

**EXTRACTION, ISOLATION AND CHARACTERIZATION OF NEW COMPOUND AND  
ANTI-BACTERIAL POTENTIALS OF THE CHEMICAL CONSTITUENTS COMPOUND  
FROM *LEPTADENIA HASTATA* LEAF EXTRACT****Isaac John Umaru<sup>\*1,2</sup>, Fasihuddin A. Badruddin<sup>1</sup> and Hauwa A. Umaru<sup>3</sup>**<sup>1</sup>Faculty of Resource Science and Technology, Universiti of Malaysia Sarawak, Kota Samarahan Malaysia.<sup>2</sup>Department of Biochemistry Federal University Wukari Taraba State.<sup>3</sup>Department of biochemistry, Modibo Adama University of Technology Yola Adamawa state. Nigeria.**Received on: 14/01/2020****Revised on: 04/02/2020****Accepted on: 25/02/2020****\*Corresponding Author****Isaac John Umaru**Faculty of Resource Science  
and Technology, Universiti of  
Malaysia Sarawak, Kota  
Samarahan Malaysia.**ABSTRACT**

This study was carried out with the aim of exploring the chemical constituents medicinal plant *Leptadenia hastata*. Identification of the compounds were based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The name, molecular weight and structure of the components of the test materials were ascertained using Nuclear Magnetic Resonance and Fourier Transform Infra-Red Spectrometry (FTIR). Phytochemicals were isolated from the leaves extracts of *Leptadenia hastata*, after the extraction from solvent, dichloromethane. The chemical compounds isolated include Benzyl alcohol (1), 3-Pyridine carboxylate (2), 2-Methoxy-4-vinylphenol (3). This study revealed that this medicinal plant *Leptadenia hastata* extract from dichloromethane had some potential phytochemicals. These chemical constituents were isolated and characterised for the first in the leaf extract of *Leptadenia hastata*.

**KEYWORDS:** Extraction, TLC, Isolation, Characterization, *Leptadenia hastata*, NMR, FT-IR, UV.**1. INTRODUCTION**

*Leptadenia hastata* (Pers.) Decne is a perennial plant of the family of Asclepiaceae. The plant is edible non-domesticated vegetable and it is collected in wild throughout Africa. It is a valuable herb with creeping latex stems, glabrescent leaves, glomerulus and racemes flowers as well as follicle fruits as shown in Figure 1. The leaves are up to 10 cm long, mostly ovate and light green. The flowers are cream or yellowish green. They are commonly known in Nigeria and other west African countries because of its potentials in traditional medicine.<sup>[1]</sup>

Plant is widely distributed throughout the world. This are source of medicine; the plants parts are used in various applications especially for medicinal purposes. They are significant element of the world cultural heritage; they resort for treating health problems. This knowledge is passed down from generation to the next generation with or without little written information was available on the active, safety and effectiveness of this medicine.<sup>[2]</sup>

The study of these plants focuses on the leaves, since these parts are usually associated to the medicinal properties and scientific literature related are still less explored.<sup>[3]</sup> Many research efforts by scientist have been directed towards the provision of empirical proof to back

the use of many tropical plants in traditional-medical practice.<sup>[4]</sup> However, there still exist a vast number of tropical plants with tremendous medicinal potentials but with no proof to support their claims of efficacy.

It is well known that phytochemicals confirm the pharmacological relevance on plants and the growing interest in herbal medicine generally.<sup>[5]</sup> This demand of information on various plants preparations used in the treatment and management of ailments or disease depends on those components. Hence scientific evaluations of medicinal plants are important in the treatment and prevention of disease as well as the discovery of novel drugs and help to assess toxicity risk associated with their uses.<sup>[6]</sup>



Figure 1: Leaves and flowers of *Leptadenia hastata* (Haruna *et al.*, 2017).

## 2. MATERIAL AND METHODS

**2.1. Chemicals.** All chemicals used in this investigation were of analytical grade and were obtained from Sigma Chemical Co., St Louis, USA. Standard were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK.

### 2.2 Plant collection and identification

The leaves of *Leptadenia hastata* were plucked at Michika Local Government Area, Adamawa state. The plant part was authenticated in the Botanical laboratory of the department of Botany Modibo Adama University of Technology Yola Adamawa state. Nigeria. The leaves are washed and air-dried and ground into fine powder using mortar and pestle in the laboratory.

### 2.3 Preparation of extract of leaves of *Leptadenia hastata*

*Leptadenia hastata* leaves were prepared by cold maceration. The extracts of the leaves of *Leptadenia hastata* were prepared by soaking 500 g of finely grounded powder of leaves of *Leptadenia hastata* 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 another 24 hours. The extract was then 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.

### 2.4 Determination of Percentage Yield

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

Percentage (%) yield =

$$\frac{\text{Weight of the extracted oil (g)}}{\text{Dried weight of sample (g)}} \times 100$$

### 2.5 Identification of Compounds

Identification of the compounds were based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained.

### 2.6 Column Chromatography (CC)

Prior to isolation process, the crude extract was examined on TLC plate (Silica Gel 60 F254 Merck) to find the best solvent systems for column chromatography (Section 3.3.2.1). The solvent used were hexane, hexane-dichloromethane, dichloromethane, dichloromethane-chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol.

The basic principle of column chromatography is to separate a mixture of metabolites based on their molecular weight and polarity. A glass column of size 40/34 (large) was used for chromatography, and the sorbent used was silica gel 60 (Merck 70-230 Mesh @ 0.063-0.200 mm). Silica gel slurry was prepared by dissolving silica gel (150 g) with suitable solvent, usually hexane. The column was prepared by pouring a slurry mixture of silica gel and solvent, into a glass column and allow it to settle down.<sup>[7]</sup> The packed column was left over night before 4-10 g of sample was introduced onto the top of the packed column via wet-packing method. The column was eluted with suitable solvent systems with increasing polarity.<sup>[8]</sup> The column's valve was then opened and about 10-30 mL fraction of the solvent coming out from the column was collected in test tubes.<sup>[9]</sup> The procedure was repeated by using different solvent systems, based on increasing polarity. Samples from the column fractions were examined by using TLC plates in few suitable solvent systems to obtain the

retention factor ( $R_f$ ) of any components that appeared as spots. Fractions with similar  $R_f$  values were combined.<sup>[9]</sup> Fractions which contain more than one components were further isolated and purified by using smaller glass column of sizes 24/29 (medium) or 14/23 (small) with suitable solvent systems.

Fraction with single component (one spot) that appeared in TLC plate was treated as possible pure secondary metabolite. The combined fractions which contain the same single component was then allowed to air-dried or evaporated to dryness to obtain a pure secondary metabolite.

## 2.7 Chemical Structure Elucidation

Identification of the isolated secondary metabolite was made by various 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.*<sup>[8]</sup> The elucidation of chemical structural for the extracted secondary metabolite was made based on the data obtained from various spectroscopy methods and also comparison with published information if available.

## 2.8 Gas Chromatography - Mass Spectrometry (GC-MS)

The combined fractions eluted from column chromatography that showed single spot in TLC were further analysed by GC-MS performed on a Shimadzu model QP 2010 Plus to obtain molecular mass of pure and semi-pure compounds according to mass per charge ( $m/z$ ) ratio. The GC-MS used was equipped with BPX-5 column (5% phenyl polysilphenylene-siloxane) of 30 m length, 0.25  $\mu$ m of film thickness and 0.25 mm internal diameter. GC-MS was performed based on the method as described by Kalaiselvan *et al.*<sup>[10]</sup> The electron ionization energy system with ionization energy of 70 eV was used for GC-MS detection. The carrier gas, helium (99.999%) was used at a constant flow rate of 1 mL per minute and 1  $\mu$ L purified sample was introduced into GC-MS using syringe for analysis (by using split mode with split ratio of 25:1). Injector temperature was set at 260 °C. The temperature of oven was programmed from 60 °C (isothermal for 5 minutes), with an increase of 10 °C per minute to 280 °C, and ending with 10 minutes' isothermal at 280 °C. At 70 eV, mass spectra were taken; a 71 scan interval of 0.5 second and fragments from 45 to 450 Da. By matching its average peak area to the total areas, the relative percentage quantity of each component

was acquired. By matching the retention times with those of authentic compounds, compound identification was obtained and the mass spectral obtained from library data of the corresponding compounds.<sup>[10]</sup>

## 2.9 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 Efdi *et al.*<sup>[11]</sup> and Danelutte *et al.*<sup>[12]</sup>  $^1\text{H}$  and  $^{13}\text{C}$  spectra were measured at 500 and 125 MHz, respectively. Sample was dissolved in 0.8 mL chloroform D1 ( $\text{CDCl}_3$ ) and placed into NMR tube to make sample depth around 3.5  $\text{cm}^{-1}$  to 4  $\text{cm}^{-1}$  and ready to be analysed by NMR spectrometer. Chemical shifts were reported as  $\delta$  units (ppm) with tetramethylsilane (TMS) as internal standard and coupling constants ( $J$ ) in Hz.

Integration of the  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR data was performed by using DELTA version 5.0.4 software by JEOL. Identification of the type of each  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR detected was based on the Table of characteristic NMR absorptions that published in Organic Chemistry [13] and with the guide of the possible proposed structure given by NIST library.

## 2.10 Fourier Transform Infra-Red Spectrometry (FTIR)

The chemical bonds (functional groups) of the compounds were detected by using Fourier Transform Infra-Red spectrometry (FTIR) (Thermo Scientific, Nicolet iS10 SMART iTR). The semi solid, crystalline and powdered samples were introduced directly into FTIR. Scan range employed was from 400  $\text{cm}^{-1}$  to 4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ , based on the method described by Shalini & Sampathkumar.<sup>[14]</sup> 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.<sup>[13]</sup>

## 4. RESULT AND DISCUSSION

Compound 1 was obtained from a fraction of 170.0 mg of dark brown colour of dichloromethane leaf extract. TLC analysis of the fraction was carried out in the following solvent ratio as shown in Table 1. It was observed under UV (Long and short wave) and recorded.

**Table 1: TLC and  $R_f$  value of fraction (A) of different solvent ration system under UV light.**

| Solvent system (v/v)        | Number of spots | $R_f$ value | Stained TLC colour |
|-----------------------------|-----------------|-------------|--------------------|
| Hexane : Chloroform (3:7)   | 2               | 0.42 0.21   | Brown              |
| Hexane: Ethyl acetate (1:9) | 2               | 0.55 0.13   | Brown              |

A dark coloured spots was seen under UV light with the same  $R_f$  value from fraction (A) which was targeted and combined and was labelled as (A2). A2, was subjected to

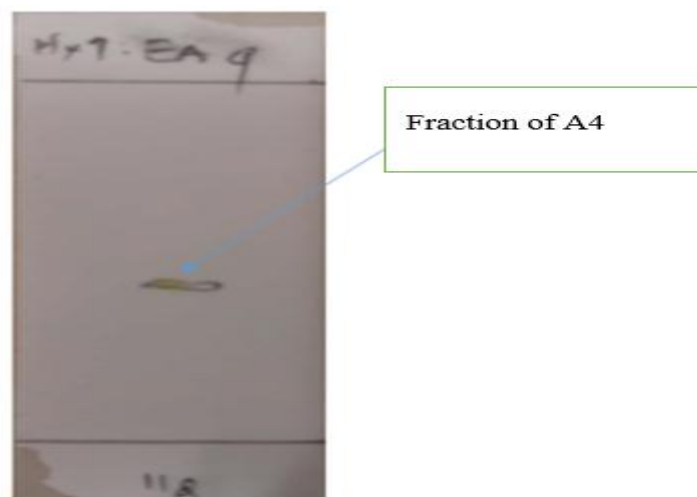
smaller column for further separation and combined fraction of (A3) was obtained. After preparative TLC of the combined fraction (A3) which gave a good

separation from other spots. The targeted spot was then separated using smaller column and a fraction of (A4)

was obtained which showed one spot under UV light and vanillin staining as shown Table 2.

**Table 2: TLC and  $R_f$  value of A4 in different solvent ration system under UV light.**

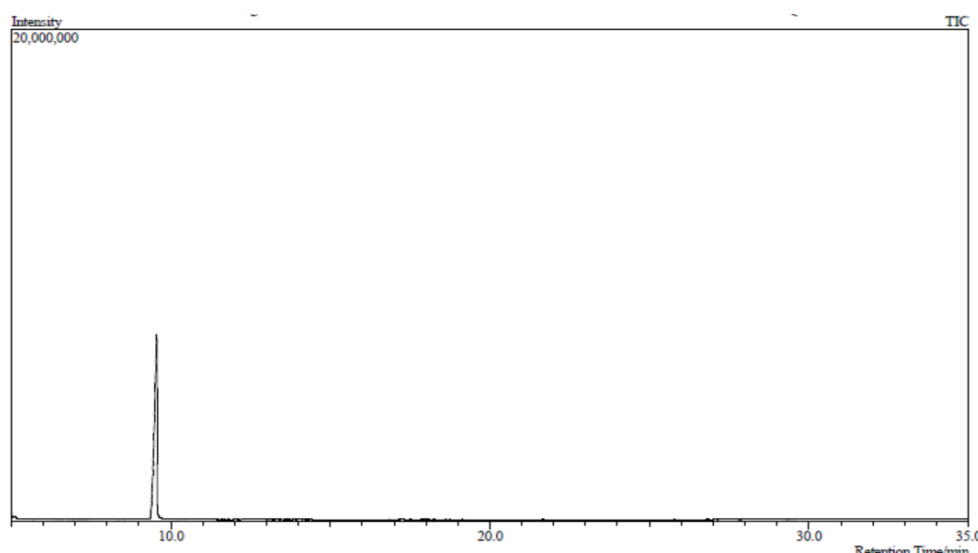
| Solvent system (v/v)        | Number of spots | $R_f$ value | Stained TLC colour |
|-----------------------------|-----------------|-------------|--------------------|
| Hexane: Ethyl acetate (1:9) | 1               | 0.56        | Light brown        |



**Figure 2: TLC plate showing one sport from the of A4 in hexane and ethyl acetate (1:9).**

Figure 2: TLC plate showing the spot of fraction A4 in Hexane: Ethyl acetate (1:9) The GC analysis of fraction A4 of Figure 2 was obtained and confirm from the chromatogram (Figure 3) showed one single peak at the

retention time of 9.55 min. This suggest that A4 is a pure compound and was named as Compound 1 with a weight of 10.2 mg.



**Figure 3: Gas chromatogram of compound 1.**

### Structural Elucidation

Compound 1 was isolated from dichloromethane leaf crude extract of *Leptadenia hastata*. It was eluted with hexane: ethyl acetate 1:9 with its physical appearance as brown powder. The mass spectrum of the compound in Figure 4 showed a similarity index of 96.00% with the mass spectrum of the suggested structure of compound 1

in Figure 5 by NIST library. On the mass spectrum of compound 1 one of the molecular ion peak was observed at  $m/z$  108 and it was found to correspond with the molecular ion peak and molecular ion weight of compound 1 suggested by the NIST library with chemical formula  $C_6H_5CH_2OH$ . The mass spectrum at Figure 5 also shows base peak for compound 1 at  $m/z$  79.

This was also observed in the mass spectrum of the suggested compound 1.

Spectrum of the isolated compound 1 figure 4 and spectrum of the suggested structure of Benzyl alcohol figure 5.

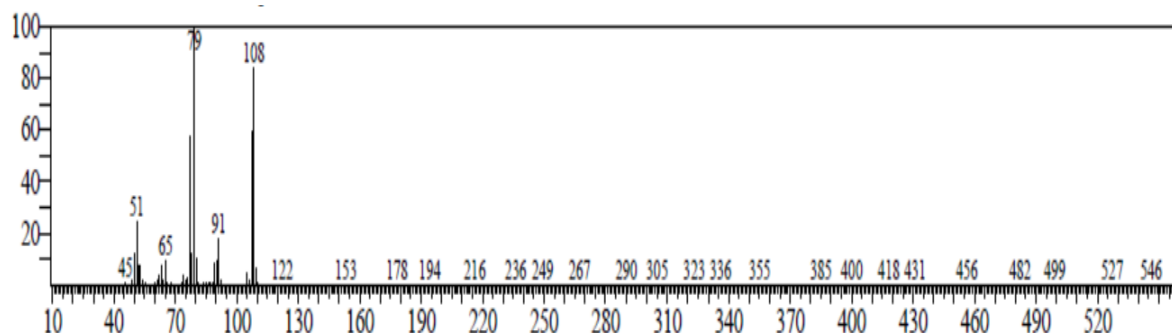


Figure 4: Mass spectrum of Compound 1.

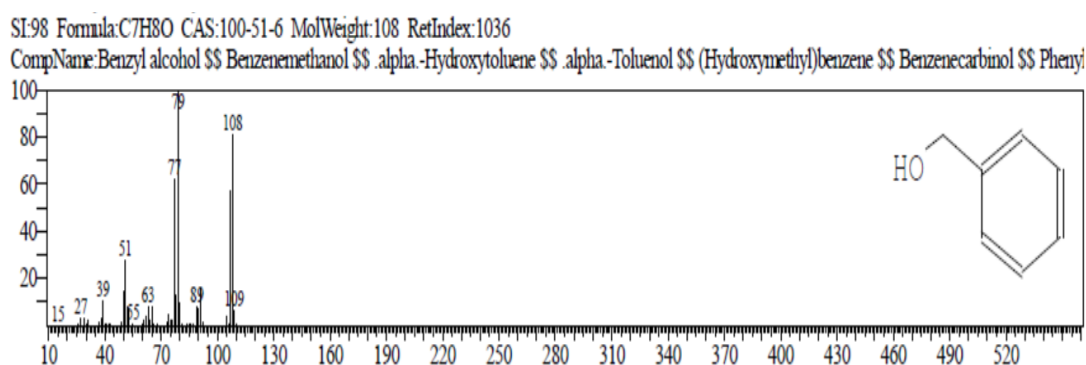


Figure 5: Mass spectrum of suggested structure of compound 1 by NIST Library.

The absorption band of C-H of the chemical structure of compound 1 was observed at 2974 cm<sup>-1</sup> in the IR spectrum (Figure 6) which indicated the presence of methyl alkyl carbon in the chemical structure. Double bond C=C signal was observed at 1646 cm<sup>-1</sup> and 1454

cm<sup>-1</sup> this may represent the three double bond in the ring in the suggested structure of Benzyl alcohol (1). OH signal was also observed at 3340 cm<sup>-1</sup> and a single bond of C-C stretching at 879 cm<sup>-1</sup> were observed in the IR spectrum of compound 1 (Figure 6)

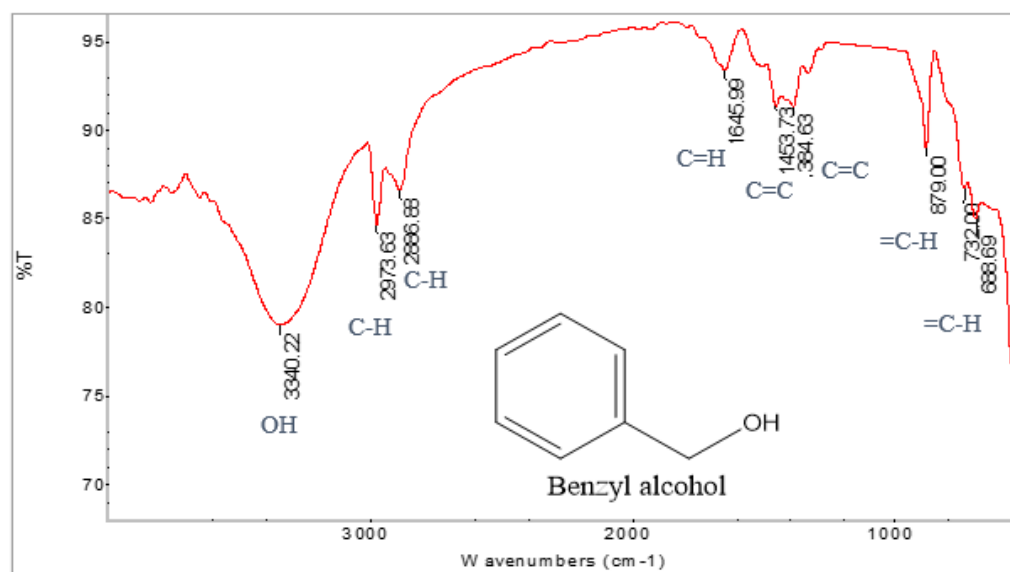


Figure 6: IR spectrum of Compound 1.

NMR analysis of compound 1 chemical structure was further elucidated and the result are shown in Figure 7 ( $^1\text{H}$ -NMR) and Figure 8 ( $^{13}\text{C}$ -NMR). With the result obtained based on the table of  $^1\text{H}$ -NMR characteristic absorption and  $^1\text{H}$ -NMR peaks splitting pattern as reported in spectrometric identification of Organic compounds by Silverstein *et al.*,<sup>[16]</sup> the proton signals were integrated and was assigned to every proton NMR of compound 1 as the proposed chemical structure. It was understood that the  $^1\text{H}$ -NMR spectrum of compound 1 in Table 3 showed five aromatic protons and two non-aromatic protons with oxygen neighbour. Two doublet proton signal were observed at  $\delta$  7.25 (2H, d) and 4.70

(2H, d) indicating the presence of methane group of the structure, therefore assigned to H-6 and H-7 respectively.

A multiplet proton signal was observed at  $\delta$  7.32 (3H, m) and was assigned to H-4, this proton signal showed the proton that was attached to the symmetry carbon attached to hydroxyl group of compound 1. Two singlet proton was observed at  $\delta$  7.25 (2H, s) and therefore assigned to H-1 and H-3 indicating the presence of methane group.

A chemical shift at 4.60 (1H, s) a singlet proton signal was observed which appeared to be attached to the alcohol group and was assigned to OH.

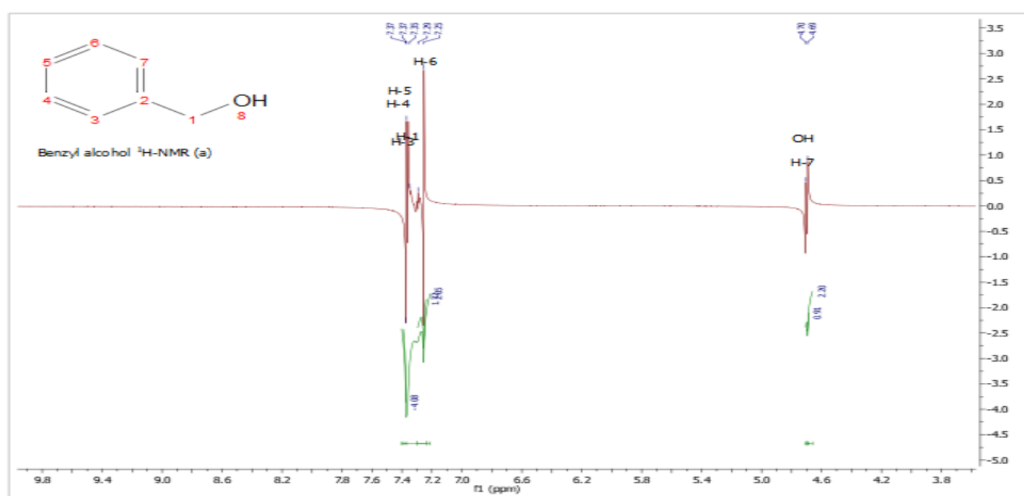


Figure 7a:  $^1\text{H}$ -NMR spectrum of Compound 1 (500MHz,  $\text{CDCl}_3$ ).

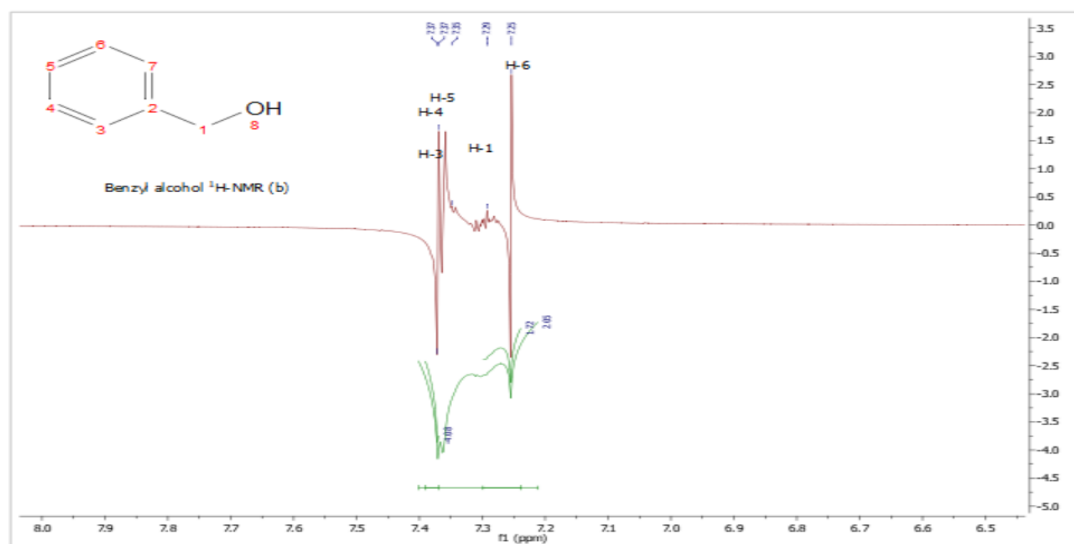


Figure 7b:  $^1\text{H}$ -NMR spectrum of Compound 1 (500MHz,  $\text{CDCl}_3$ ).

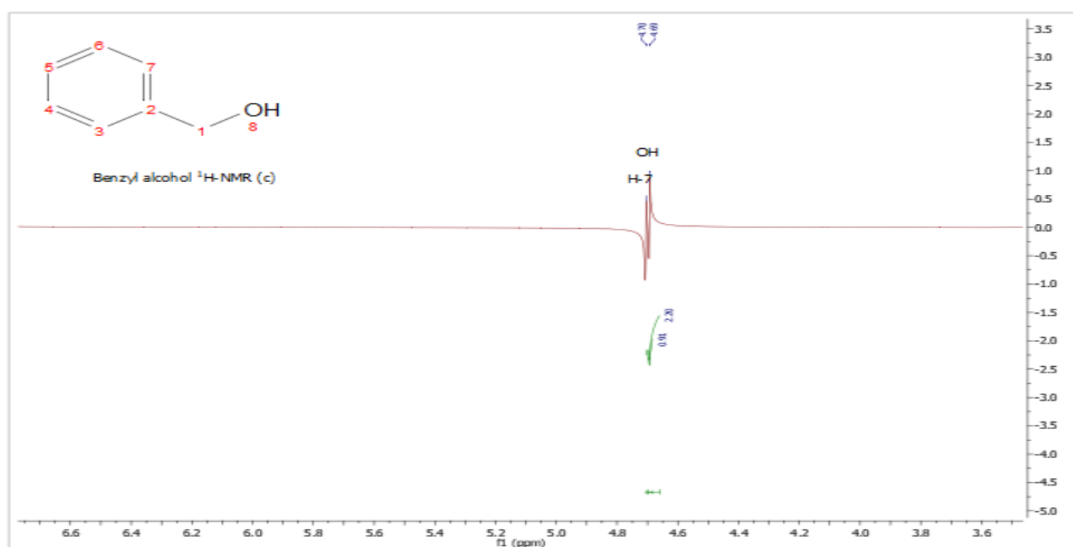


Figure 7c:  $^1\text{H}$ -NMR spectrum of Compound (500MHz,  $\text{CDCl}_3$ ).

Figure 8 shows the result of Compound 1  $^{13}\text{C}$ -NMR spectrum. The result indicated signal of every carbon that was observed and assigned to the proposed chemical structure of compound 1 which is based on the table of  $^{13}\text{C}$ -NMR characteristic absorption as in spectrometric identification of Organic compounds by Silverstein *et al.*,<sup>[16]</sup>

$^{13}\text{C}$ -NMR spectrum of compound 1 as shown in Figure 8, the result from every carbon NMR signal was as assigned to the proposed chemical structure of compound 1 which is based on the table of  $^{13}\text{C}$ -NMR characterization absorption as reported in spectrometric identification of Organic compounds by Silverstein *et al.*,<sup>[16]</sup>

From the observed spectrum, a total of 7 carbon resonates was obtained in the spectrum as shown in

Table 4. The down field showed six signal at  $\delta$  127.08,  $\delta$  127.77,  $\delta$  127.75,  $\delta$  128.67,  $\delta$  127.06, and  $\delta$  141.94 which was identified as methine carbons and were assigned to C-7, C-6, C-5, C-4, C-3, and C-2 respectively. A signal at up field region a signal appeared at  $\delta$  65.51 which indicated the presence of methylene (aliphatic) carbon and was assigned to C-1 in the chemical structure of Compound 1. The presence of an oxygenated quaternary carbon at  $\delta$  65.51 in the  $^{13}\text{C}$ -NMR spectrum as indicative of the presence of a C-O bond. Thus suggested a hydroxyl group as the substituent.

The chemical structure of carbon NMR for Compound 1 is shown in Table 4 as well as the comparison with the reference data of similar compound reported by Shaari & Waterman.<sup>[15]</sup>

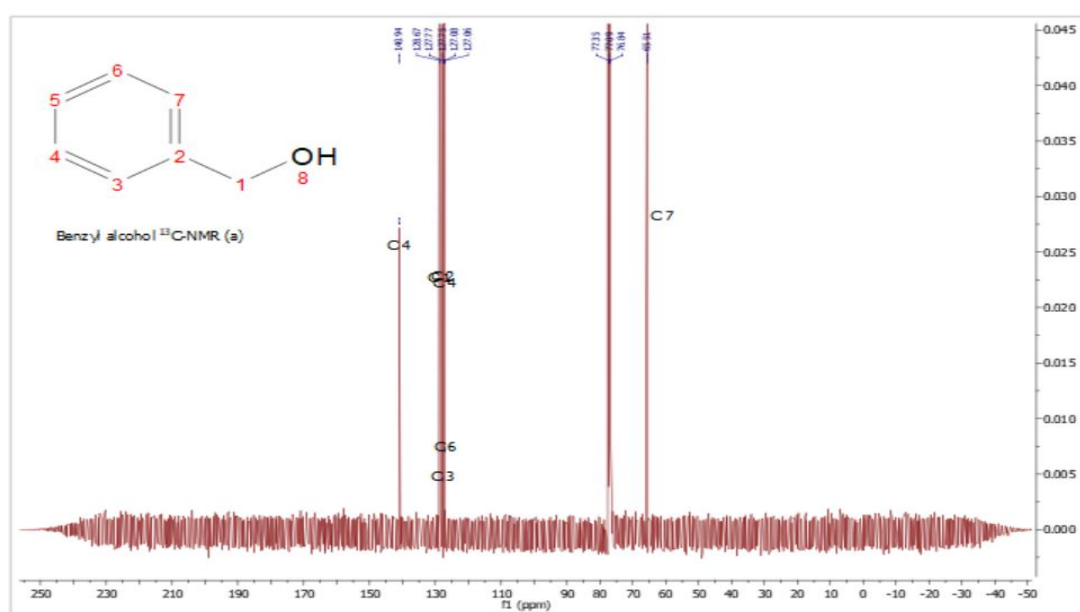


Figure 8a:  $^{13}\text{C}$ -NMR spectrum of Compound 1 (125MHz,  $\text{CDCl}_3$ ).

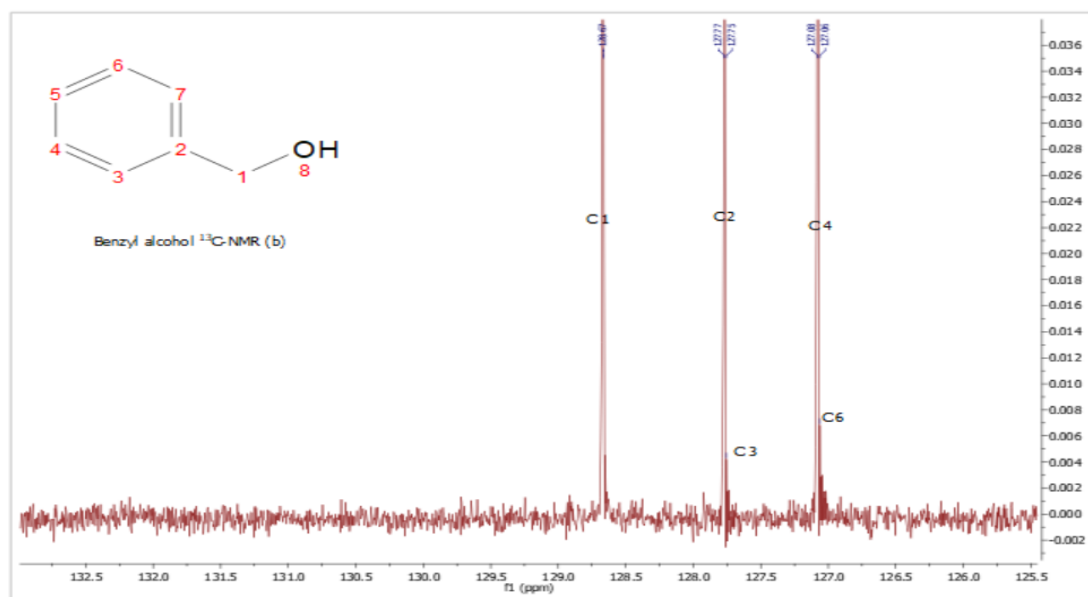


Figure 8b:  $^{13}\text{C}$ -NMR spectrum of Compound 1 (125MHz,  $\text{CDCl}_3$ ).

The  $^{13}\text{C}$ -NMR spectrum of compound 1 showed the presence of 7 carbons, which indicated the presence of methylene signal and one signal of alcohol (OH).

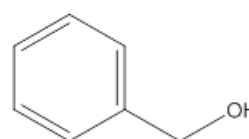
Table 3: Proton NMR signal of compound 1 and that reported by Shaari & Waterman. (1995).

| Proton assigned to Compound 1 | Proton chemical shift (ppm) of compound 1 | Proton assigned to Benzyl alcohol (Shaari & Waterman. 1995) | Proton chemical shift (ppm) of Benzyl alcohol (Shaari & Waterman.1995) |
|-------------------------------|-------------------------------------------|-------------------------------------------------------------|------------------------------------------------------------------------|
| H-1/H-3                       | 7.25 (s)                                  | H-3                                                         | 7.59 (d, 8.8)                                                          |
| H-4                           | 7.37 (m, J 7.32)                          | H-4                                                         | 7.07 (dd, 8.8, 2.9)                                                    |
| H-6                           | 7.25 (dd, J 7.27)                         | H-6                                                         | 7.38 (d, 2.0)                                                          |
| H-7                           | 4.70 (d, J4.70)                           | H-7                                                         | 5.75 (d,13.2)                                                          |
| OH                            | 4.60 (s)                                  | OH                                                          | 4.72 (s)                                                               |

Table 4: Carbon NMR signal of compound 1 and that reported by Shaari & Waterman. (1995).

| Carbon assigned to Compound 1 | Carbon chemical shift (ppm) of compound 1 | Carbon assigned to Benzyl alcohol (Shaari & Waterman. 1995). | Carbon chemical shift (ppm) of Benzyl alcohol (Shaari & Waterman. 1995). |
|-------------------------------|-------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------|
| C-1                           | 128.67                                    | C-1                                                          | 128.40                                                                   |
| C-2                           | 127.77                                    | C-2                                                          | 129.80                                                                   |
| C-3                           | 127.75                                    | C-3                                                          | 129.20                                                                   |
| C-4                           | 127.08                                    | C-4                                                          | 126.90                                                                   |
| C-5                           | 141.94                                    | C-5                                                          | 154.80                                                                   |
| C-6                           | 127.06                                    | C-6                                                          | 126.60                                                                   |
| C-7                           | 65.51                                     | C-7                                                          | 63.00                                                                    |

From the data obtained, the GCMS analysis of Compound 1 gave similarity index of 96.00% with the mass spectrum of the compound proposed in the NIST library which match with Benzyl alcohol (1) with chemical formula  $\text{C}_6\text{H}_5\text{CH}_2\text{OH}$ . The melting point of Compound 1 is  $239^\circ\text{C}$  (Lit  $238^\circ\text{C}$ ). it was also observed that the mass spectrum of Compound 1 is similar to the mass spectrum as suggested in NIST library as Benzyl alcohol (1). Report from the IR data by Shaari & Waterman,<sup>[15]</sup> was found to match the IR data of Compound 1 as Benzyl alcohol (1).



Benzyl alcohol (1) is an aromatic alcohol with formula  $\text{C}_6\text{H}_5\text{CH}_2\text{OH}$  exhibited a wide range of bioactivity on bacterial growth. This coupled with the findings from the bioassay studies of the crude extract, justifying the use of

*Leptadenia hastata* in the traditional medicine (Umaru *et al* 2018), furthermore the use of the leaves of *Leptadenia hastata* as an anti-inflammation and for pain reliever as well as a preservative in pharmaceutical topical preparation improve the half-life of the medicinal potential of the plant extract. It also acts as protein stabilizers work either by interacting directly with the protein or by altering the solvent properties of the surrounding medium, hence altering the protein-solvent interactions.<sup>[17]</sup>

The biological activity of lysozyme was found to be the highest in the presence of benzyl alcohol. It was also

**Table 5: TLC and  $R_f$  values of combined fraction of B 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 (7:3) | 3                      | 0.42 0.21   | Light brown        |
| Dichloromethane: Ethyl acetate (8:2) | 2                      | 0.55 0.13   | Light brown        |
| Dichloromethane: Ethyl acetate 9:1   | 2                      | 0.60 0.20   | Light brown        |

A fraction from dichloromethane and Ethyl acetate ration as shown in Table 5 containing a light brown colour was collected from a fraction B was targeted and combined, it was labelled B1, it was then subjected for separation using small column and similar TLC fractions was collected and combined fractions was labelled as B2. TLC which gave a good separation was performed in a solvent system dichloromethane: ethyl acetate gave good

reported that compound 1 causes protein aggregation and therefore, decreases protein stability.<sup>[18]</sup>

Compound 2 was obtained from the combined fractions of (B) of 300.16 mg extract of *Leptadenia hastata* in dichloromethane with a Dark green colour. The TLC analysis of the fraction B, was performed in a different solvent system after which the result was observed under UV light and recorded as shown in Table 5.

separation from the other spots. The targeted spots were further purified in a smaller column using solvent ratio of dichloromethane: ethyl acetate (9:1) and dichloromethane: ethyl acetate (8:2). Each fraction was collected and labelled as B3 and B4. The fractions were subjected to UV light and those with same  $R_f$  value and colour similarity were combined separately. The result was as shown in the Table 6.

**Table 6: TLC and  $R_f$  values of combined fraction of B3 and B4 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 (9:1) | 2                      | 0.73 0.69   | colourless         |
| Dichloromethane: Ethyl acetate (9:1) | 2                      | 0.60 0.58   | colourless         |

The combined fractions showing similar  $R_f$  value and colour were combined and labelled B5 and subjected to small column using Dichloromethane: Ethyl acetate

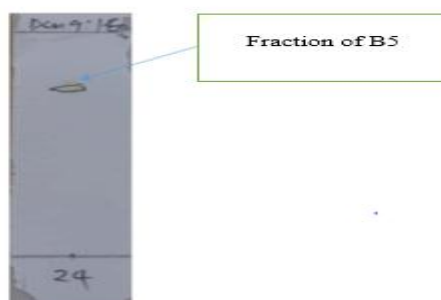
(9:1). TLC of the fractions was collected and examined under UV and Vanillin stain which shows a single spot as shown in Table 7.

**Table 7: TLC and  $R_f$  values of combined fraction of B5 in different solvent system under UV light.**

| Combined fraction | Solvent system (v/v)     | Number of spot on TLC | $R_f$ value | Stained TLC Colour |
|-------------------|--------------------------|-----------------------|-------------|--------------------|
| LHDCM8-A3         | DCM: Ethyl acetate (9:1) | 1                     | 0.60        | colourless         |

Figure 9: shows the TLC profile for the combined fraction, labelled as B5 in dichloromethane: ethyl acetate

(9:1) with a single sport which suggest a pure compound with a weight of 9.0 mg.



**Figure 9: TLC plate with one spot of combined fraction of B5 in dichloromethane: ethyl acetate (9:1).**

The GC result of B5 Figure 9 was then carried out and the report from the chromatogram indicated one peak at a

retention time of 10.73 min as shown in figure 10, which indicated that B5 is a pure compound and was renamed as Compound 2.

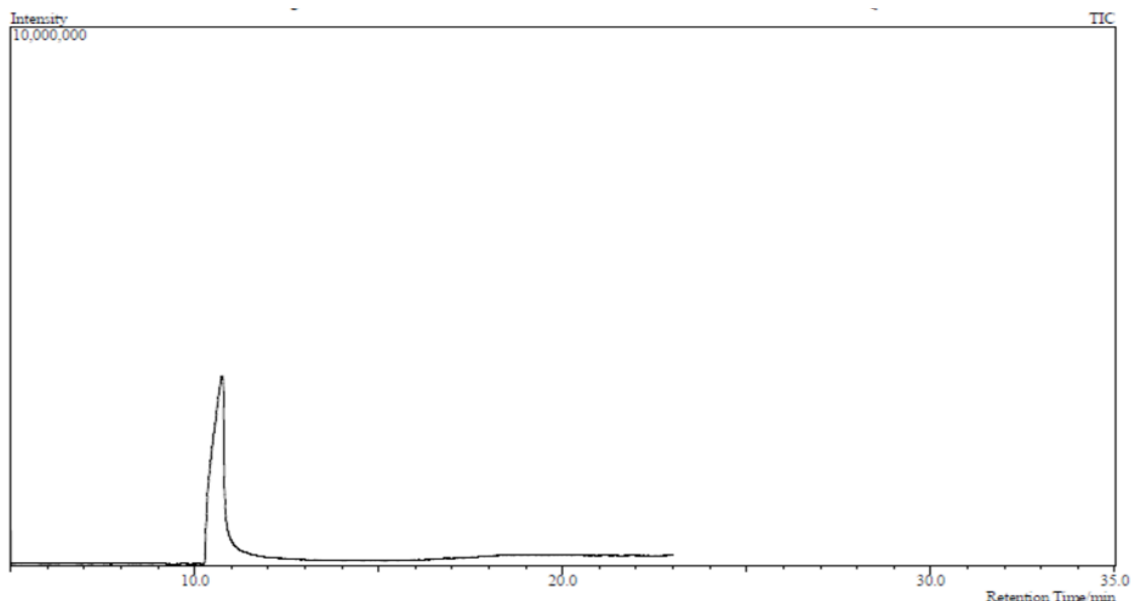


Figure 10: Gas chromatogram of Compound 2.

### Structural Elucidation

Compound 2 was obtained as a colourless solid fraction from dichloromethane leaf extract of *Leptadenia hastata* and a melting point at 253-254 °C. Figure 11 shows the mass spectrum of Compound 2 with one of its molecular ion peak observed at  $m/z$  123 which correspond to the

same molecular ion peak and molecular ion weight as suggested structure of Compound 2 by NIST library with the chemical formula of  $C_6H_5NO_2$ . Figure 12 also indicated a base peaks of Compound 2 at  $m/z$  105 which was also obtained on the mass spectrum of the suggested spectrum of 3-Pyridine carboxylic acid.

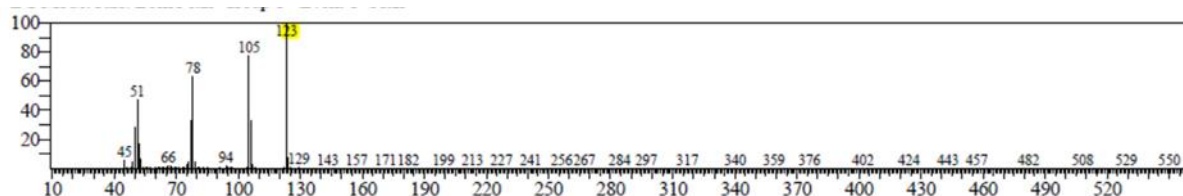


Figure 11: Mass spectrum of Compound 2.

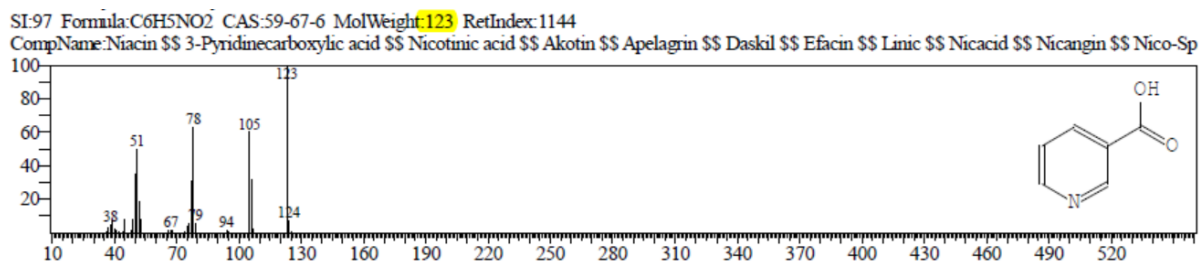
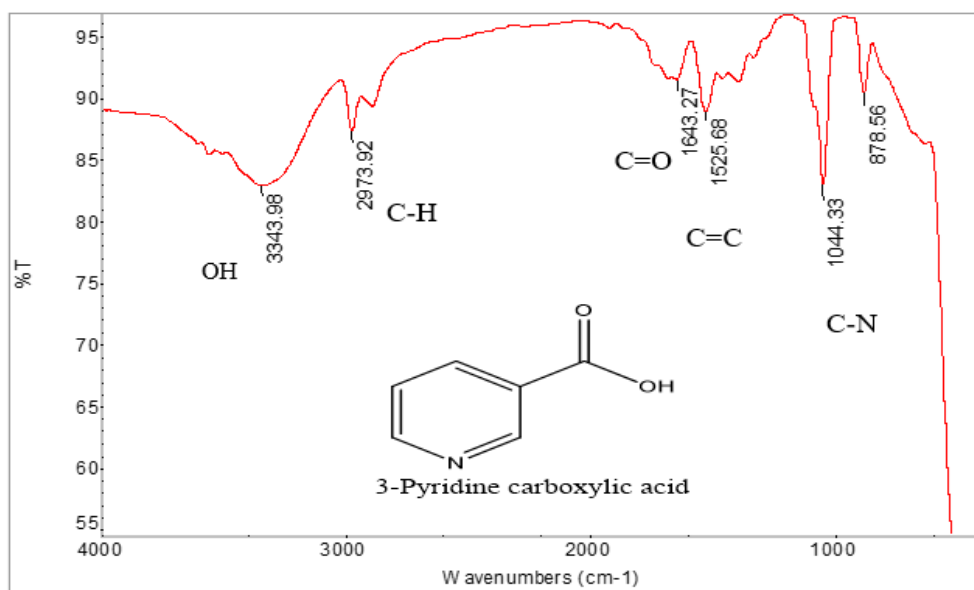


Figure 12: Mass spectrum of suggested structure of compound 2 by NIST Library.

IR spectrum of Compound 2 (Figure 13) showed the absorption band of C=C which was observed at  $1526\text{ cm}^{-1}$  and a signal was observed at  $1643\text{ cm}^{-1}$  which was seen to represent the C=O bond and another one at  $1526\text{ cm}^{-1}$  which may represent the C=C bond in the ring in the suggested structure of 3-Pyridine carboxylic acid and a signal bond of C-C stretching at  $879\text{ cm}^{-1}$  was observed on the IR spectrum of Compound 2 (Figure 13). IR

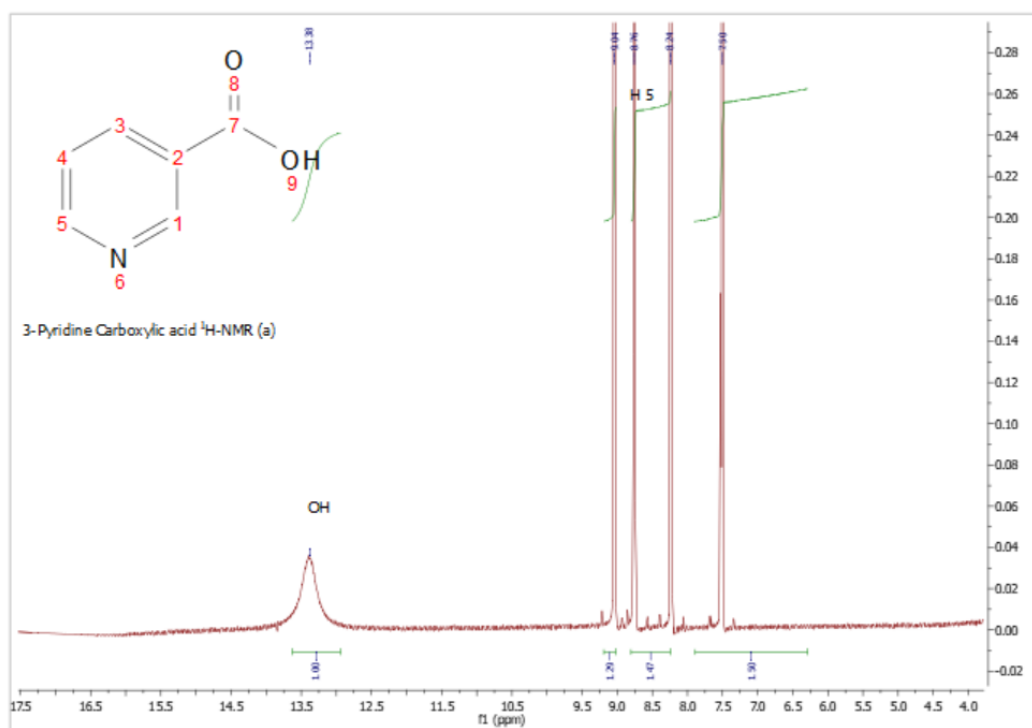
spectrum of Compound 2 is similar with the IR spectrum as reported by Venkateswarlu *et al.*<sup>[19]</sup>



**Figure 13: IR spectrum of Compound 2.**

NMR analysis of Compound 2 was further performed for the elucidation of the chemical structure and the result are as shown in Figure 14a and b ( $^1\text{H}$ -NMR) and Figure 15 ( $^{13}\text{C}$ -NMR). The proton of Compound 2 was based on the Table of  $^1\text{H}$ -NMR characteristic absorption as well as the  $^1\text{H}$ -NMR splitting pattern as reported in spectrometric identification of Organic compounds by Silverstein *et al.*,<sup>[16]</sup> the proton signal was all integrated and are assigned to every proton NMR of Compound 2 as the suggested chemical structure.

From the result it was observed that  $^1\text{H}$ -NMR spectrum of compound 2 is composed of 5 proton resonates, two doublet proton signals were observed at  $\delta$  8.24 (2H, d,  $J=8.24$ ), 8.24 (2H, d,  $J=8.24$ ), and 8.76 (2H, d,  $J=8.50$ ) indicating the presence of methane group of the structure, however, was assigned to H-3, H-4 and H-5 respectively. A singlet was observed at 9.04 (1H, s) and was assigned to H-1 indicate the existence of alcohol group.



**Figure 14a:  $^1\text{H}$ -NMR (a) spectrum of Compound 2 (500 MHz,  $\text{CDCl}_3$ ).**

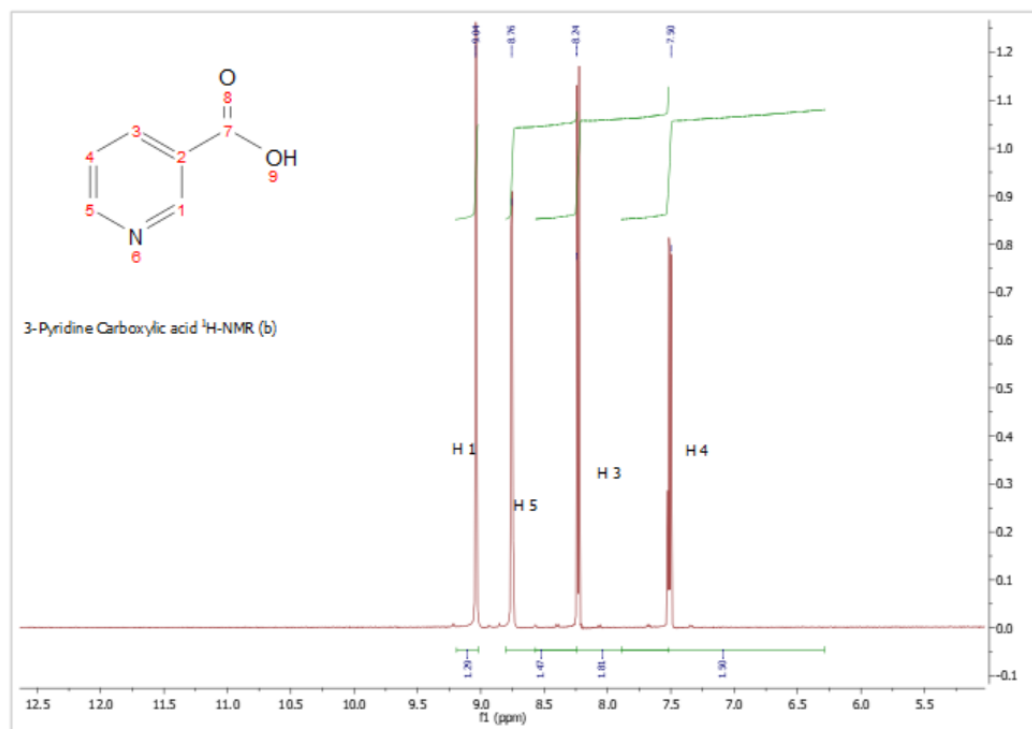


Figure 14b:  $^1\text{H}$ -NMR (b)spectrum of Compound 2 (500 MHz,  $\text{CDCl}_3$ )

The  $^{13}\text{C}$ -NMR of Compound 2 as shown in Figures 15 presented the result of carbon NMR signal result, was assigned to the proposed chemical structure which is based on the Table of  $^{13}\text{C}$ -NMR characteristics absorption as reported in spectrometric identification of Organic compounds by Silverstein *et al.*<sup>[16]</sup>

A total of 6 carbon resonates was observed in the spectrum presented. The down field region showed six

signals at  $\delta$  150.19,  $\delta$  123.90,  $\delta$  154.23 and  $\delta$  167.23 were identified as methane Carbon and were assigned as C-1, C-4, C-5 and C-7, respectively. Another signal was observed at  $\delta$  127.70 was assigned C-3 which was identified as C=O group. A signal was also observed to appear at  $\delta$  137.60 and was assigned to C-3. Six signals appeared at the down field which indicated the chemical structure of Compound 2.

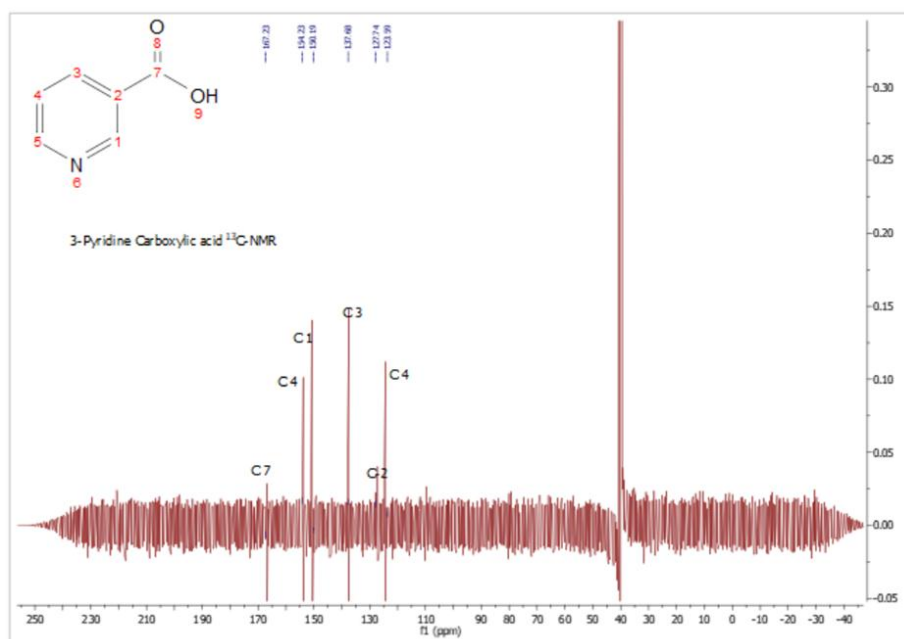


Figure 15:  $^{13}\text{C}$ -NMR spectrum of Compound 2 (125 MHz,  $\text{CDCl}_3$ ).

Compound 2 chemical shift of every  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR are shown in Tables 8 and 9 together with

reference data to similar NMR Compound reported by Venkateswarlu *et al.*<sup>[16]</sup>

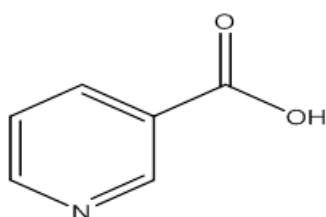
**Table 8: Proton NMR signal of compound 2 and that reported by Venkateswarlu *et al.* (2015).**

| Proton assigned to Compound 2 | Proton chemical shift (ppm) of compound 2 | Proton assigned to 3-Pyridine carboxylic acid (Venkateswarlu <i>et al.</i> , 2015) | Proton chemical shift (ppm) of 3-Pyridine carboxylic acid (Venkateswarlu <i>et al.</i> , 2015) |
|-------------------------------|-------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| H-1                           | 9.04 (1H,).                               | H-1                                                                                | 9.02 (1H, s)                                                                                   |
| H-3                           | 8.24 (2H, d, J=8.24,)                     | H-3                                                                                | 8.32 (d, 2H, J=2.9)                                                                            |
| H-4                           | 7.50 (d, 2H, J=7.50)                      | H-4                                                                                | 7.48 (d, 2H, J= 7.48)                                                                          |
| H-5                           | 8.76 (2H, d, J=8.50)                      | H-5                                                                                | 8.64 (2H, d, J=3.6)                                                                            |

**Table 9: Carbon NMR signal of compound 2 and that reported by Venkateswarlu *et al.* (2015).**

| Carbon assigned to Compound 2 | Carbon chemical shift (ppm) of compound 2 | Carbon assigned to 3-Pyridine carboxylic acid (Venkateswarlu <i>et al.</i> , 2015) | Carbon chemical shift (ppm) of 3-Pyridine carboxylic acid (Venkateswarlu <i>et al.</i> , 2015) |
|-------------------------------|-------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| C-1                           | 150.19                                    | C-1                                                                                | 151.20                                                                                         |
| C-2                           | 127.70                                    | C-2                                                                                | 128.70                                                                                         |
| C-3                           | 137.60                                    | C-3                                                                                | 139.30                                                                                         |
| C-4                           | 123.90                                    | C-4                                                                                | 125.30                                                                                         |
| C-5                           | 154.23                                    | C-5                                                                                | 153.60                                                                                         |
| C-7                           | 167.23                                    | C-7                                                                                | 167.70                                                                                         |

Therefore, based on the spectrum data of Compound 2 which include similarity of the mass spectrum with the suggested structure by the NIST library, this matched the characteristic of 3-Pyridine carboxylic (2) with a chemical formula  $\text{C}_6\text{H}_5\text{NO}_2$ . The melting point of Compound 2 is  $235^\circ\text{C}$  thus, the mass spectrum of Compound 2 is similar to the mass spectrum of the suggested structure by the NIST library which was identified as 3-Pyridine carboxylic acid reported by Venkateswarlu *et al.*<sup>[16]</sup>



The mass spectrum of IR,  $^1\text{H}$ -NMR and  $^{13}\text{NMR}$  based on the published literature (Venkateswarlu *et al.*, 2015), Compound 2 was identified as 3-Pyridine carboxylic acid (2) and it has been reported as fungal metabolite from phycomyces blakesleenu. the compound is widely used as vitamin, coenzyme co-factor, Vasodilator and anti-hyperglycaemic agent. 3-pyridine carboxylic acid is also used as plant intermediate and in the treatment of Lipid disorder as well as the compound for treatment of HIV.<sup>[20]</sup>

Compound 3 was isolated from the combined fraction of C, from 177.9 mg with light yellow colour (*Leptadenia hastata* DCM extract). TLC analysis of the combined fraction was subjected to a different solvent ratio and then observed under UV light and recorded as shown in Table 10.

**Table 10: TLC and  $R_f$  values of combined fraction of C, 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 (7:3) | 3                      | 0.42 0.21   | yellow             |
| Hexane: Ethyl acetate (8:2) | 4                      | 0.55 0.13   | yellow             |
| Hexane: Ethyl acetate (9:1) | 2                      | 0.36 0.47   | yellow             |

From the TLC, fraction of the yellow colour where collected, combined and labelled as C1. The combined fractions were then purified in small column using a sweet-able solvent system, Hexane: Ethyl acetate (8:2). Each fraction collected were observed under UV light and those with yellow colour and similar  $R_f$  value were

combined and labelled as C2 was then subjected to TLC and observed for possible one sport under UV as shown in Table 11.

**Table 11: TLC and  $R_f$  values of combined fraction of C2 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 (7:3) | 2                      | 0.83        | yellow             |
| Hexane: Ethyl acetate (8:2) | 2                      | 0.82        | yellow             |

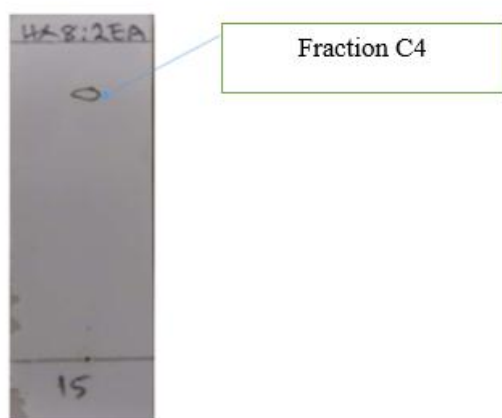
Further column chromatography of the combined fraction of C3 (8:2). The TLC analysis of the fraction resulted in one single spot as shown in Table 12.

**Table 12: TLC and  $R_f$  values of combined fraction of C3 in different solvent system under UV light.**

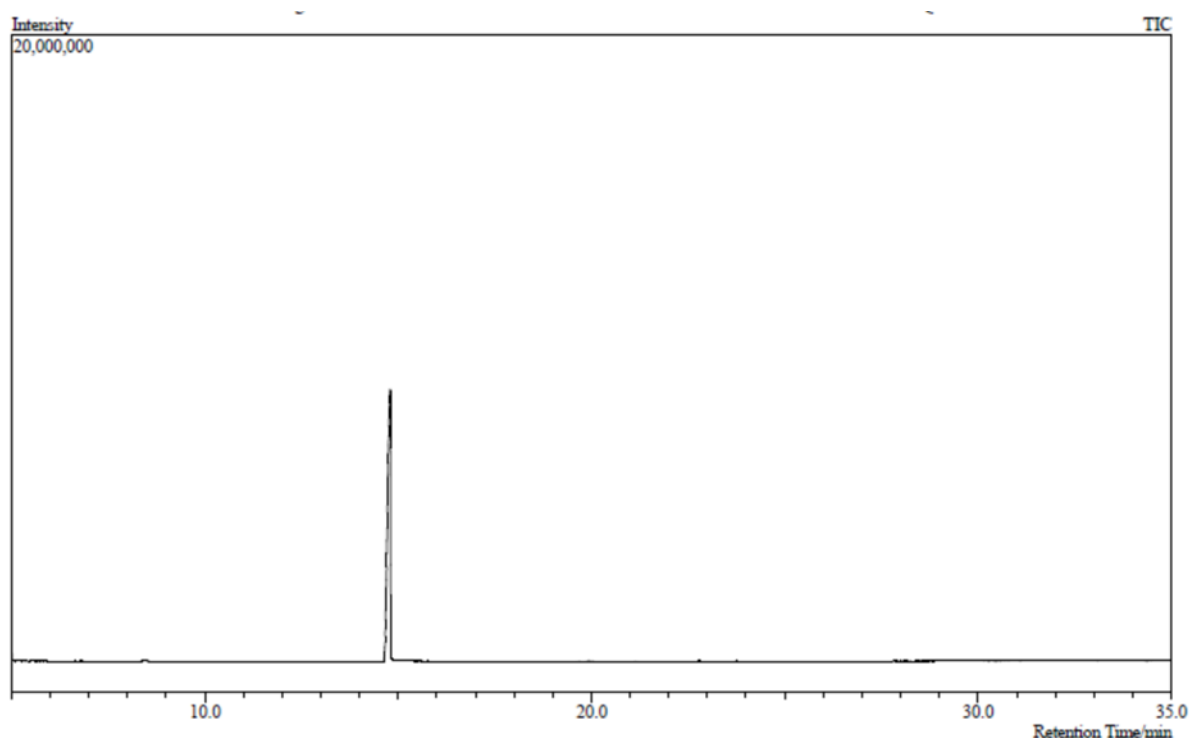
| Solvent system (v/v)        | Number of spots on TLC | $R_f$ value | Stained TLC Colour |
|-----------------------------|------------------------|-------------|--------------------|
| Hexane: Ethyl acetate (8:2) | 1                      | 0.82        | yellow             |

The combined fraction of C3 was observed to have one spot and was renamed as C4

Figure 16: TLC plate showing the single spot of fraction C4, Hexane: Ethyl acetate (8:2)



This shows that C4 is a pure compound. Gas chromatography analysis of C4 was carried out and the result showed a single peak indicating a pure compound at retention time of 14.792 min as shown in the chromatogram figure 17. This shows that C4 is a pure compound of yellow colour and thus, was renamed Compound 3 with 10.5 mg.

**Figure 16: Shows the TLC profile for C4 (8:2) as a single spot.****Figure 17: Gas chromatogram of Compound 3.**

Compound 3 was obtained from dichloromethane crude extract of *Leptadenia hastata*, its physical appearance as a light yellowish compound with a melting point of 28°C. The mass spectrum of Compound 3 (Figure 18) showed a similarity index of 94.42 % with the mass spectrum of compound suggested by the NIST library in Figure 19. The mass spectrum of Compound 3 showed an ion base

peak at  $m/z$  150 and a molecular ion peak of  $m/z$  150 was observed in the spectrum of the suggested structure of Compound 3. One of the molecular ion peak of compound 3 mass spectrum was observed at  $m/z$  135 this corresponded with the ion peak and molecular weight of the suggested structure of compound 3 in the NIST library figure 19 with a chemical formula of  $C_9H_{10}O_2$ .

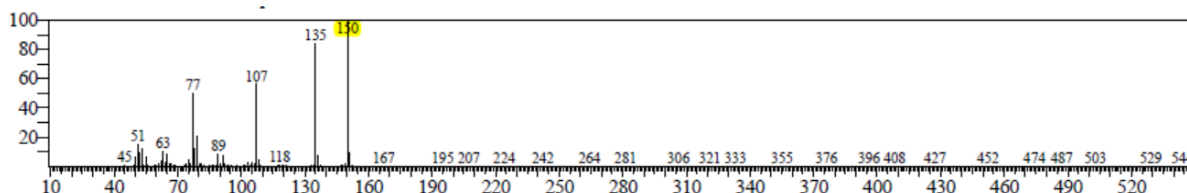


Figure 18: Mass spectrum of compound 3.

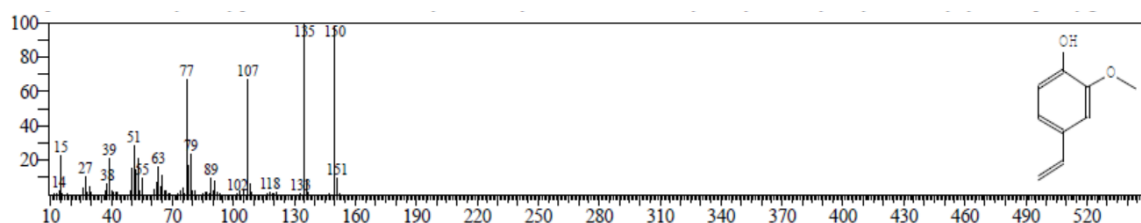


Figure 19: Mass spectrum of suggested structure of compound 3 by NIST Library.

IR spectrum of Compound 3 (Figure 20) showed functional groups and absorption bands which appeared at 33746  $cm^{-1}$  as O-H stretch as illustrated in the IR spectrum. An absorption at 1681  $cm^{-1}$  for C=C stretching which illustrated the presence of double bond in the chemical structure of Compound 3. The absorption of C-

H was observed at 2975  $cm^{-1}$  which indicated the presence of methyl carbon in the chemical structure. A signal was observed at 1042  $cm^{-1}$  as C-O bond and single bond stretching was observed at 878  $cm^{-1}$  in the IR structure of Compound 3.

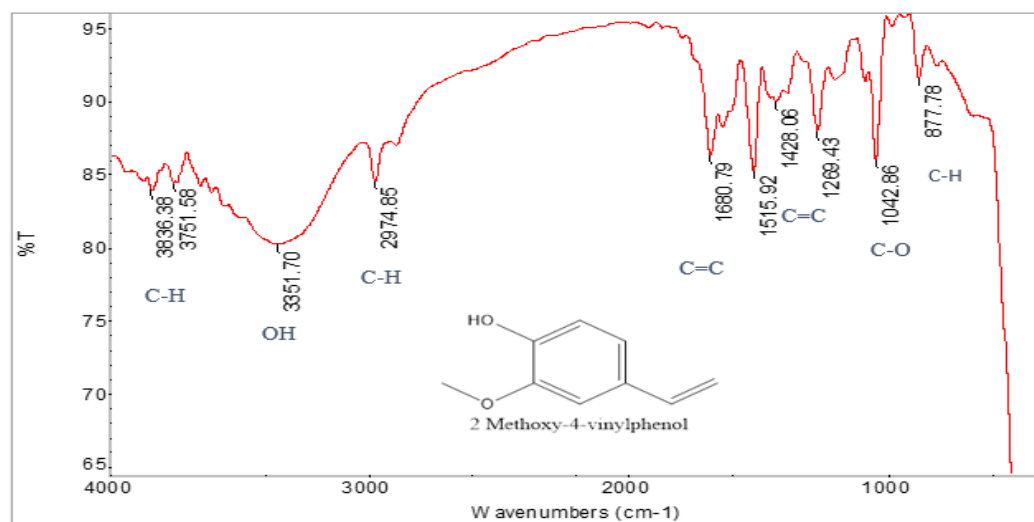


Figure 20: IR Spectrum of Compound 3.

In NMR analysis, integration and assignation of every proton and carbon of Compound 3 is based on the  $^1H$ -NMR and  $^{13}C$ -NMR analysis. The analysis of the chemical structure of Compound 3 and the result are as shown in Figure 21 for  $^1H$ -NMR and Figure 22 for  $^{13}C$ -NMR. Observation of the  $^1H$ -NMR revealed a total 10 proton resonance, where the proposed chemical structure is based on the splitting pattern as reported in

spectrometric identification of Organic compounds by Silverstein *et al.*,<sup>[16]</sup>

The  $^1H$ -NMR spectrum of Compound 3 exhibited 10 proton resonates. A singlet proton signal was observed at  $\delta$  2.17 (3H, s) indicating that they had no neighbouring proton and the methyl group is attached to pi-bonded carbon. A set of two doublet signal at 7.06 (2H, d)

$J=6.93$  and  $6.94$  (2H, d)  $J=6.93$  indicating they are neighbours attached to the same alkenic carbon. A singlet was observed at  $\delta$  3.48 indicating the presence of a methoxy group and was assigned as 11-OMe. A singlet proton signal was observed at  $\delta$  9.37 (1H, s) indicating the presence of an O-H group (hydroxyl) of the structure and was assigned to H-7.

A proton was also observed at the chemical shift  $\delta$  6.92 (H, s),  $\delta$  2.17 (3H, s) which represent a singlet of methane group in the structure of the compound and was assigned to H-6 and H-11. At chemical shift of  $\delta$  7.45 a multiplet proton signal was observed as to methane group of the structure and was assigned to H-5 (7.45 9 (2H, m,  $J=7.44$ ). A signal was also observed of chemical shift at 5.29 (H, s) and was assigned to H-13.

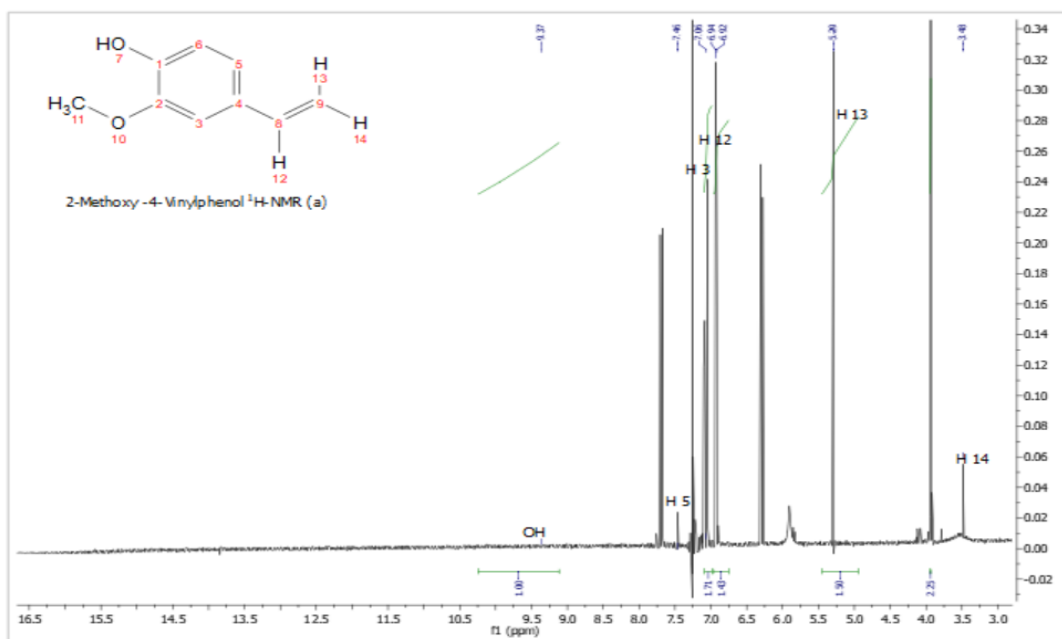


Figure 21a:  $^1\text{H}$ -NMR spectrum of Compound 3 (500 MHz,  $\text{CDCl}_3$ ).

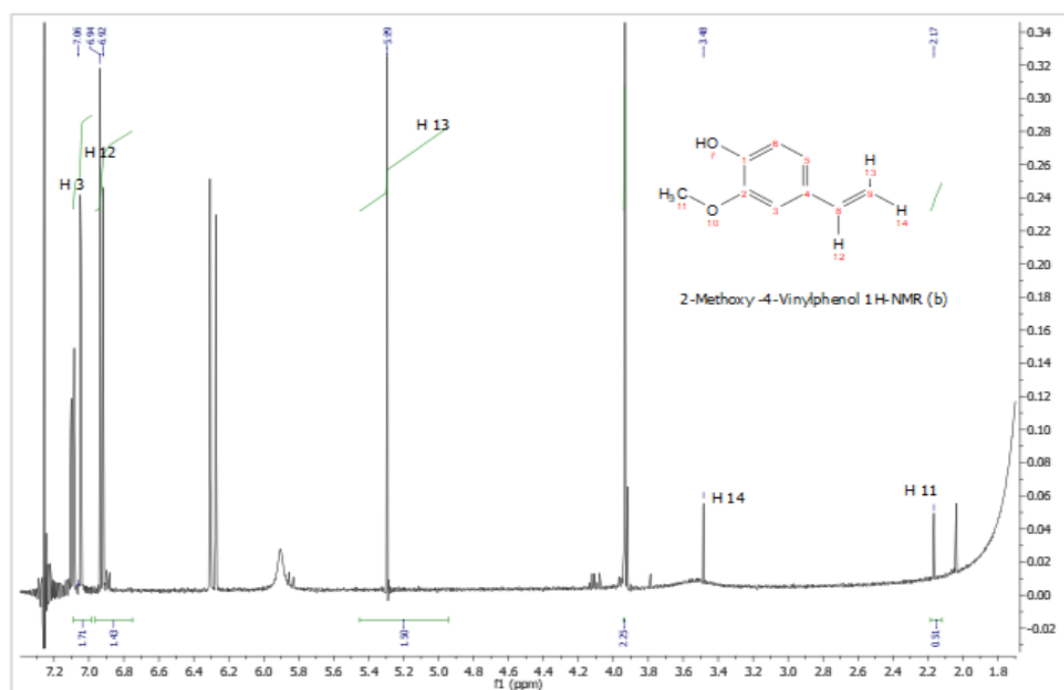


Figure 21b:  $^1\text{H}$ -NMR spectrum of Compound 3 (500 MHz,  $\text{CDCl}_3$ ).

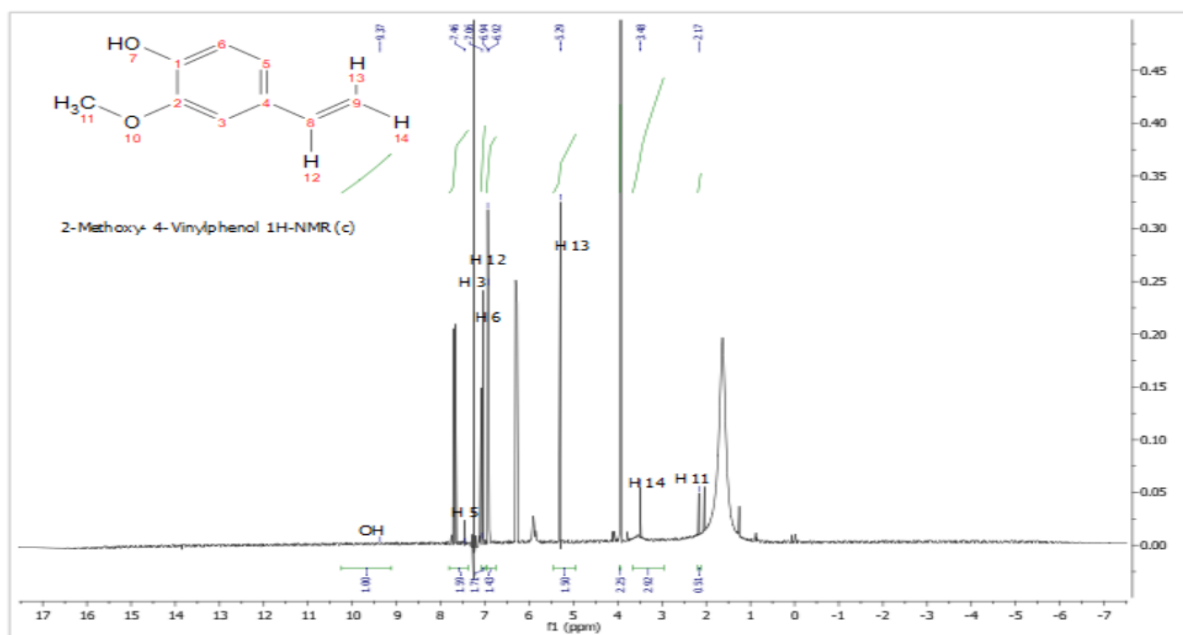


Figure 21c:  $^1\text{H}$ -NMR spectrum of Compound 3 (500 MHz,  $\text{CDCl}_3$ ).

The  $^{13}\text{C}$ -NMR result of Compound 3 indicated every Carbon bond signal and was assigned to the proposed chemical structure of Compound 3 which is based on the table of  $^{13}\text{C}$ -NMR characteristics absorption as reported in spectrometric identification of Organic compounds by Silverstein *et al.*<sup>[16]</sup>

A total of 9 carbon resonates were observed in the carbon spectrum of Compound 3. The down field region of the spectrum of Compound 3 indicated a signal which was observed as  $\delta$  147.00,  $\delta$  148.42,  $\delta$  109.55,  $\delta$  126.81,  $\delta$  123.44,  $\delta$  114.33,  $\delta$  146.89,  $\delta$  114.86 and were assigned to C-1, C-2, C-3, C-4, C-5, C-6, C-8 and C-9

respectively. C-1 was found to be attached to the hydroxyl group, C-2 indicated the presence of methane group of the structure and was found to be attached to the methoxy group.

At the up field region a signal was observed at  $\delta$  55.78 that was identified as the carbon attached to the methoxy group and was assigned to C-11 in the chemical structure of Compound 3. The chemical shift of all the proton and carbon NMR for Compound 3 are shown in Table 13 and Table 14 and comparison was made with the NMR data of similar Compound as reported by Easwaran *et al.*<sup>[21]</sup>

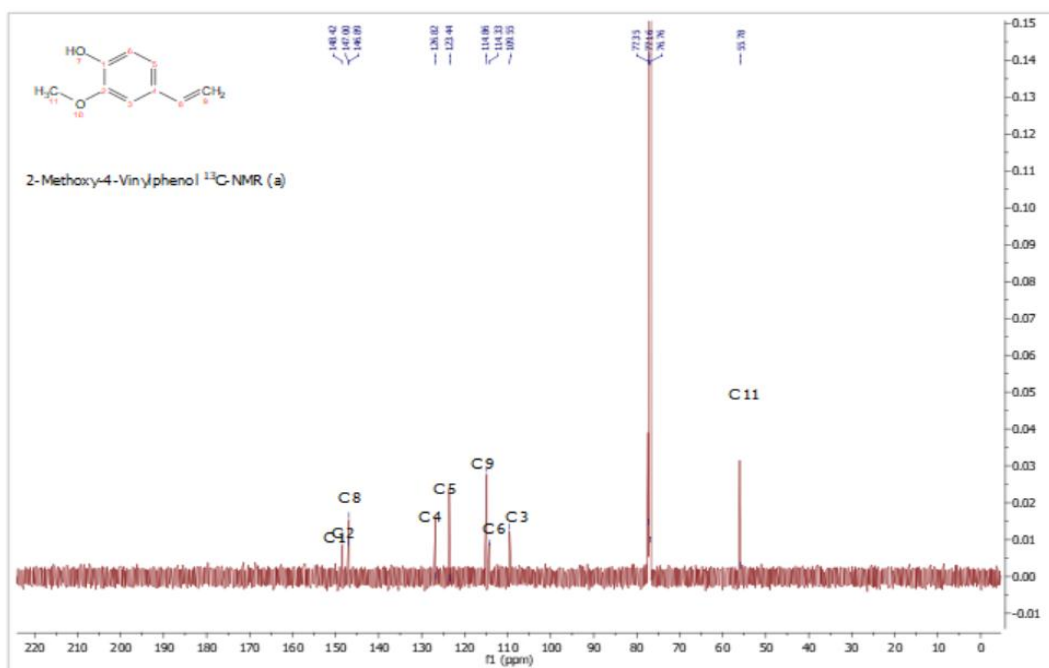
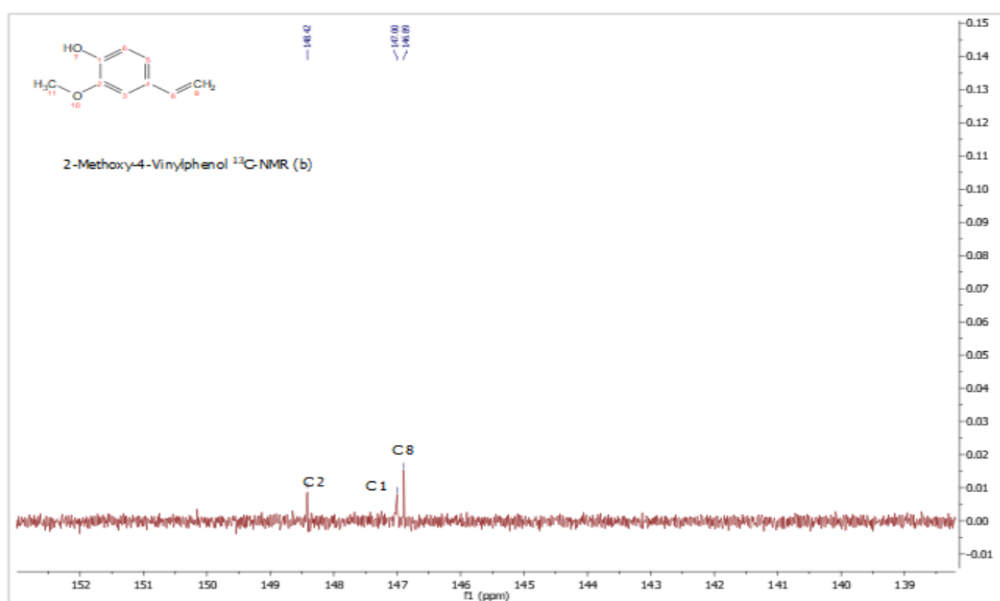
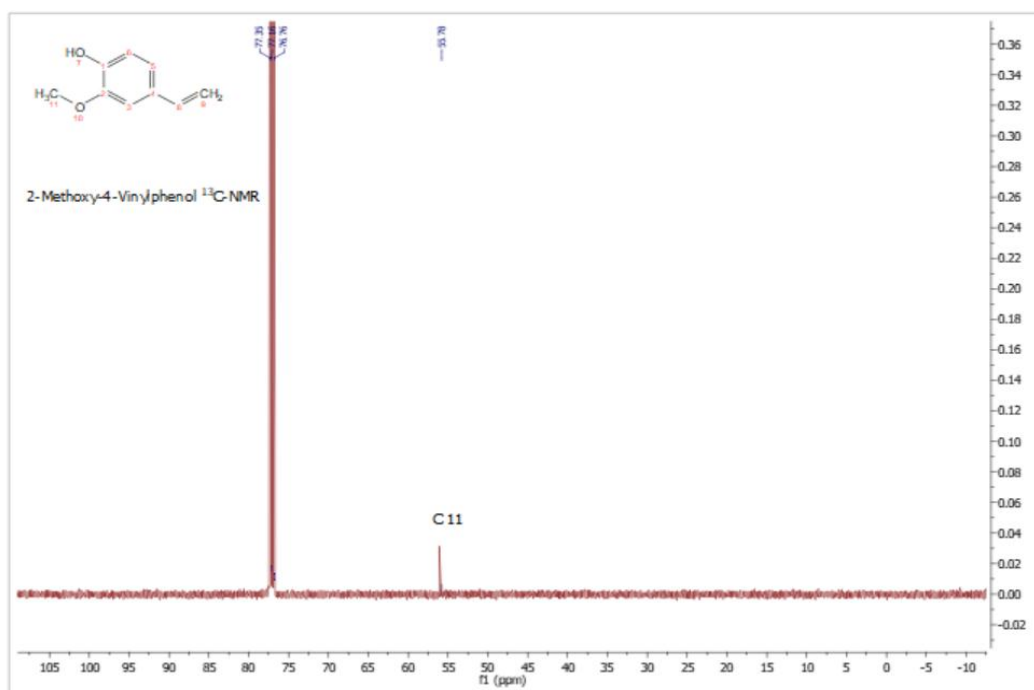


Figure 22a:  $^{13}\text{C}$ -NMR spectrum of Compound 3 (500 MHz,  $\text{CDCl}_3$ ).

Figure 22b:  $^{13}\text{C}$ -NMR spectrum of Compound 3 (125 MHz,  $\text{CDCl}_3$ ).Figure 22c:  $^{13}\text{C}$ -NMR spectrum of Compound 3 (500 MHz,  $\text{CDCl}_3$ ).Table 13: Proton NMR signal of compound - and that reported by Easwaran *et al.*, (2014).

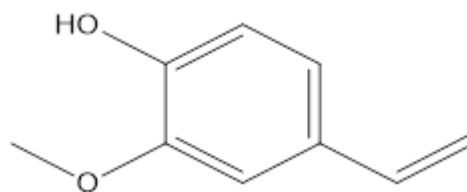
| Proton assigned to Compound 3 | Proton chemical shift (ppm) of compound 3 | Proton assigned to 2-Methoxy-4-Vinylphenol (Easwaran <i>et al.</i> , 2014). | Proton chemical shift (ppm) of 2-Methoxy-4-Vinylphenol (Easwaran <i>et al.</i> , 2014). |
|-------------------------------|-------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| H-3                           | 7.06 (2H, d, J=6.93)                      | H-3                                                                         | 6.561 (d)                                                                               |
| H-5                           | 7.45 (2H, m) J=7.44                       | H-5                                                                         | 6.734 (d)                                                                               |
| H-6                           | 6.92 (H, s)                               | H-6                                                                         | 6.560 (d)                                                                               |
| H-7                           | 9.37 (H, s)                               | H-7                                                                         | 9.412 (s)                                                                               |
| H-11                          | 2.17 (3H, s)                              | H-11                                                                        | 2.016 (s)                                                                               |
| H-12                          | 6.94 (2H, d, J=6.93)                      | H-12                                                                        | 6,72 (d)                                                                                |
| H-13                          | 5.29 (H, s)                               | H-13                                                                        | 5.337 (d)                                                                               |
| H-14                          | 3.48 (3H, m, J 3.94)                      | H-14                                                                        | 4.892 (d)                                                                               |

**Table 14: Carbon NMR signal of compound 3 and that reported by Easwaran *et. al.*, (2014).**

| Carbon assigned to Compound 3 | Carbon chemical shift (ppm) of compound 3 | Carbon assigned to 2-Methoxy-4-Vinylphenol (Easwaran <i>et. al.</i> , 2014). | Carbon chemical shift (ppm) of 2-Methoxy-4-Vinylphenol (Easwaran <i>et al.</i> , 2014). |
|-------------------------------|-------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| C-1                           | 147.00                                    | C-1                                                                          | 142.92                                                                                  |
| C-2                           | 148.42                                    | C-2                                                                          | 155.90                                                                                  |
| C-3                           | 109.55                                    | C-3                                                                          | 107.55                                                                                  |
| C-4                           | 126.81                                    | C-4                                                                          | 139.91                                                                                  |
| C-5                           | 123.44                                    | C-5                                                                          | 120.83                                                                                  |
| C-6                           | 114.33                                    | C-6                                                                          | 114.21                                                                                  |
| C-8                           | 146.89                                    | C-8                                                                          | 142.14                                                                                  |
| C-9                           | 114.86                                    | C-9                                                                          | 115.43                                                                                  |
| C-11                          | 55.78                                     | C-11                                                                         | 56.51                                                                                   |

From the result obtained the spectrum for Compound 3 gave a similarity index of 94.42 % with the mass spectrum of the proposed structure by the NIST library, which matched the characteristic of 2-Methoxy-4-Vinylphenol (3) with the chemical formula  $C_9H_{10}O_2$  the melting point of Compound 3 is (28 °C). The Compound 3 proton and carbon NMR data were mostly identical to match the NMR signal of 2-Methoxy -4-Vinylphenol (3) as reported by Easwaran *et. al.*<sup>[21]</sup>

Based on the reported results of IR,  $^1H$ -NMR and  $^{13}C$ -NMR and comparisons with published literature.<sup>[21]</sup> Compound 3 was identified as 2-Methoxy -4-Vinylphenol (3).



2-Methoxy -4-Vinylphenol is a compound containing a methoxy group attached to the benzene ring of a phenol moiety. 2-Methoxy-4-Vinylphenol (3) was found to contain antibacterial potential.<sup>[21]</sup> It was found to induce a spicy odour quality and 2-Methoxy-4-vinylphenol can induce cell cycle arrest by blocking the hyperphosphorylation of retinoblastoma protein in benzo[a]pyrene-treated NIH3T3 cells.<sup>[22]</sup>

**Table 15:**

| Concentration (ppm)          |                              |                        |           |           |           |
|------------------------------|------------------------------|------------------------|-----------|-----------|-----------|
| Isolated Compound            | Organism                     | Control (Tetracycline) | 25 ppm    | 50 ppm    | 100 ppm   |
|                              |                              | (mm)                   | (mm)      | (mm)      | (mm)      |
| Benzyl alcohol (1)           | <i>Escherichia coli</i>      | 3.17±0.43              | 0.94±0.08 | 1.14±0.08 | 1.17±0.04 |
|                              | <i>Klebsiella pneumonia</i>  | 2.95±0.17              | 0.97±0.04 | 1.37±0.04 | 1.77±0.04 |
|                              | <i>Staphylococcus aureus</i> | 3.21±0.58              | 2.11±0.31 | 2.70±0.01 | 2.24±0.35 |
|                              | <i>Escherichia coli</i>      | 3.17±0.43              | 1.04±0.05 | 1.14±0.05 | 1.94±0.09 |
| 3-Pyridinecarboxylate (2),   | <i>Klebsiella pneumonia</i>  | 2.95±0.17              | 0.89±0.01 | 2.57±0.04 | 2.72±0.02 |
|                              | <i>Staphylococcus aureus</i> | 3.21±0.58              | 2.39±0.02 | 1.87±0.09 | 2.57±0.09 |
|                              | <i>Escherichia coli</i>      | 3.17±0.43              | 1.04±0.05 | 1.47±0.04 | 1.69±0.05 |
|                              | <i>Escherichia coli</i>      | 3.17±0.43              | 1.04±0.05 | 1.47±0.04 | 1.69±0.05 |
| 2-Methoxy-4-vinylphenol (3). | <i>Klebsiella pneumonia</i>  | 2.95±0.17              | 1.14±0.08 | 1.52±0.02 | 1.97±0.04 |
|                              | <i>Staphylococcus aureus</i> | 3.21±0.58              | 0.82±0.02 | 1.04±0.05 | 2.47±0.04 |

Result is in Mean ±SD. N=3. \* = significant activity was observed

Figures are in mm and include the diameter of the paper disc (5mm). Data are means of triplicate determinations.

The *in vitro* antibacterial activity of isolated Pure compound from *Leptadenia hastata*, extracts was carried out for 24 hrs culture of three selected bacteria. The bacteria organisms used were *Staphylococcus aureus*,

*Escherichia coli*, and *Klebsiella pneumoniae*. All the test organisms were obtained from stock cultures at virology Laboratory, Faculty of resource and Technology Universiti Malaysia Sarawak.

With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The sensitivity of each test microorganism to the pure compounds was determined using the Disc Diffusion Technique.

The growth inhibitory concentration of the pure compounds were significantly active on all the selected pathogen. From the result, greater antibacterial activity was shown against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* suggesting that the isolated compounds of *Leptadenia hastata* could be used in the treatment of bacteraemia infections.

The Table 15 shows the mean value of zone of inhibition of the antibacterial activity of the isolated compounds from *Leptadenia hastata* against the three selected bacteria. Significant activity was observed in all the fractions at 25 ppm, 50 ppm and 100 ppm in all the bacteria except of Pyridine carboxylic acid was there observe to less growth inhibition on *Klebsiella pneumoniae* at 25 ppm.

Strong growth inhibition of Benzyl alcohol (1) activity was observed on *Staphylococcus aureus* at all the concentration. Higher inhibition was on concentration 50 ppm of  $2.70 \pm 0.01$  mm and weaker inhibition of *Staphylococcus aureus* was at 25 ppm of  $2.11 \pm 0.31$ .

*Klebsiella pneumoniae* was observed to be inhibited strongly by Pyridine Carboxylic acid (2) at 50 ppm and 100 ppm of  $2.57 \pm 0.04$  mm and  $2.72 \pm 0.02$  mm. there was no inhibition observed at 25 ppm.

This compound 2-Methoxy-4-vinyl phenol (3) exhibited strong inhibition rate on *Klebsiella pneumoniae* at 25 ppm, *Escherichia coli*, *Klebsiella pneumoniae* at 50 ppm. Higher inhibition on *Staphylococcus aureus* at 100 ppm of  $2.47 \pm 0.04$  mm was as shown in Table 15. Weaker growth inhibition rate was observed at 25 ppm of  $0.82 \pm 0.02$  mm against *Staphylococcus aureus* when compared to the control tetracycline of  $3.21 \pm 0.58$  mm.

## 5. CONCLUSION

This study revealed that this medicinal plant *Leptadenia hastata* extract from dichloromethane had some potential phytochemicals. The structure of the pure compound isolated were elucidated using various spectroscopic methods especially Gas Chromatography and Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance and Fourier Transformer Infrared (FT-IR). A total of three secondary phytochemicals were isolated and characterised. They are Benzyl alcohol (1), 3-pyridine carboxylic acid (2) and 2-Methoxy -4-Vinylphenol (3). It was interesting to note that the three compound isolated from the extract of *Leptadenia hastata* was observed in Table 15 to have a significant activity on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## ACKNOWLEDGEMENT

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