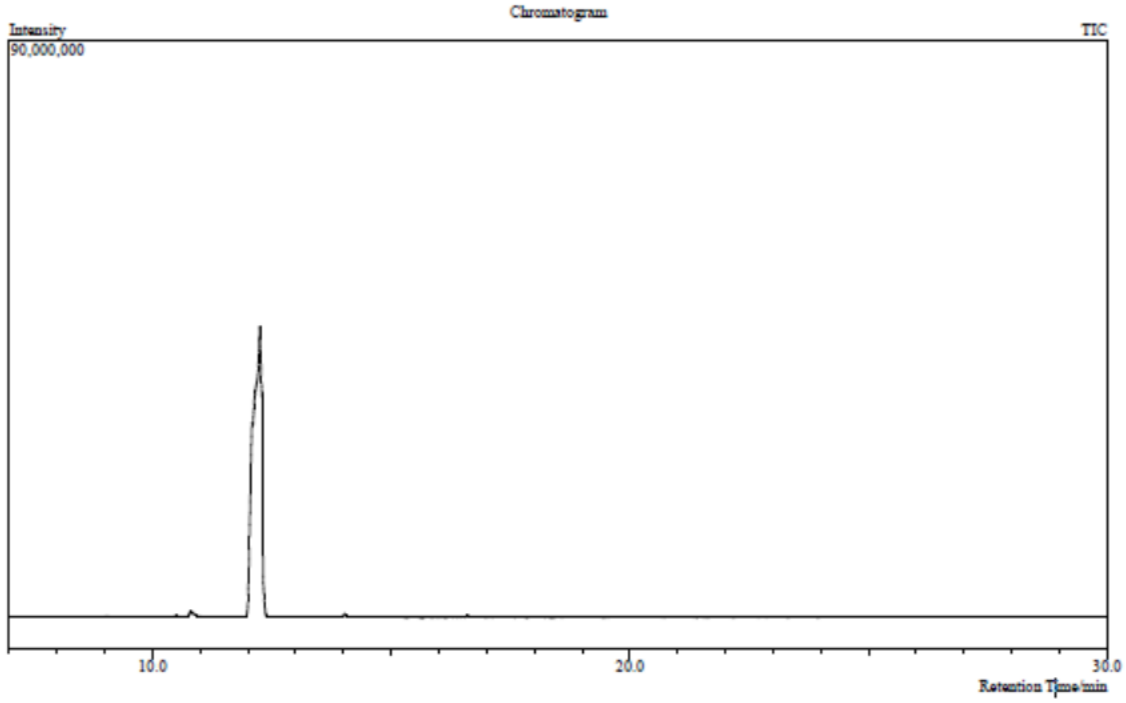


Figure 1: Gas Chromatogram of Compound 1

Structural Elucidation

The compound 1 was isolated from ethyl acetate crude extract of *Adonsonia digitata* yellow in colour and a melting point of 74.35oc. The mass spectrum of Compound 1 in Figure 2 shows a similarity index of 98.91 % with mass spectrum of the suggested structure of Compound 1 by the NIST library in Figure 3. A common peak was observed at m/z 136 which was found to correspond with the molecular ion peak and molecular ion weight of the suggested structure of Compound 1 and that of NIST library with a chemical formula C10H16.

A base peak of Compound 1 at m/z 68 was common on both spectrum Figure 2 and Figure 3 of the suggested structures for Compound 1.

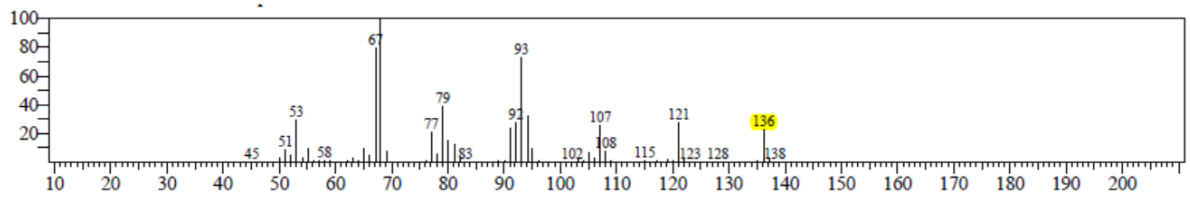


Figure 3: Mass Spectrum of Compound 1

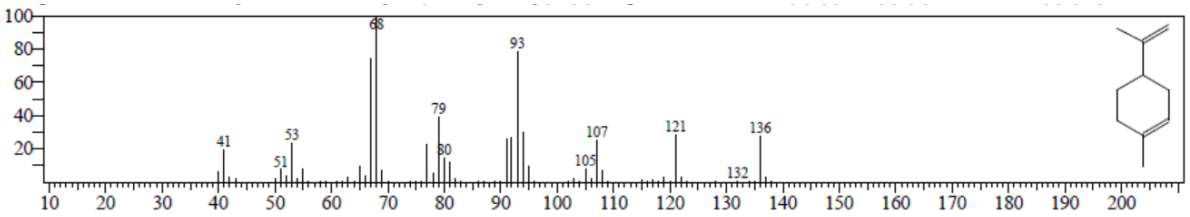


Figure 4: Mass Spectrum of Suggested Structure of Compound 1 by NIST Library

The IR structure of Compound 1 indicated an absorption band at 2973.76 cm^{-1} representing a C-H band. A signal which indicated the presence of double bond as variable bending was observed at 1680.23 cm^{-1} and 1525.02 cm^{-1} medium stretch as the double bond on the chemical ring structure of Compound 1. A single bond was observed at 1381.56 cm^{-1} as –C-H variable bending. Another single bond C-C strong bending at 878.88 cm^{-1} was observed in the IR spectrum of the suggested structure of Compound 1.

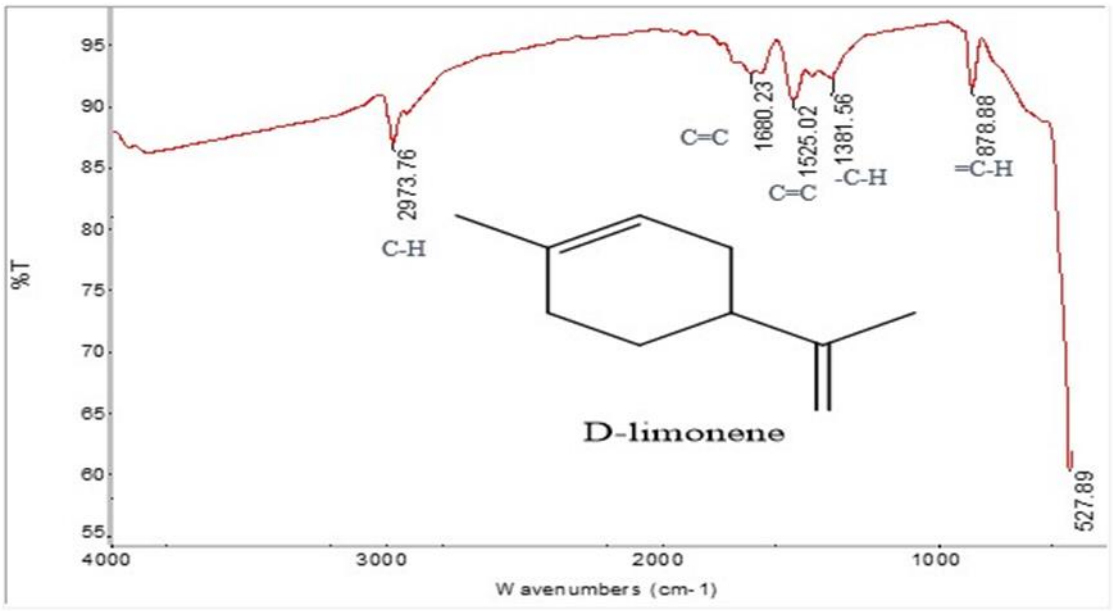


Figure 5: IR spectrum of Compound 1

NMR analysis was further performed to elucidation the chemical structure of Compound 1 through the chemical shift of every proton and NMR for Compound 1 and the report was as shown in Table 6 (1H-NMR) and Table 7 (13C-NMR). Based on the table in Organic Chemistry by Janice (2008) and Silverstein et al., (2005), the proton signals were all integrated and were assigned to every proton NMR of Compound 1 as the proposed chemical structure.

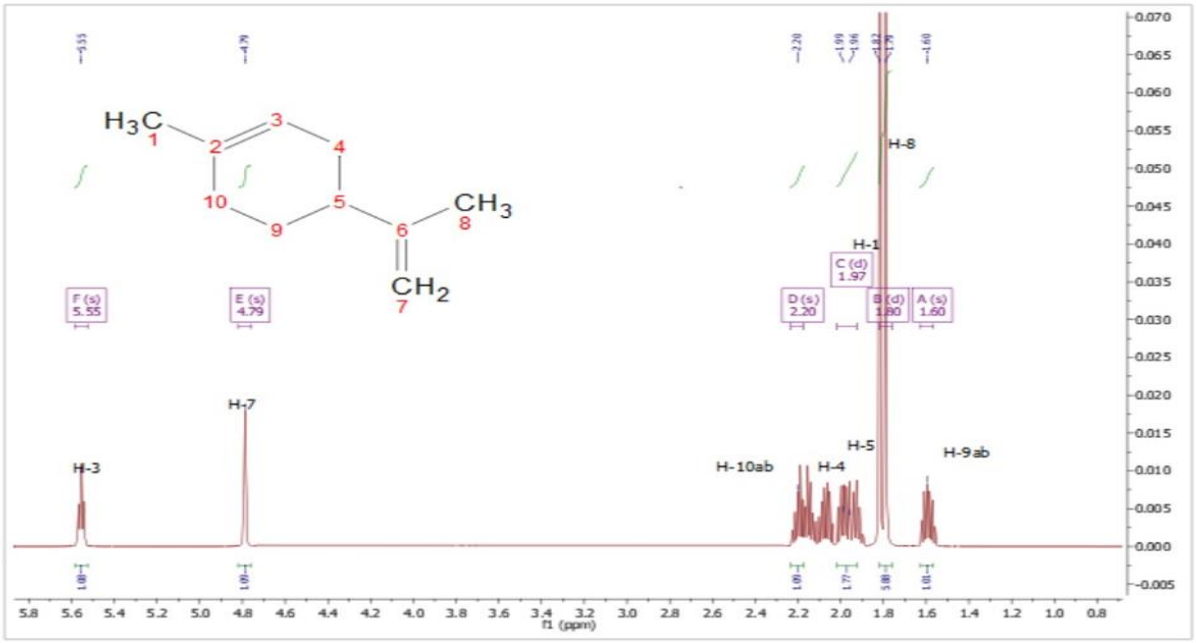


Figure 6: 1H-NMR spectrum of Compound 1 from 0.8 to 5.8 (500 MHz $CDCl_3$)

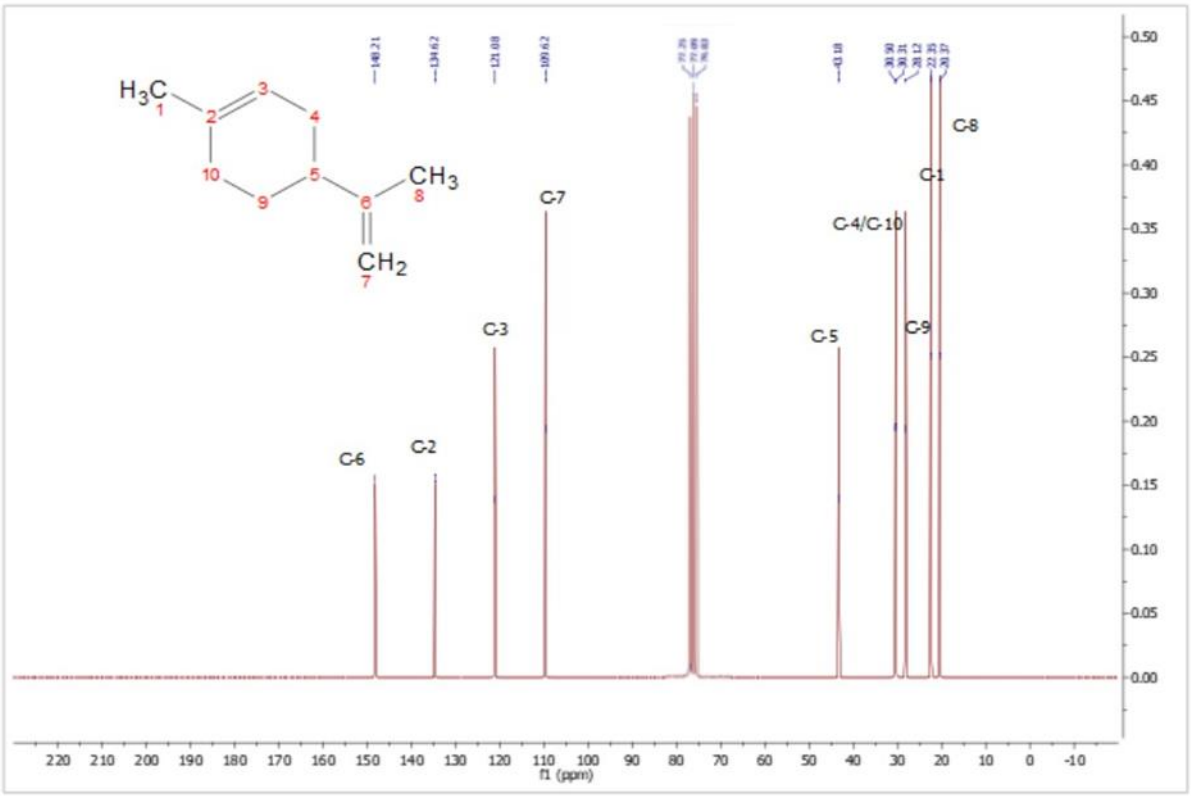


Figure 7: ^{13}C-NMR spectrum of Compound 1 from -1.0 to 220 (125 MHz $CDCl_3$)

1H-NMR of Compound 1 exhibited 10 proton resonates a signal was observed at δ 1.82 (3H, m) indicating the presence CH3 terminal and was assigned to H-1. A doublet proton signal was observed at δ 5.55 (1H, d) also indicating CH and was assigned to H-3. A signal proton was also observed at δ 1.99 (2H, q) and was assigned to H-4.

A chemical shift at δ 1.96 (1H, d) was identified to contain methine and was assigned to H-5. A signal was observed which consist of two protons (2H, m) and identified as methylene group and was assigned to H-7. At a chemical shift δ 1.79 (3H, m) identified as CH3 and was assigned to H-8. A doublet was observed at δ 1.60; δ 1.60 and δ 2.20; 2.20 were assigned to H-9 and H-10, respectively.

The 13C-NMR spectrum showed a total of 10 signals. Four signals were observed at a down field with chemical shift of δ 134.62, δ 121.08, δ 148.21 and δ 109.62, indicating the presence of methane group of the ring structure and were assigned to C-2, C-3, C-6 and C-7, respectively.

Six other signals were observed at chemical shift of δ 22.35, δ 30.50, δ 43.18, δ20.37, δ 28.12, and δ 30.31, respectively. They were assigned to C-1, C-4, C-5, C-8, C-9 and C-10 which indicated the methyl group of the compound ring.

From the result it was observed that the signals correspond to the four methyl groups (CH3) and six methylene group (CH2). The chemical shift of every proton NMR and carbon NMR for Compound 1 is shown in Table 6 and Table 7 and was in comparison with the NMR data reported by Farias et al., (2019).

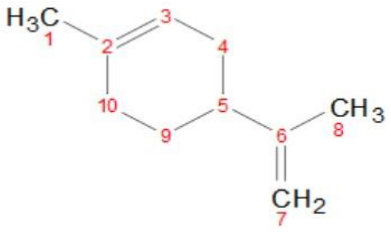
Table 6: Proton NMR Signal of Compound 1 and that Reported by Farias et. al. (2019).

Proton assigned to Compound 1	Proton chemical shift (ppm) of Compound 1	Proton assigned to D-Limonene (Farias *et. al.*, 2019).	Proton chemical shift (ppm) of D-Limonene (Farias *et. al.*, 019).
H-1	1.82 (3H, m)	H-1	2.08 5.46
H-3	5.55 (1H, d)	H-3	1.71
H-4	1.99 (2H, t)	H-4	1.673
H-5	1.96 (1H, d)	H-5	4.77
H-7	4.79 (2H, m)	H-7	1.791
H-8	1.79 (3H, m)	H-8	1.60; 1.60
H-9	(2H, m)	H-9	1.675; 1.495)
H-10	2.20; 2.20 (2H, m)	H-10	(2.288; 2.081)

Table 7: Carbon NMR Signal of Compound 1 and that Reported by Farias et. al. (2019).

Carbon assigned to Compound 1	Carbon chemical shift (ppm) of Compound 1	Carbon assigned to D-Limonene (Farias *et. al.*, 2019).	Carbon Chemical shift (ppm) of D-Limonene (Farias *et. al.*, 2019).
C-1	22.35	C-1	23.30
C-2	134.62	C-2	133.00
C-3	121.08	C-3	120.70
C-4	30.50	C-4	30.80
C-5	43.18	C-5	41.10
C-6	148.21	C-6	149.70
C-7	109.62	C-7	108.40
C-8	20.37	C-8	20.60
C-9	28.12	C-9	27.90
C-10	30.31	C-10	30.60

The mass spectrum of Compound 1 also showed a similarity index of 98.91 % with the mass spectrum of the proposed structure identified as D-Limonene (1) by NIST library. Thus, based on the spectroscopic data of Compound 1 and the comparison with the published literature



Compound 1 was identified as D-Limonene (1) with the chemical formula C10H16

D-Limonene (1) is a cyclic monoterpene (C10) present in nature as two enantiomers, the compound was reported to show contact toxicity against S. zeamais and T. castaneum of grain storage insects (Fang et al., 2019) These findings, considered together, suggest that the compound DLimonene (1) show potential for development as natural fumigants for stored products (Fang et al., 2010). D-Limonene (1) is used for the manufacture of food, beverages and cosmetics as a flavouring additive (Chiralt, et al., 2002).

Purification and Structural Elucidation of Compound 2

Purification

Compound 2 was obtained from the combined fraction of ADLEA6 of 270.6 mg of dark brown fraction Table 2. TLC analysis of the fraction was carried out in the following solvent ratio (hexane: chloroform (3:7) and hexane: ethyl acetate (1:9). The result from the TLC fraction was shown in Table 8 as ADLEA6-A

Table 8: TLC and Rf Value of ADLEA6 in different solvent ratio system under UV

Solvent system (v/v)	**Number of spots**	**R_f value**	**Stained TLC colour**
Hexane : Chloroform (3:7)	2	0.45 0.21	Brown
Hexane: Ethyl acetate (1:9)	2	0.55 0.13	Brown

Hexane: ethyl acetate (3:7) was selected for further purification. Combined fraction of similar Rf value from TLC ADLEA6A were combined labelled as ADLEA6-A. ADLEA6-A was further purified using a smaller column and the TLC of the fraction collected was performed and the result was shown in Table 9 show a single spot as while in Figure 6

Table 9: TLC and Rf Value of ADLEA6-A in different solvent ratio system under

Solvent system (v/v)	**Number spots of**	**R_f value**	**Stained TLC colour**
Hexane: Ethyl acetate (3:7)	1	0.55	Brown

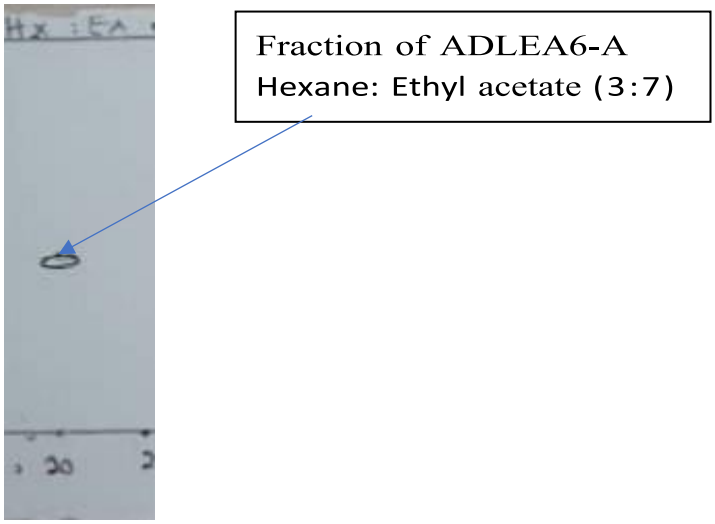


Figure 6: TLC plate showing one spot in combined fraction of Hexane: Ethyl acetate (3:7)

ADLEA6-A was subjected to GC analysis and the result obtained from the gas chromatograph revealed a single peak at the retention time 34.14 min as shown in Table 7. This confirms ADLEA6-A a pure compound and renamed as Compound 2.

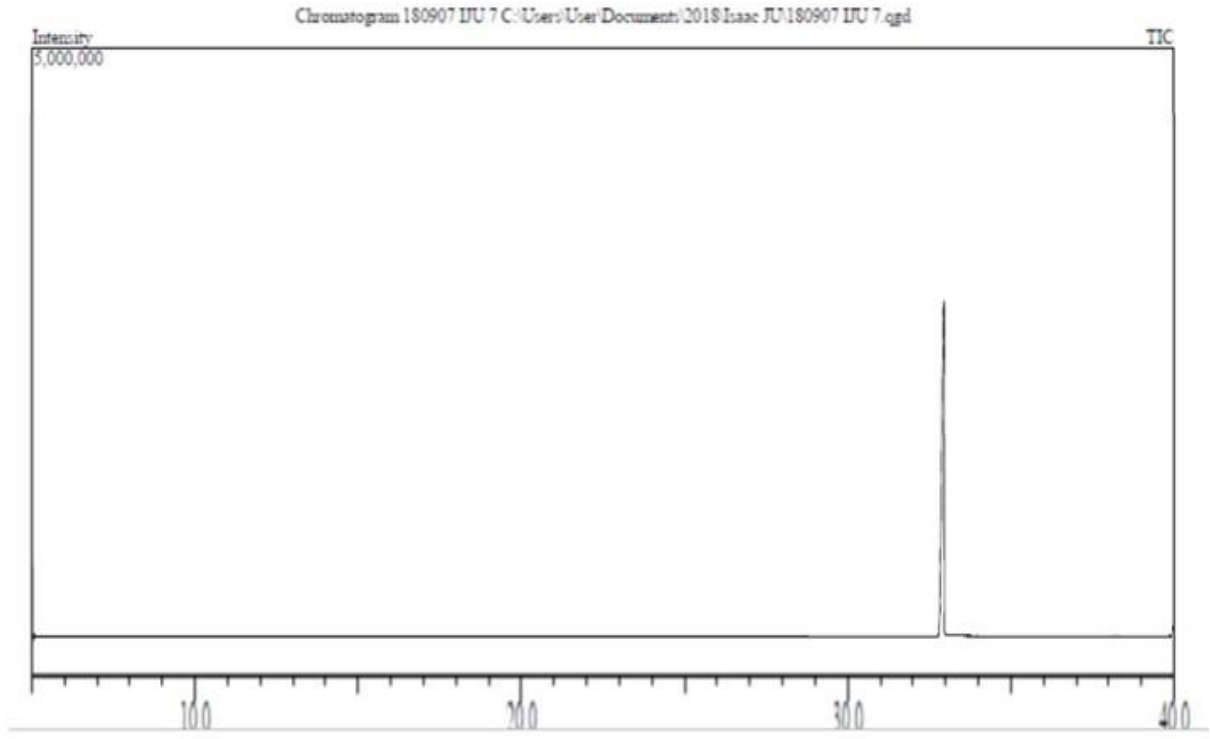


Figure 7: Gas Chromatogram of Compound 2

Structural Elucidation of Compound 2

Compound 2 was isolated from dichloromethane leaf crude extract of

Adonsonia digitate Table 2 from ADLEA 6 (270.6 mg). the physical appearance of Compound 2 (14 mg) is white solid with melting point of 76 oC. The mass spectrum of Compound 2 in Figure 8 shows a similarity index of 95% with the mass spectrum of the structure suggested by the NIST library in Figure 9. The mass spectrum of Compound 2 has an ion base peak that appeared at m/z 95, which is also observed on the mass spectrum of the suggested structure by the NIST library which corresponded with the molecular weight (290 mg) of the proposed structure of Compound 2 with chemical formula (C20H34O)

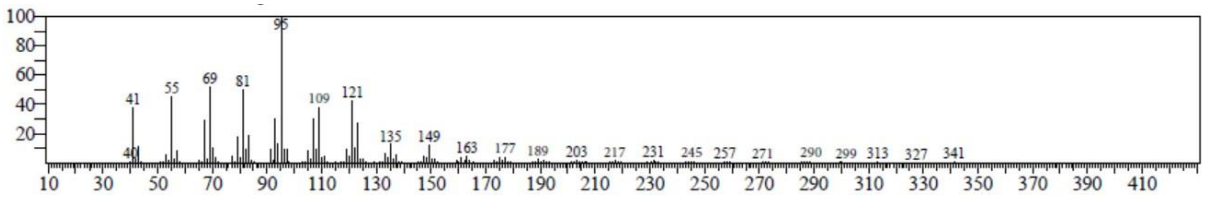


Figure 8: Mass spectrum of Compound 2

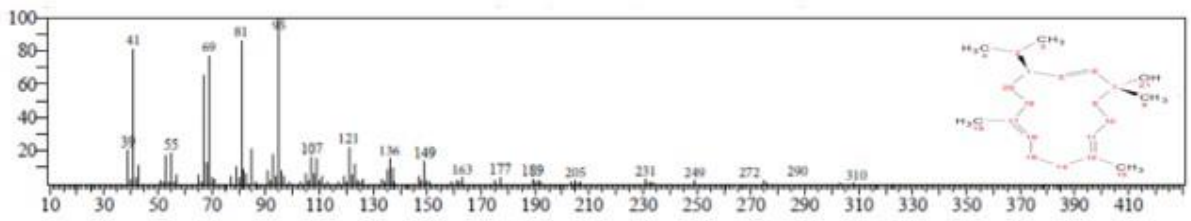


Figure 9: Mass Spectrum of Suggested Structure of Compound 2 by NIST Library

The IR spectrum of Compound 2 in Figure 10 suggest the presence of functional group which characterize the chemical compound 2 as presented in NIST library and in the literature, to confirm compound 2. A functional group of OH which appeared at 3346.4 cm-1. An absorption band which indicated a methylene at C-H was observed at 2974.15 cm-1 and a band was observed at 1652.89 cm-1indicated a double band which represented double bond in the structure of compound 2. A signal was observed at 1384.58 cm-1 which represent the methine groups in the compound and 878 cm-1 band represent the bands of methine group attached to the double in the structure. The IR structure showed similarity to the IR stretching and bending vibration of the same compound as reported by Inger et al., (1981

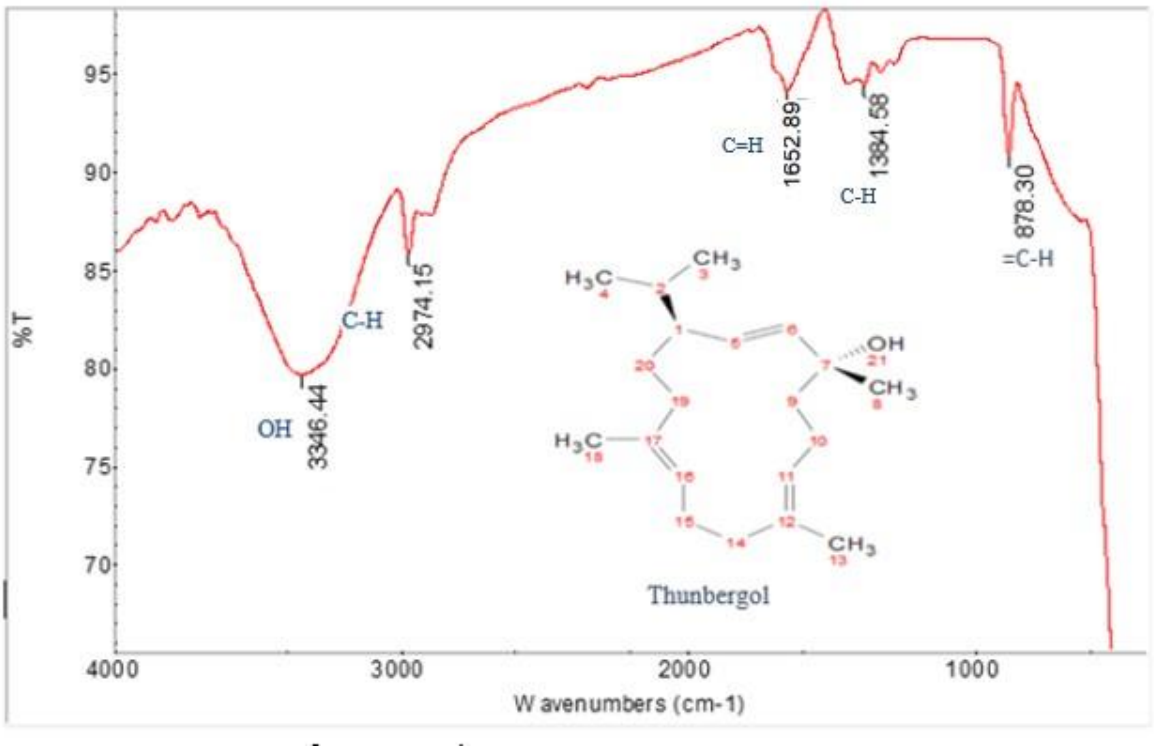


Figure 9: IR Spectrum of Compound 2

The existence of Compound 2 was further confirmed by characterization obtained from the 1H-NMR and 13C-NMR through the chemical shift of every proton and carbon of compound 2. The proton NMR was integrated and assigned to the proposed chemical of

compound 2 which was based on the Table of 1H-NMR characteristic absorption as well as the peaks splitting pattern as reported in spectrometric identification of organic compounds by Silverstein et al., (2005).

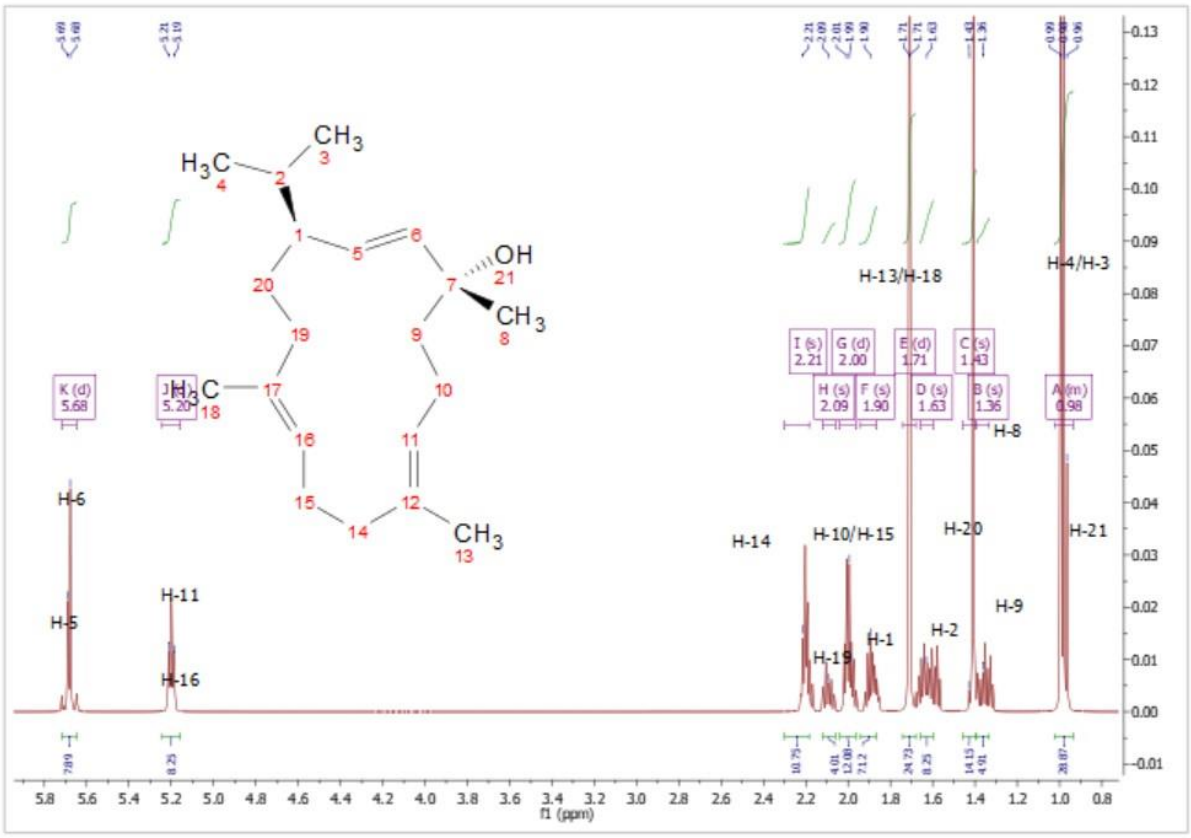


Figure 10: 1H-NMR spectrum of Compound 2 from 0.8 to 5.8 (500 MHz CDCl3)

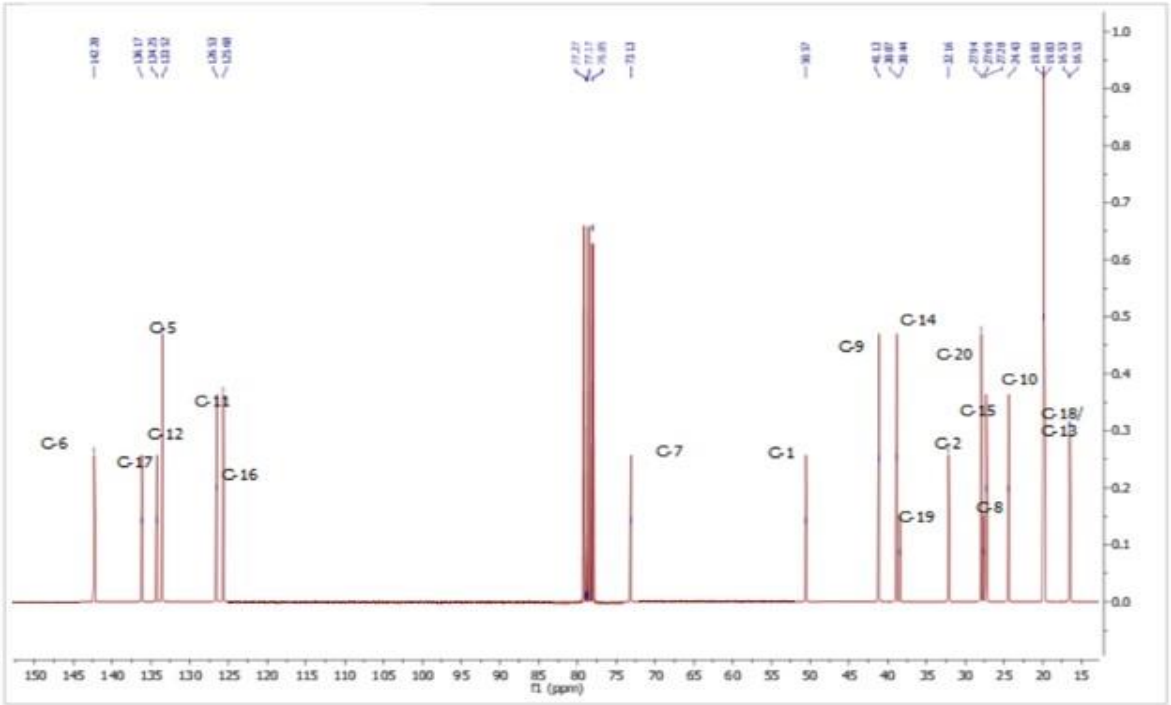


Figure 11: 13C-NMR spectrum of Compound 2 from 15 to 150 (125 MHz CDCl3)

1H-NMR of Compound 2 with chemical formula C20H34O exhibited 18 proton resonates of six methine (CH), seven ethylene (CH2), five methyl (CH3) and OH group were observed on 1H-NMR spectrum of Compound 2 from 0.8 to 5.8. six signal methine group were identified as δ 1.88, δ 1.63, δ 5.09, δ 5.67, δ 5.20 and δ 5.20 and are assigned to H-1, H-2, H-5, H-6, H-11 and H-16, respectively. A doublet proton signal of methylene was observed at δ 1.41, δ 1.99, δ2.20, δ 2.00 δ2.19 δ1.39 and δ 1.34. They were assigned to H-8, H-10, H-14, H-15, H-19, H-20 and H-9, respectively as shown in Table 10

Five methyl protons were observed on the spectrum at chemical shift of δ 0.99, δ 0.99, δ 1.41 δ 1.71 and δ 1.71 and were assigned to H-3, H-4, H-8 H-13 and H-18. Hydroxyl group was observed attached to Carbon seven demonstrated of an OH of the methanol group of structure of Compound 2. OH, was assigned to H-21 as shown in Table 10.

Table 10: Proton NMR signal of Compound 2 and that reported by Inger et al., (1981)

Proton assigned to Compound 2	Proton chemical shift (ppm) of Compound 2	Proton assigned to thunbergol by Inger et al., (1981)	Proton chemical shift (ppm) of thunbergol Inger et al., (1981)
H-1	1.88 (1H, s)	H-1	1.88
H-2	1.63 (1H, m)	H-2	1.66
H-3	0.99 (3H, m)	H-3	0.88
H-4	0.99 (3H, m)	H-4	0.88
H-5	5.69 (1H, t)	H-5	5.66
H-6	5.67 (1H, d)	H-6	5.22
H-8	1. 41(3H, s)	H-8	1.46
H-9	1.34 (2H, d)	H-9	1.20
H-10	1.99 (2H, m)	H-10	1.99
H-11	5.20 (1H, m)	H-11	5.20
H-13	1.71 (3H, s)	H-13	1.66
H-14	2.20 (2H, m)	H-14	2.20
H-15	2.00 (2H, m)	H-15	2.01
H-16	5.20 (1H, m)	H-16	5.10
H-18	1.71 (3H, s)	H-18	1.79
H-19	2. 19(2H,dd,	H-19	1.78
H-20	=16)	H-20	1.32
H-21	1.39 (2H, m)	H-21	0.86
	0.97 (1H, s)		

The data obtained from the 13C-NMR carbon signal of the compound was as reported in Table 15. The compound exhibited 20 carbon signals. The assignation and integration of the carbon signal in the chemical structure, six carbons were observed at the up field region

with signal at δ133.52, δ 142.28, δ 126.53, δ 134.25, δ 125.68 and δ 136.17 indicating the presence of ethylene carbon and were assigned to C-5, C-6, C-11, C-12, C-16 and C-17.

Fourteen signals were observed as aliphatic carbon, six were observed at δ 50.57, δ32.16, δ 73.13, δ 38.44, δ 41.13 and δ 38.87 as alkene carbon and was assigned to C-1, C-2, C-7, C-19, C-9, and C-14. Another group of the aliphatic carbon were observed at δ27.94, δ 24.43, δ 27.28 and δ 27.94 was assigned to C-8, C-10, C-15, and C-20.

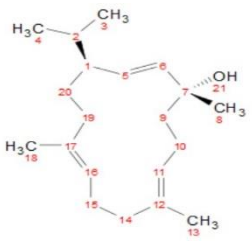
At the down field still four of the fourteen Aliphatic carbon were observed at δ 19.83, δ 19.83, δ 16.53 and δ 16.53 are assigned to carbon C-3, C-4, C-13 and C-18 respectively. The spectrum of 13C-NMR of Compound 2 as presented in Table 15 corresponds with the reference data reported by Inger et al., (1981). Thus, the chemical shift of the proton and carbon NMR of Compound 2 and the comparison with the reference was as shown in Table 10 and Table 11.

Table 11: Carbon NMR Signal of Compound 2 and that Reported by Inger et al., (1981)

Carbon assigned to Compound 2	Carbon chemical shift (ppm) of Compound 2	Carbon assigned to, thunbergol by Inger et al ., (1981)	Carbon chemical shift (ppm) of thunbergol (Inger et al ., 1981)
C-1	50.57	C-1	45.9
C-2	32.16	C-2	33.0
C-3	19.83	C-3	19.5
C-4	19.83	C-4	20.4
C-5	133.52	C-5	132.3
C-6	142.28	C-6	138.3
C-7	73.13	C-7	72.5
C-8	27.94	C-8	27.6
C-9	41.13	C-9	43.1
C-10	24.43	C-10	23.8
C-11	126.53	C-11	125.2
C-12	134.25	C-12	132.2
C-13	16.53	C-13	15.0
C-14	38.87	C-14	39.2
C-15	27.28	C-15	28.10
C-16	125.68	C-16	128.6
C-17	136.17	C-17	129.0
C-18	16.53	C-18	14.7
C-19	38.44	C-19	36.80
C-20	27.94	C-20	22.6

From the Tables, it was observed that all the data from the chemical shift of the proton and the carbon for compound 2 was reported and are in support with the 1H-NMR and 13C-NMR from the published work of Inger et al., (1981).

The mass spectrum of Compound 2 showed a similarity index with the mass spectrum from the NIST library. Therefore, based on the comparison made with published literature Compound 2 was identified as Thunbergol (C20H34O)



Thunbergol (2) isolated from the leaf of Adonsonia digitate were also identified in the Survey of Chemical Compositions and Biological Activities of Yemeni Aromatic Medicinal Plants which happened to aid in the biological activity of the Conyza bonariensis (L.) crude extract (Bhuwan et al., 2015)

Isolation and Identification of Secondary Metabolites

Isolation and Purification

Elucidation and characterization of Compound 3 showed a dark brown colour which was purified in a solvent system of hexane: ethyl acetate (3:7) and was examined under UV light, a targeted spot was further purified to obtain a single clear spot. The developing solvent polarity was changed to hexane: ethyl acetate (4:6) and the TLC examined showed a clear distant separation, spots of same Rf value were combined and named as ADLEA8. The combined fraction of ADLEA8 was further subjected to a smaller column and the result of the TLC under UV was shown in Table 12.

Table 12: TLC and Rf Values of Combined Fraction of ADLEA8 in Different Solvent System Under UV Light

Solvent system (v/v)	**Number of spots on TLC**	**R_f value**	**Stained TLC Colour**
Hexane: Ethyl acetate (3:7)	3	0.52 0.49	Colourless
		0.43	
Hexane: Ethyl acetate (4:6)	3	0.48 0.53 0.56	Colourless

ADLEA8 of hexane: ethyl acetate was subjected to column for further better separation and spots of same Rf value observed under UV light was combined and labelled as ADLEA8-B. ADLAE8-B was again subjected to smaller column for further purification in different solvent system of dichloromethane: ethyl acetate (1:4), the TLC fraction under UV light and Vanillin stain indicated two spots and a fraction of interest was labelled ADLAE8-B1 as shown in Table 13.

Table 13: TLC and Rf Values of Combined Fraction of ADLAE8-B1 in Different Solvent System Under UV Light

Solvent system (v/v)	**Number of spots on TLC**	**R_f value**	**Stained TLC Colour**
Dichloromethane: Ethyl cetate (1:4)	2	0.54 0.22	Colourless

The targeted compound from the fraction of ADLEA8-B1 was again subjected to a smaller column to further purified with a solvent system of dichloromethane: ethyl acetate 3:7 and the TLC of the fraction showed one spot under UV and vanillin stain. Fraction was labelled as ADLAE8B2 as shown in Table 14.

Table 14: TLC and Rf Values of Combined Fraction of ADLAE8-B2 in Different Solvent System Under UV Light

Solvent system (v/v)	**Number of spots on TLC**	**R_f value**	**Stained TLC Colour**
Dichloromethane: ethyl acetate (3:7)	1	0.54 0.22	Colourless

ADLAE 8-B2 with single spots suggest pure compound as shown in Figure 10.

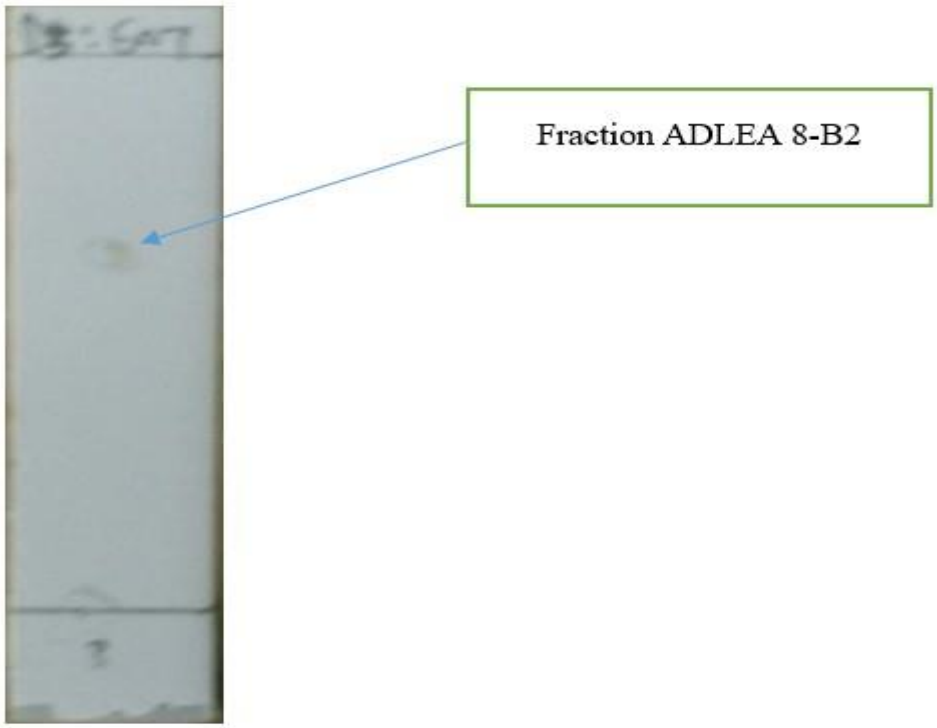


Figure 10: TLC Plate Showing One Spot from Combined Fraction of ADLAE8-B2 in dichloromethane: ethyl acetate (3:7)

The single spot fraction ADLAE8-B2 was then subjected to GC-MS and the result from the GC showed a single peak with retention time of 20.55 min. This confirms that ADLEA8-B2 is a pure compound and was renamed Compound 3.

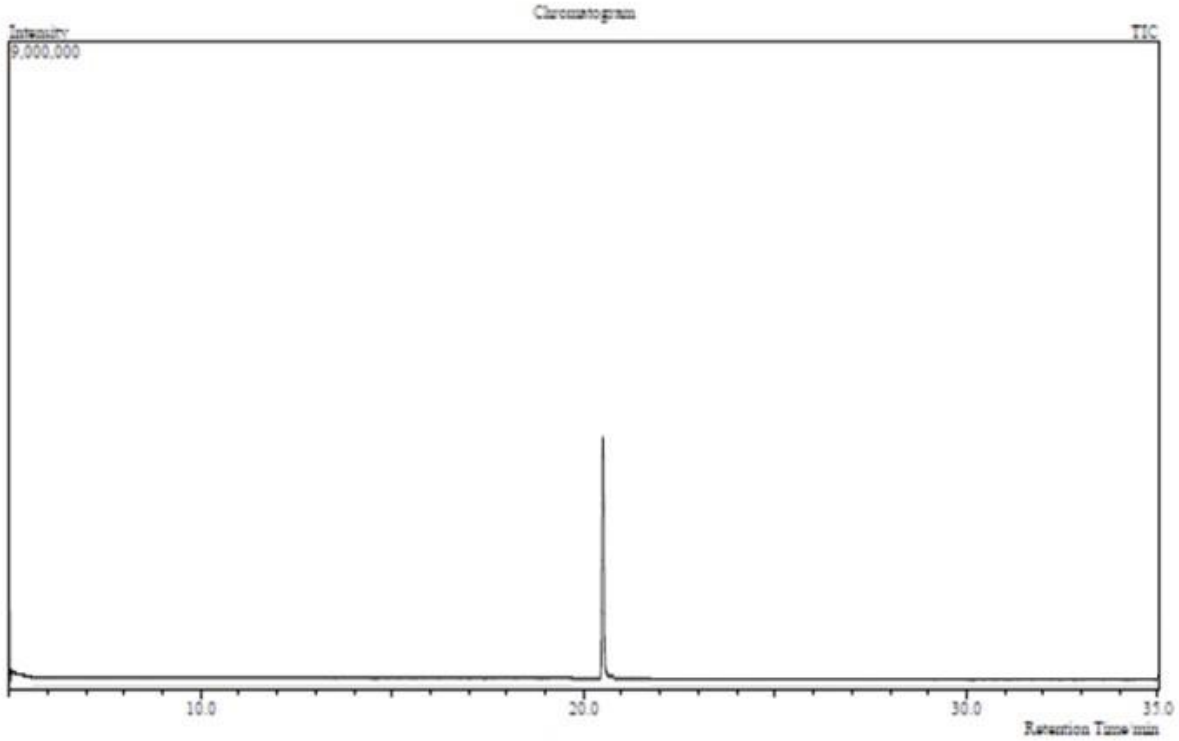


Figure 11: Gas Chromatogram of Compound 3

Structural Elucidation

The compound isolated from Adonsonia digitata Leaf ethyl acetate is as shown in Figure 11. The physical appearance of compound is white crystal solid with a weight of 20 mg. the mass spectrum of the isolated compound 3 has similarity index of 73.57 % with the mass spectrum of Compound 3 as suggested by NIST library in Figure 12. One of the molecular ion peaks on the mass spectrum of compound 3 was observed at m/z 226, this was found to correspond to the molecular ion peak and molecular weight of suggested compound 3 on the NIST library with a chemical formula of C19H38. A common base peak was observed in Figure 12 and Figure 13 at m/z 56.

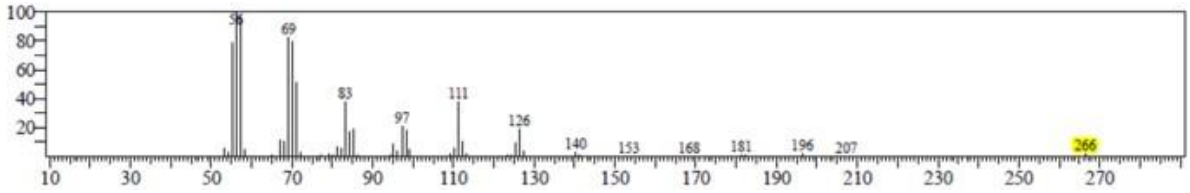


Figure 12: Mass Spectrum of Compound 3

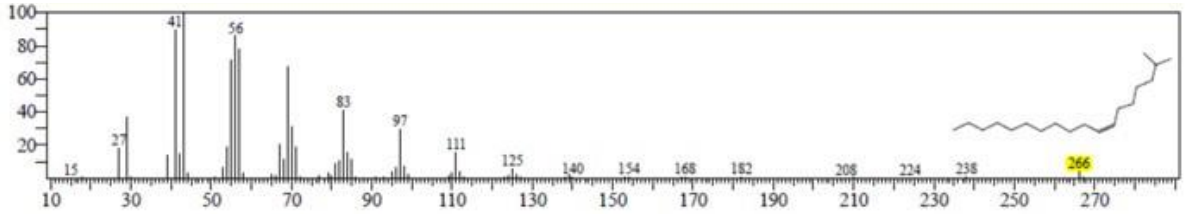


Figure 13: Mass Spectrum of Suggested Structure of Compound 3 by NIST Library.

IR spectrum showed the presence of a bending variable at 1484.44 cm-1, which suggest the double bond of the compound. An absorption band was also observed at 2955 cm-1 and 2890.96 cm-1 with a strong stretching which suggest the presence of C-H. However, a single bond of methine with a bending variable of the double bond of the compound (=C-H) at 879 cm-1 was also observed as shown in the spectrum of Compound 3.

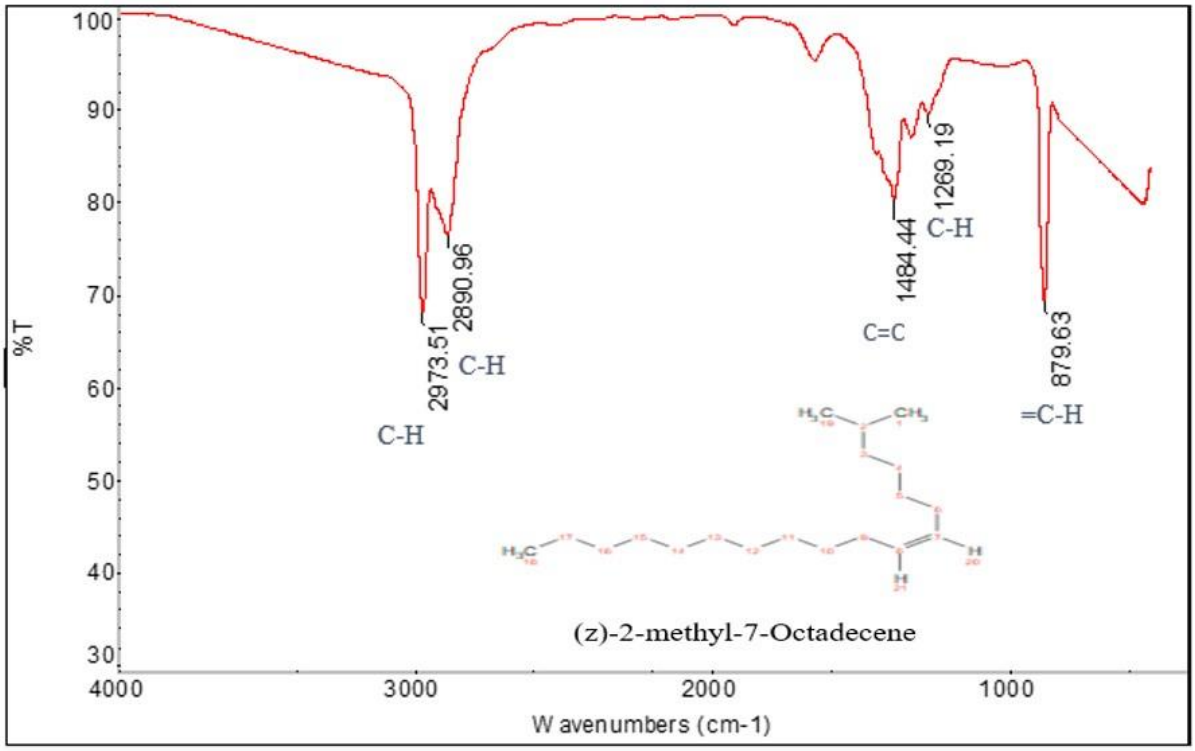


Figure 14: IR Spectrum of Compound 3.

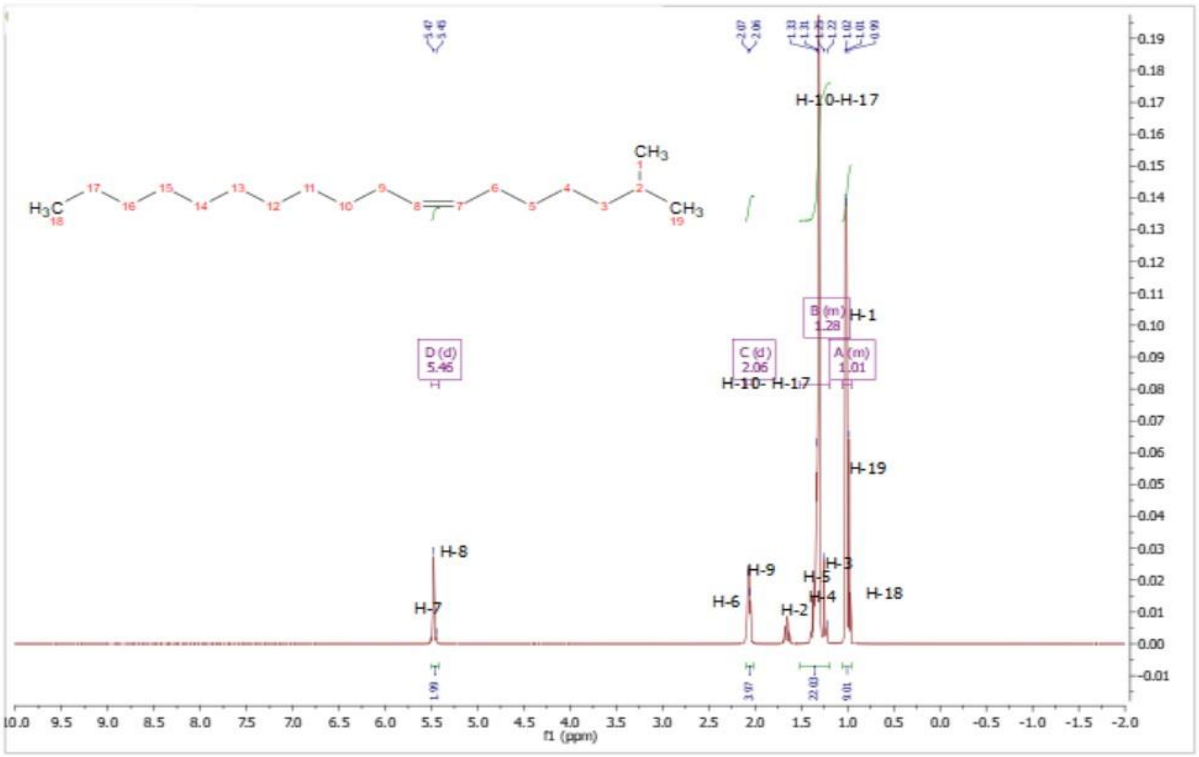


Figure 15: 1H-NMR spectrum of Compound 3 from -2.0 to 10.0 (500 MHz CDCl3)

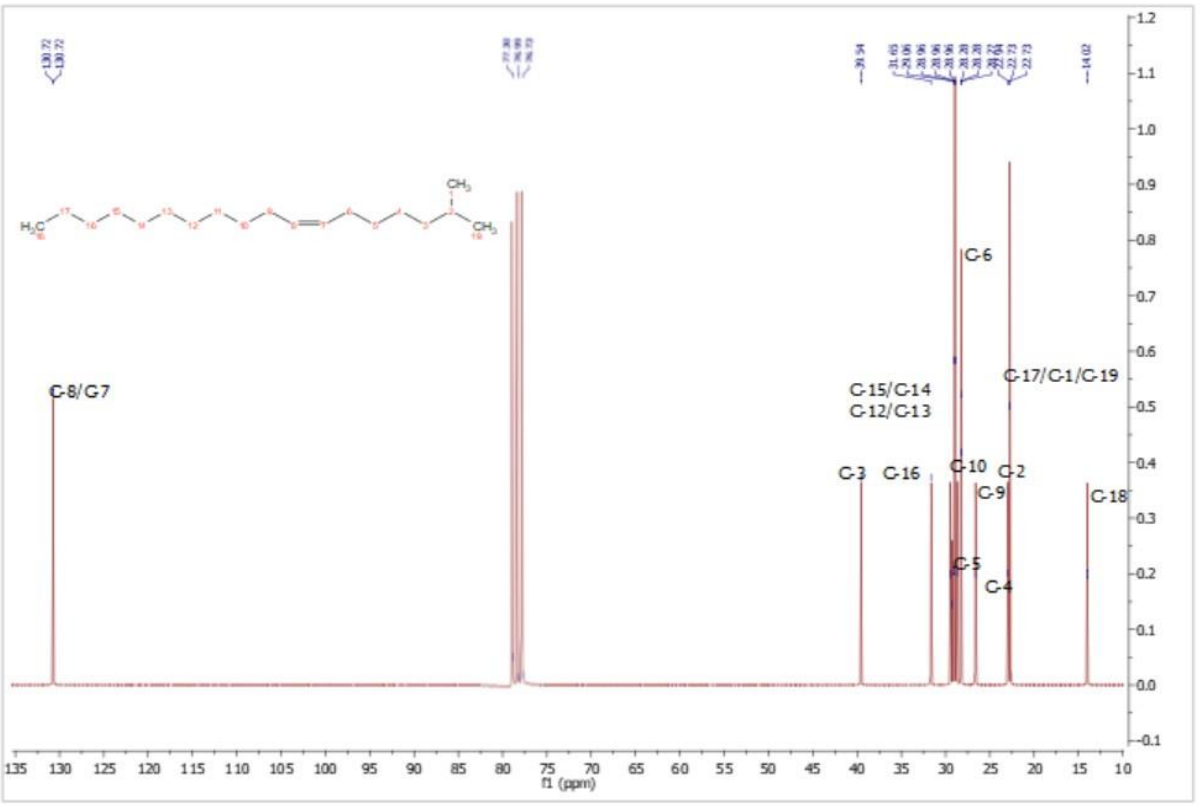


Figure 16: 13C-NMR spectrum of Compound 3 from 10 to 135 (125 MHz CDCl3)

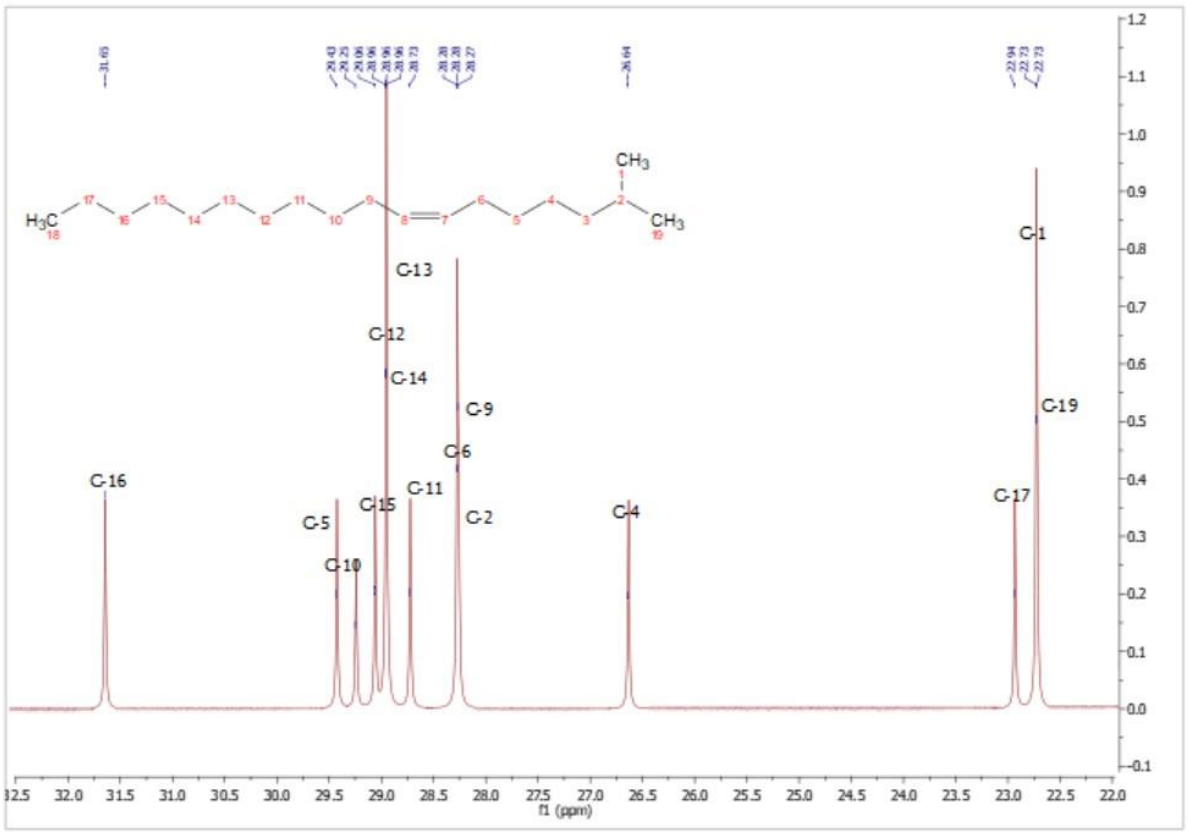


Figure 17: 13C-NMR spectrum of Compound 3 from 22.0 to 32.5 (125 MHz CDCl3)

Further elucidation and characterization of Compound 3 was done in the NMR analysis of 1H-NMR and 13C-NMR as shown in Table 15 and Table 16. Its peak splitting was as reported in the spectrometric identification of organic compounds by Silverstein et al. (2005).

The 1H-NMR splitting of Compound 3 was as shown in Table 16 which exhibits 12 protons signal and 19 carbons (C19H38). A signal proton was observed at δ 5.47-5 and 5.45 indicating the presence of methine group with double bond and was assigned to H-8 and H-9. A singlet was observed at δ 1.37- δ 1.31 (16H, d) which indicated the presence of methylene group in the structure of the isolated Compound 3 and was assigned to H-10 to H-17 of the long chain.

A proton was observed at δ 2.09 (2H, m) and δ 2.06 (2H, m) which indicated the presence of methylene group and was also assigned to H-6 and H-9 respectively. This corresponds with the ones obtained in the literature. Also observed on the spectrum was δ102 (3H, m), δ 1.01(3H,

m), and 0.99 (3H, m), indicating the presence of terminal methyl group of Compound 3 and was assigned to H-1, H-19 and H-18

From the result of the 13C-NMR spectrum of Compound 3 in the table15 and 16, every carbon NMR signal that was observed were assigned to the proposed chemical structure of the Compound 3 isolated which was based on the Table of 13C-NMR as reported in the spectrometric identification of organic compounds by Silverstein et al. (2005).

In the tables, a total of 19 carbon resonates were observed in the 13C-NMR spectrum of Compound 3. At the down field region signals were observed at δ 130.72, and δ 130.72 was identified as methine, this was assigned to C-7 and C-8. Whereas at the up field three signal were observed at δ 22.73, δ 22.73 and δ 14.02 as methyl carbon of the compound terminals as was assigned to H-2, H-6 and H-7. Thirteen signal was observed as methylene carbon at δ 39.54, δ 26.64, δ 29.43 δ 28.28, δ 130.72, δ 130.72, δ 28.28, δ 29.25, δ 28.73, δ 28.96, δ 29.96, δ 28.96, δ 29.06, δ 31.65 and δ 22.94, as the carbon of the longest carbon chain of Compound 3 and was assigned to C-3, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16 and C-17.

Chemical shift of every proton and carbon NMR for Compound 3 is shown in Table 15 and Table 16 and was in comparison with the NMR data of the similar compound reported by Russell et al., (2003).

Table 15: Proton NMR Signal of Compound 3 and that Reported by Russell et al. (2003).

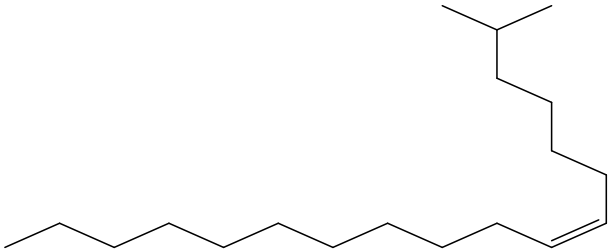
Proton assigned to Compound 2	Proton chemical shift (ppm) of Compound 2	Proton assigned to (z)-2-methyl-7-octadecene (Russell et al. (2003).	Proton chemical shift (ppm) of (z)-2-methyl-7-octadecene (Russell et al ., 2003).
H-1	1.02 (3H, m)	H-1	1.05 (3H, m)
H-2	1.66 (2H, m)	H-2	1.52 (1H, s)
H-3	1.25 (2H, m)	H-3	1.17 (2H, m)
H-4	1.33 (2H, m)	H-4	1.31 (2H, m)
H-5	1.33 (2H, m)	H-5	1.32 (2H, m)
H-6	2.09 (2H, m)	H-6	2.02 (2H, m)
H-7	5.47 (1H, m)	H-7	5.41 (1H, m)
H-8	5.45 (1H, m)	H-8	5.29 (1H, m)
H-9	2.06 (2H, m)	H-9	2.10 (2H, m)
H-10-H-17	1.30-1.37 (16H, m)	H-10-H-17	1.44-1.21 (16H, m)
H-18	0.99 (3H, m)	H-18	0.86 (3H, m)
H-19	1.02 (3H, m)	H-19	1.03 (3H, m)

Table 16: Carbon NMR Signal of Compound 3 and that Reported by Russell et al. (2003).

Carbon assigned to Compound 2	Carbon chemical shift (ppm) of Compound 2	Carbon assigned to (z)-2-methyl-7-octadecene by Russell et al. (2003).	Carbon chemical shift (ppm) of (z)-2-methyl-7-octadecene (Russell et al. (2003).
C-1	22.73	C-1	22.7
C-2	28.27	C-2	28.0
C-3	39.54	C-3	38.9
C-4	26.64	C-4	26.8
C-5	29.43	C-5	31.9
C-6	28.28	C-6	27.7
C-7	130.72	C-7	129.8
C-8	130.72	C-8	129.9
C-9	28.28	C-9	27.2
C-10	29.25	C-10	29.8
C-11	28.73	C-11	29.7
C-12	28.96	C-12	29.6
C-13	29.96	C-13	29.5
C-14	28.96	C-14	29.4
C-15	29.06	C-15	29.3
C-16	31.65	C-16	31.9
C-17	22.94	C-17	22.6
C-18	14.02	C-18	14.1
C-19	22.73	C-19	22.73

The data obtained for compound 3 reported by the GC-MS spectrum gave similarity index of 73.57 % with the mass spectrum of the proposed structure of the compound in NIST library. This matched the characteristic of the compound identified as (z) 2-Methyl-7-Octadecene (3) with the formula C19H38 as reported by Russell et al. (2003). The IR data of Compound 3 was observed also to match the IR data of Compound 3 which was reported as (z) 2-Methyl-7-Octadecene (3). The 1H-NMR and 13C-NMR data also matched the suggested chemical structure of Compound 3 to the data on the 1H-NMR and 13C-NMR signals of the published information of Russell et al. (2003).

Based on the data on mass spectrum, IR, 1H-NMR and 13C-NMR and in comparison, with the published journal, Compound 3 was identified as (z)-2-Methyl-7-Octadecene (3) as pheromones compound identified for the first time in Adonsonia digitata plant.



Antioxidant potential (IC 50) of the isolated pure Compound

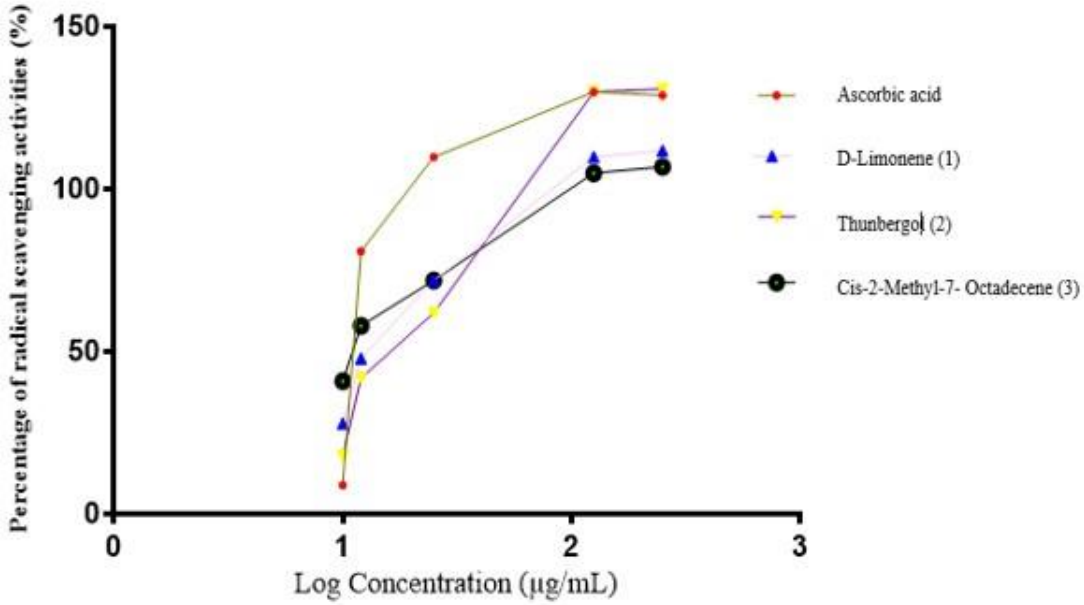


Figure 15: Graph IC50 Value of the Isolated Pure Compound from *Adonsonia digitata*

Table 17: Antioxidant IC_{50} of Pure Compound Isolated from *Adonsonia digitata*

Pure Compound	IC_{50} (µg/mL)	R2
D-Limonene (**1**),	82.51	0.9836
Thunbergol (**2**)	77.79	0.9772
Cis-2-Methyl-7octadecene (**3**)	85.51	0.9991
Ascorbic acid	61.96	0.9657

Antioxidant are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals.

Antioxidant help prevent oxidation which can cause damage to cells and contribute to aging. The free radical scavenging activity was measured in the terms of hydrogen donation or radical scavenging ability using the stable radical 1,1-Diphenyl-2-picrylhydrayl (DPPH) (Abubakar et al., 2014). This compound is readily destroyed by proton radical scavengers. Exposure to proton radical scavengers of the tested sample significantly decreased its characteristic absorption at 510-520nm ultra violet region (Isaac et al., 2018), and the results was used to indicate the antioxidant properties of the pure compounds isolated and characterised from Adonsonia digitata leaf extract.

The Isolated Pure Compounds D-Limonene (1), Thunbergol (2) and Cis-2-Methyl-7-octadecene (3) exhibited some antioxidant activity. In this study, the absorbance was measured at 517 nm using UV spectrophotometer. The antioxidant activity was evaluated with DPPH radical scavenging assay.

This was determined from the IC50 value, which were statistically determined using Log dose inhibition curve in computerized PRISM programme, based on a percentage of DPPH scavenging of the chemical constituents. The value of IC50 of the isolated compound is shown in Figure 15 and Table 20, as D-Limonene (1) 82.51μg/mL, Thunbergol (2) 77.79μg/mL and Cis-2-Methyl-7-octadecene (3) 85.51μg/mL and the control Ascorbic acid 61.96 μg/mL.

CONCLUSION

In summary, plant extracts from Adonsonia digitata showed an important isolate of D-Limonene (1), Thunbergol (2) and Cis-2-Methyl-7-octadecene (3) which can be used as an agent for disease cure. They can be considered a good source of natural antioxidants. Natural bioactive compounds have been found to interfere with and prevent all kinds of diseases like cancer. The potentials of this chemical constituents as an antioxidant can work as an agent involving a free radical quenching mechanism on OH and ROO. In fact, many studies have shown that antioxidant phytochemicals play significant multiple roles in curtailing the menace of dangerous disease-causing organisms including mutagenic, cell damage, and carcinogens due to their acceleration of different aging factors. In addition to antioxidant activity, the inhibition of cancer development by compounds relies on a number of basic cellular mechanisms. More comprehensive studies related to these

compounds; D-Limonene (1), Thunbergol (2) and Cis-2-Methyl-7-octadecene (3) will enhance pharmaceutical exploration in the field of endemic disease control.

Availability of data and materials: All supplementary materials regarding this work are available on request from the authors at Isaac.j62@yahoo.com

Competing interest

The authors declare no competing of interest

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Authors Contributions

This work was carried out in collaboration among all authors. Author FBA and HAU designed the study and wrote the protocol, Authors HAU, IJU and FAB wrote the draft of the manuscript, design and analysis the results. All authors read and approved the manuscript.

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