International Journal of Chemistry Studies
ISSN: 2581-348X
Impact Factor: RJIF 5.44
www.chemistryjournal.in
Volume 1; Issue 2; December 2017; Page No. 35-43

Isolation and identification of components from the leaf and stem bark extracts of *Borreria verticillata*

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Abstract

Borreria verticillata (youngest mature) leaves were collected from their natural habitat of coastal plain sands in Calabar Municipality. The method of cold solvent was used in the extraction. This was to prevent the escape of some volatile of the sample. Infrared spectroscopic (IR) and Gas chromatography / Mass spectroscopic (GC/MS) techniques were employed to analysis the structure of the active purified components (LEAF1, LHF1and LHF2) of the Borreria verticillata extract. Infrared spectroscopic and gas chromatography/mass spectroscopic analysis of these active components (ethyl acetate fraction LEAF1) shows that LEAF1 is heptanal while hexane extract LHF1 is heptanone. Heptanal or heptanaldehyde is an alkyl aldehyde with a strong fruity odor which is used as an ingredient in cosmetics, perfumes, and flavors. Industrially, heptanal is used in the manufacture of 1- heptanol and ethyl heptanoate.

Keywords: *Centella asiatica*, threatened, pharmacology, perennial and ancient

Introduction

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases (Rita Nath *et al*., 2015) [14]. The medicinal plants are those that provide people with medicines to treat illness, maintain and promote health (Sivasankari *et al*., 2010) [11]. The phytochemicals are the wide variety of compounds produced by plants manipulated wisely in the pharmacognostic drug development and treatment of the major ailments (Sivasankari *et al*., 2010) [11]. The rural inhabitants use the leaves and stem bark of *Borreria verticillata* for curative purposes. For this reasons therefore, it could be argued that further investigation into medicinal plants, like *Borreria verticillata* leaves and stem bark. The plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered. *Borreria verticillata* is one of the plants which have been used in traditional medicine for many years.

Column chromatography provide means of isolation and purification of compound in large amount than is possible with thin layer chromatography (TLC). Infrared spectroscopy is generally useful in elucidation of structure of mainly organic molecules (Ogugbuaja, 2000). It provides a simple and rapid instrumental technique that can give evidence of various functional groups. IR spectroscopy depends on the interaction of molecules or atoms with electromagnetic radiation. It causes atoms and groups of atoms of organic compounds to vibrate with increased amplitude about the covalent bonds that connect them. Since the functional groups of organic molecules include specific arrangement of bonded atoms, absorption of IR energy by an organic molecule will occur in a manner characteristics of the type of bonds and atoms present in the specific functional of that molecule (Solomon and Fryhle, 2004).

To the best of my knowledge little or no work has been done on the plant *Borreria verticillata* in this part of the world. The present work is designed to enrich the available scientific data on efficacy of *Borreria verticillata*. The study is limited to the leaves and stems of *Borreria verticilata* and the sampling was done in Calabar. The isolation of the components of the extracts from the leaves and stems with the aid of chromatographic methods:- thin layer chromatography and column chromatography. The spectroscopic analyses of the isolated active components, were carried out to determine the structures using IR and GC-MS

Materials and Methods

Sample Collection and Preparation

Borreria verticillata (youngest mature) leaves were collected from their natural habitat of coastal plain sands in Calabar Municipality (04o 15oN; 08o 25oE), Nigeria. The samples were air-dried for about two weeks and then milled into fine powder using a Thomas-Willey milling machine.

Method of Extraction

The method of cold solvent was used in the extraction. This was to prevent the escape of some volatile of the sample. The extracts of the leaf and stem bark were prepared by soaking 100g of each in 250 ml hexane for four days. The resulting mixtures were filtered by gravity filtration and the filtrate will be concentrated by evaporation in a water bath, dried and weighed. The procedure was repeated on the residue using the following solvents: ethyl acetate, acetone chloroform and methanol sequentially in order of polarity. The extracts were stored in a desiccator.

Thin Layer Chromatography (TLC)

Before a complete isolation and purification of the active component in the leaves and stem bark of *Borreria verticillata*, a qualitative examination of the mixture was carried out using the thin layer chromatography. This was aimed at establishing three facts:

a. Revealing the number the various components in the extracts,
b. Showing how easy or difficult to separate the various components in the extracts and
c. Helping to establish the best absorbent and solvent (or solvents mixture) to be used in column chromatography.

Commercially made micro plates were used for TLC plates. Some fractions were spotted on the silica TLC plates and developed using various solvent mixtures. Thus, the extracts were purified with the aid of preparatory thin layer chromatography. This was carried out by preparing a solution (slurry) of the crude extract using the solvent used in their extraction. With the aid of a micropipette, the crude extract to be separated was applied as a thin line of material 1 cm from the bottom of the preparatory TLC plate. Mixture of solvents were used to developed the plates on which the various extracts were applied on. The mixture which is in 3:2:1 or the reverse (1:3:2) are as indicated below:

Table 1

Solvent extract	**Solvent mixture**
Hexane extract	Hexane and methanol
Methanol extract	Methanol and hexane
Chloroform extract	Chloroform and acetone
Acetone extract	Acetone and chloroform
Ethyl acetate extract	Ethyl acetate and hexane

After development, the zones of the various coloured bands were outlined in pencil. To determine the retarding factor value (Rf) of different spots traveled from the initial point of the original spotting were measured. The solvent front distance that was marked was also marked measured.

The distance moved by the solvent from the base line would be calculated as

$$R_f = \frac{\text{Distance moved by component (spots).}}{\text{Distance moved by solvent.}}$$

Having identified the zones, the absorbent in those bands were scraped from the plate and extracted with hexane (for the hexane extracts) to remove the adsorbed materials. Filtration removed the adsorbent and evaporation of the solvent gave the recovered component from the mixture. This was kept in a dessicator.

Column Chromatography

In column chromatography, the sample is applied in a very narrow plug at the top of the column and solvent is allowed to flow through the adsorbent. This process developed the chromatogram into bands containing the individual compound. The bands can be eluted (washed off) in sequence by further solvent and collected in separate fractions.

The column was prepared in the following ways;

1. A wad of glass wool was placed in the bottom of tube and a layer of anhydrous magnesium sulphate over this. The anhydrous magnesium sulphate retains fine particles and also provides a flat horizontal base for the adsorbent column.
2. The tube was filled with first the (hexane) solvent to be used and the dry adsorbent in a fine stream, shaking or tapping the to dislodge air bubbles, and draining solvent out at the bottom to make room as needed, but keeping the solvent level above the adsorbent.
3. When the adsorbent had settled into a compact column, another layer of anhydrous magnesium sulphate was added at the top of the adsorbent to prevent disturbance of the surface when the solvent was added.
4. The solvent was allowed to drain down to just above the top of the magnesium sulphate. The adsorbent column was kept covered with solvent during the chromatography to prevent the development of channels and racks.
5. A uniform sized adsorbent (silica gel or alumina) was mixed into a solvent (hexane, for instance) in a ratio of 1:10 to produce a slurry. The slurry was poured through a funnel into the column that is clamped in a vertical position,
6. The adsorbent was allowed to settle evenly and free from air bubble, by gentle tapping of the column tube with a wood rod or rubber hose.
7. The sample was dissolved in a minimum volume of the solvent (hexane) and the solution was added to the column with pipette and bulb. The solution was allowed to drain on to the column and and immediately more hexane was added.
8. The solvent (hexane) was removed via the tap fitted to the adaptor at the column.
9. More slurry was added until the required length of the column is obtained.
10. At no time during the column preparation or in subsequent use was the level of liquid allowed to fall below the level of the adsorbent.
11. Fractions were collected at regular interval and each fraction was monitored by TLC analysis.

Structural Elucidation

Infrared spectroscopic (IR) and Gas chromatography / Mass spectroscopic (GC /MS) techniques were employed to analysis the structure of the active purified components (LEAF1, LHF1and LHF2) of the *Borreria verticillata* extract. These were done in NARICT, Zaria.

Infrared Spectroscopy (IR)

A spectrophotometer was used to take infra-red measurements of the isolates from the hexane, chloroform and ethyl acetate extracts of the leaves of *Borreria verticillata*. The various absorption bands in cm-1 were observed, measured and recorded. The analyses involve the use of KBr cell. The sample was applied on the cell and placed in the machines sample pot and get started. An IR spectrometer operates by passing a beam of IR radiation through the sample and compares the ratio transmitted through the sample with that transmitted in the absence of the sample.

Any frequency absorbed by the sample will be apparent by the difference. The spectrometer plots the result as a graph showing absorbance verses frequency. The location of an IR absorption band (peaks) can be specified in frequency related unit by its wave number (cm -1). The excitation of a molecule from one vibrational energy level to another occurs only when the compound absorbs IR radiation of a particular energy i.e. particular wave number.

Gas Chromatography/Mass Spectroscopy (GC/MS)

A very useful innovation in sample introduction is the use of GC coupled with MS. In effect the mass spectrometer acts in the role of detector (Donald *et al*., 2001). The sample was first injected into the GC where it vaporized in a heated chamber.

The gas mixture travels through a GC column where the compounds become separated as they interact with the column. Those separated compounds then immediately enter the mass spectrometer to cation by loss of an electron. In order to measure the characteristics of individual molecule, a mass spectrometer converts them to ions so that they can be moved and manipulated by external electric and magnetic fields. The three essential functions of a mass spectrometer and the components associated are;

1. A small sample is ionized, usually to cation by loss of electron, the ion source.
2. The ions are sorted and separated according to their mass to charge ratio.
3. The separated ions are then detected and tallied, and the results are displayed on the chart.

Because ions are very reactive and short lived, their formation and manipulation is conducted in a vacuum at a pressure of 100.2 KPa and temperature of 60.0 oc, the in temperature was about 250 oc. the trace from the recorder is the spectrum. A graph of the number of particles detected as function of mass to charge ratio (vertical bar). Mass spectra first display the molecular ion peak which is a radical cation, M+, as a result of removing one electron from the molecule. The length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100 and it is referred to as the base peak.

Results

Thin Layer Chromatography Results

Result of Qualitative TLC of Hexane Leaf Extract

Table 1 below shows the result of the qualitative TLC performed on the hexane crude extracts of leaves of *Borreria verticillata.* As indicated in table 1, there are four spots detected on the TLC plates for the hexane extracts with Rf values ranging from 0.309 to 969. This implies that there are four different components in the hexane extracts.

Table 2: Result of qualitative TLC of hexane leaf extract

Distance moved by the solvent = 9.70 cm		
No. of spots	**Spots distance.(cm)**	**Rf values**
1	9.40	O.969
2	6.60	0.680
3	4.20	0.432
4	3.00	0.309

Result of Qualitative TLC Chloroform Leaves Extracts

Table 2 shows the result of the qualitative TLC performed on chloroform extract of the leaves of *Borreria verticillata.* There are three spots detected on the TLC plates with Rf values ranging from 0.821 to 0.989 implying that there are three components.

Table 3: Result of qualitative TLC of chloroform leaf extract

Distance moved by solvent = 9.50cm		
No of spots	**Spots distance**	**Rf value**
1	9.40	0.989
2	8,50	0.894
3	7.80	0.821

Result of Qualitative TLC Ethyl Acetate Leaves Extracts

Table 3 shows the result of the qualitative TLC performed on ethyl acetate extract of the leaves of *Borreria verticillata.* There are two spots detected on the TLC plates with Rf values ranging from 0.777 to 0.922 implying that there are two components.

Table 4: Result of qualitative TLC of ethyl acetate leaf extract

Distance moved by solvent = 9.00cm		
No of spots	**Spots distance(cm)**	**R f value**
1	7.00	0.777
2	8.30	0.922

Result of qualitative TLC Acetone Leaf Extract

Table 4 shows the result of the qualitative TLC performed on methanol extract of the leaves of *Borreria verticillata.* There are two spots are four spots detected on the TLC plates with Rf values ranging from 0.108 to 0.891 implying that there are two components

Table 5: Result of qualitative TLC of acetone leaf extract

Distance moved by solvent = 9.20 cm		
No of spots	**Spots distance**	**Rf value**
1	9.00	0.108
2	8.20	0.891

Result of Qualitative TLC Methanol Leaves Extracts

Table 5 shows the result of the qualitative TLC performed on methanol extract of the leaves of *Borreria verticillata.* There are four spots are four spots detected on the TLC plates with Rf values ranging from 0.238 to 0.988 implying that there are four components.

Table 6: Result of Qualitative TLC of Msthanol Leaf Extract

Distance moved by the solvent = 8.40 cm		
	Spots distance cm	**Rf values**
1	2.00	0.238
2	6.70	0.797
3	7.50	0.892
4	8.30	0.988
1	2.00	0.238

Result of qualitative TLC of the fractions from the Column chromatography Fractions of Hexane Leaf Extract

The column fractions leaves hexane extract (LHF) *Borreria verticillata* collected were concentrated and dried in Desiccators. After subjecting to analytical TLC, their retention factors (Rf values) were found as recorded in table 6 below. These results are almost like the ones of the Rf values from the analytical TLC of the crude extracts.

Table 7: shows the result of the qualitative TLC performed on the fractions of the column chromatography of chloroform extract of leaves of *Borreria verticillata.*

Fractions	Solvent front	Component front	Rf values
LHF1	6.50	2.50	0.384
LHF2	7.30	7.00	0.958
LHF3	6.00	3.90	0.650
LHF4	6.50	5.90	0.907

Fractions of Chloroform Leaf Extract

The column fractions leaves chloroform extract (LCF) *Borreria erticillata* collected were concentrated and dried in desiccators. After subjecting to analytical TLC, their retention factors (Rf values) were found as recorded in table 7 below. These results are in conformity with the Rf values from the analytical TLC of the crude extracts.

Table 8: below shows the result of the qualita**tiv**e TLC performed on the fractions of the column chromatography of chloroform extract of leaves of *Borreria verticillata.*

Fractions	Solvent front(cm^3)	Component front(cm^3)	Rf value.
LCF1	8.10	7.90	0.987
LCF2	6.00	5.00	0.833
LCF3	6.10	5.50	0.901

Fractions of Ethyl Acetate Leaves Extract

The column fractions leaves chloroform extract (LEAF) *Borreria verticillata* collected were concentrated and dried in desiccators. After subjecting to analytical TLC, their retention factors (Rf values) were found as recorded in table 8 below. These results are almost like the ones of the Rf values from the analytical TLC of the crude extracts.

Table 9: below shows the result of the qualita**tiv**e TLC performed on the fractions of the column chromatography of ethyl acetate extract of leaves of *Borreria verticillata*

Fractions	Solvent front (cm^3)	Component front(cm^3)	Rf value
LEAE1	6.40	0.50	0.781
LEAE2	6.70	6.20	0.925
LEAE3	6.20	5.80	0.935
LEAE4	7.00	6.50	0.928

Results of the Structural Elucidation of the Isolated /Purified Active Components IR Spectra for LEAF 1

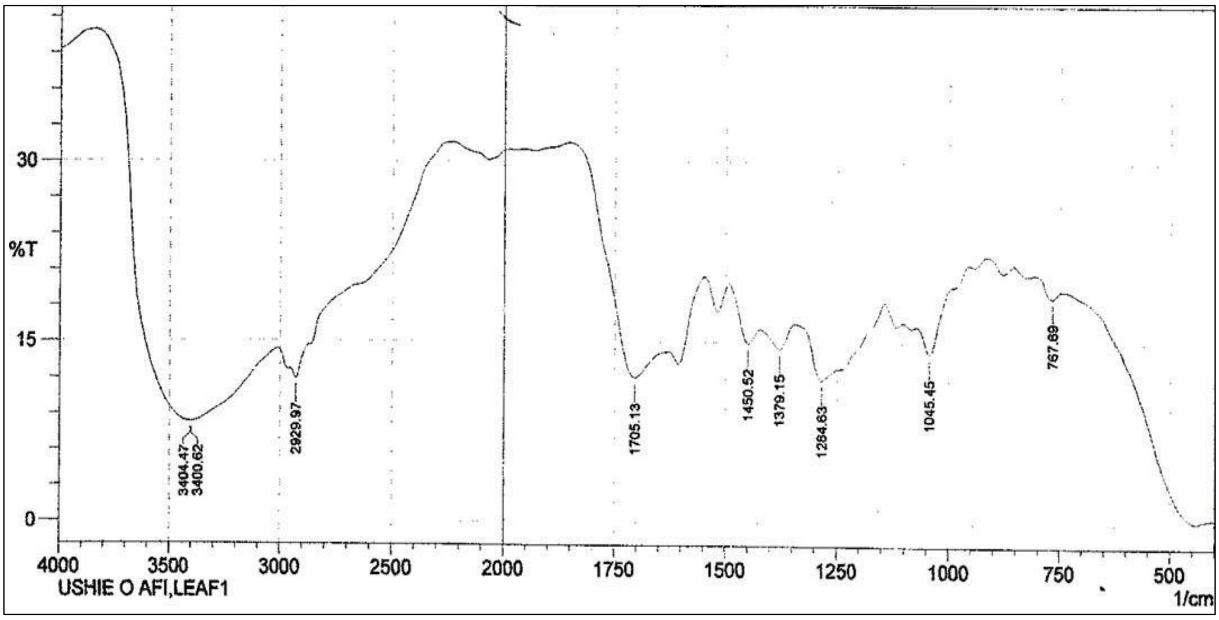


Fig 1: FTIR Analysis Result Narict, Zaria

Table 10: The IR Spectral Data and Interpretation of LEAF1.

Frequency range,cm -1	functional group	interpretation
2929.97	C-H stretching	aliphatic
2850.00	C – H stretch	aldehyde
1450.15	H-C=O stretch	aldehyde
1450.52	CH_3 deformation	aliphatic
1379.15	CH_3 deformation	aliphatic

IR Spectra for LHF 1

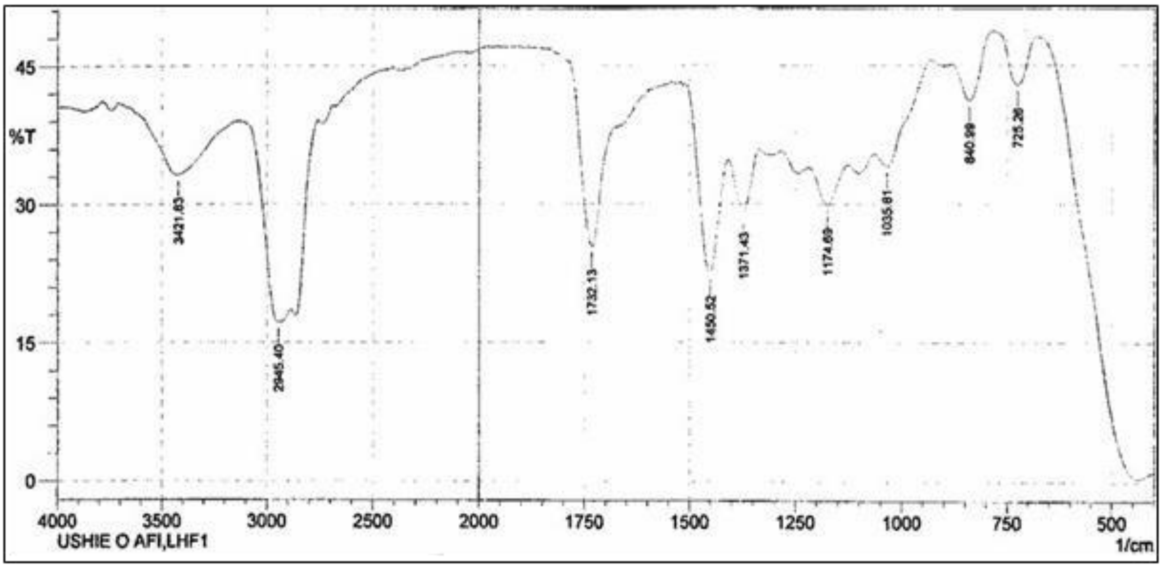


Fig 2: FTIR Analysis Result Narict, Zaria

Table 11: The IR Spectral Data and Interpretation of LHF1.

Frequency range,cm -1	functional group	interpretation
2945.40	C-H stretching	aliphatic
1732.13.	C=O stretch	ketone
1450.42	H-C=O stretch	aldehyde
1450.52	CH3 deformation	aliphatic
1371.43	CH3 deformation	aliphatic

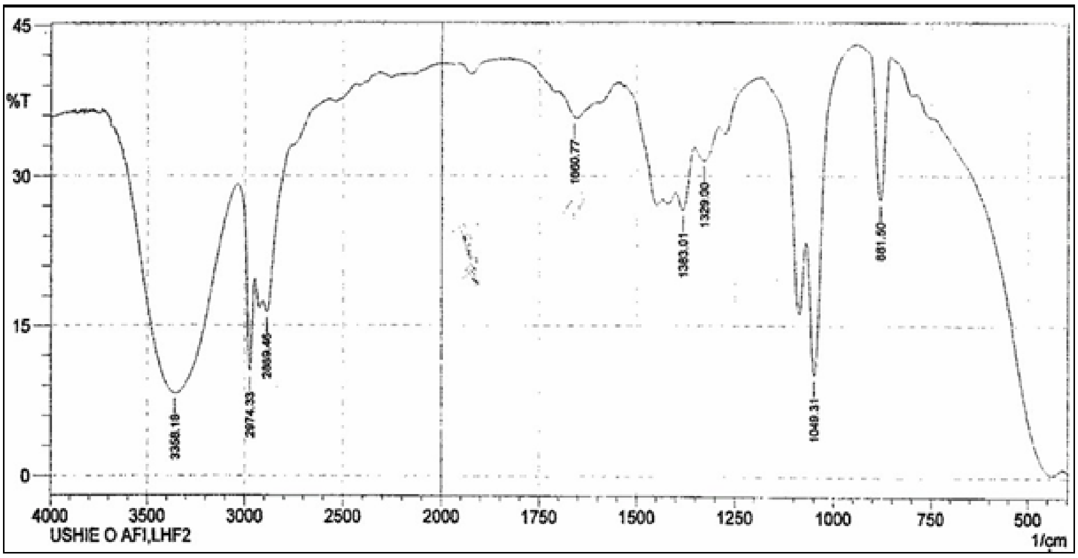


Fig 3: FTIR Analysis Result Narict, Zaria

Table 12: The IR Spectral Data and Interpretation of LHF2.

Frequency Range, cm -1	Functional Group	Interpretation
1660.77	C=O stretch	unsaturated ketone
1660.77	C=C stretch	alkene
2889.46	CH3 stretching	aliphatic

IR Spectra for LCF 2

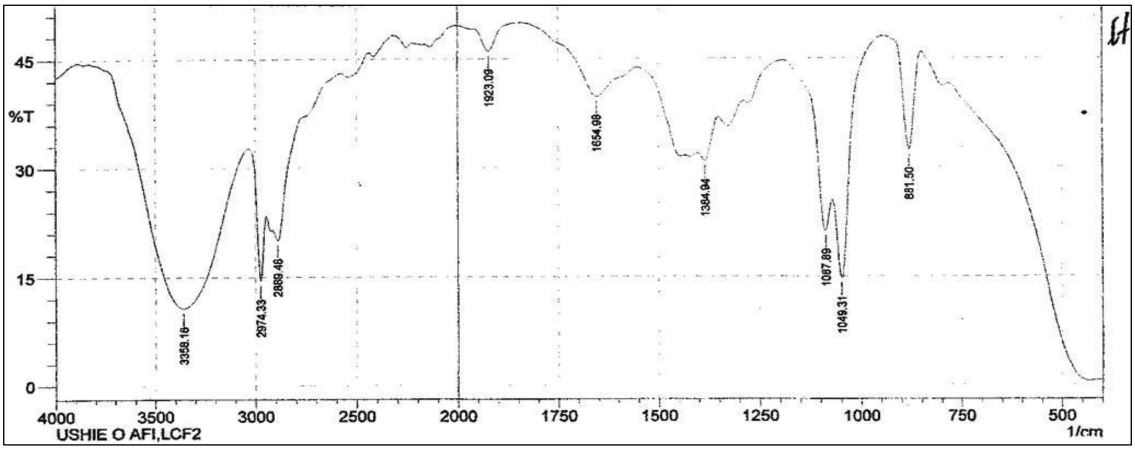


Fig 4: FTIR Analysis Result Narict, Zaria

Gas Chromatography / Mass Spectroscopy (GC /MS) Result of LEAF1

The GC /MS analysis of LEAF1 reveals compound with molecular ion peak of 113 and base peak 43. The retention time was 38.9 (scan number 4304). From the spectral data the compound is believed to be heptanal. The molecular ion peak of aliphatic aldehyde is observable. The peak due to the loss of hydrogen atom is a very characteristic of aldehyde to yield acylium ion, this peak is observed at m/z 113 followed by gradual loss of alkyl or alkenes. Beta cleavage gives rise to m/z 43. The following is the fragmentation pattern of the compound.

Gas Chromatography / Mass Spectroscopy (GC /MS) Result of LEAF1

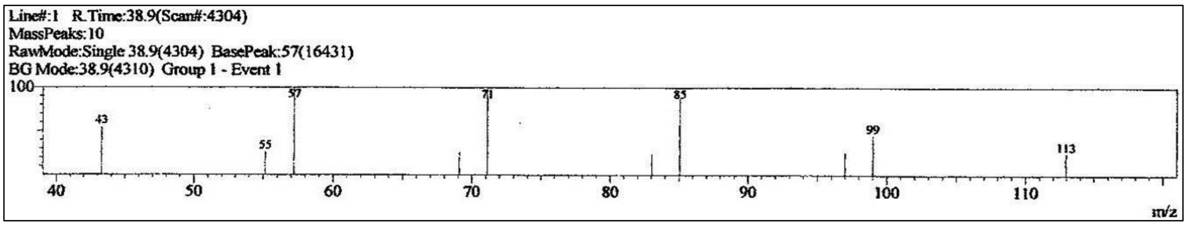
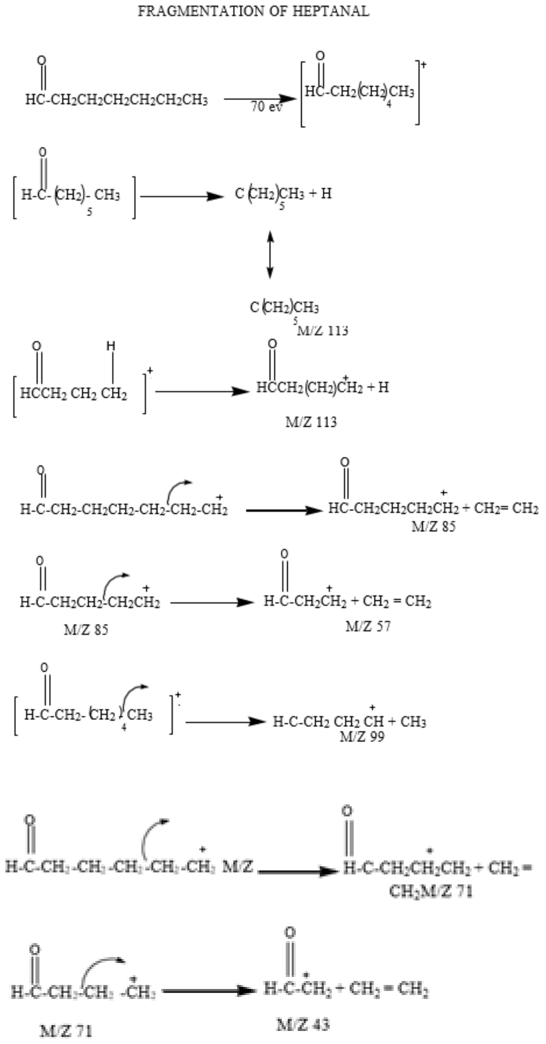


Fig 5



Gas Chromatography / Mass Spectroscopy (GC /MS) Result of LHF1

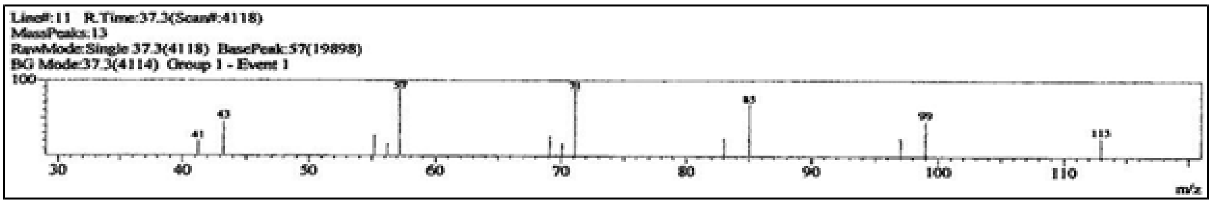
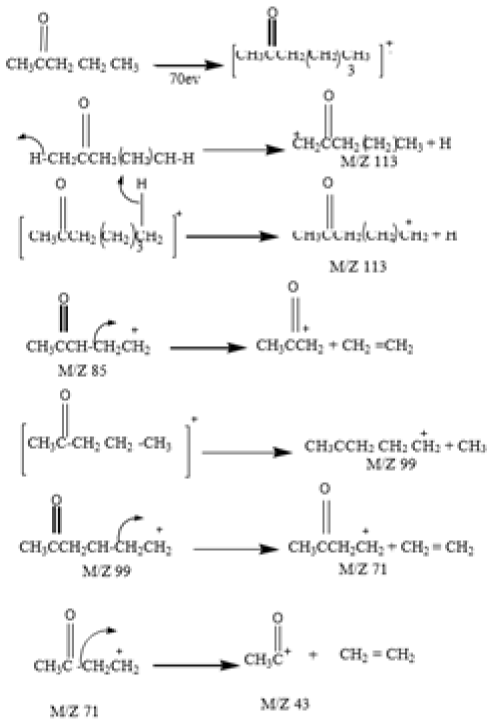


Fig 6

The GC /MS analysis of LHF1 reveals compound with molecular ion peak of 113 and base peak 43. The retention time was 37.3 (scan number 4118). From the spectral data the compound is believed to be heptanone The molecular ion peak of aliphatic ketone is observable. The peak due to the loss of hydrogen atom is a very characteristic of ketone to yield acylium ion, this peak is observed at m/z 113 followed by gradual loss of alkyl or alkenes. Beta cleavage gives rise to m/z 43. The following is the fragmentation pattern of the compound.

FRAGMENTATION OF METHYL PENTYL KETONE (HEPTANONE)



Gas Chromatography / Mass Spectroscopy (GC /MS) Result of LHF2

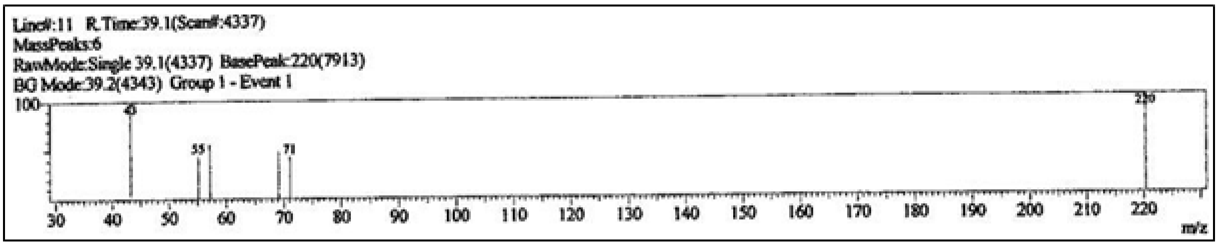
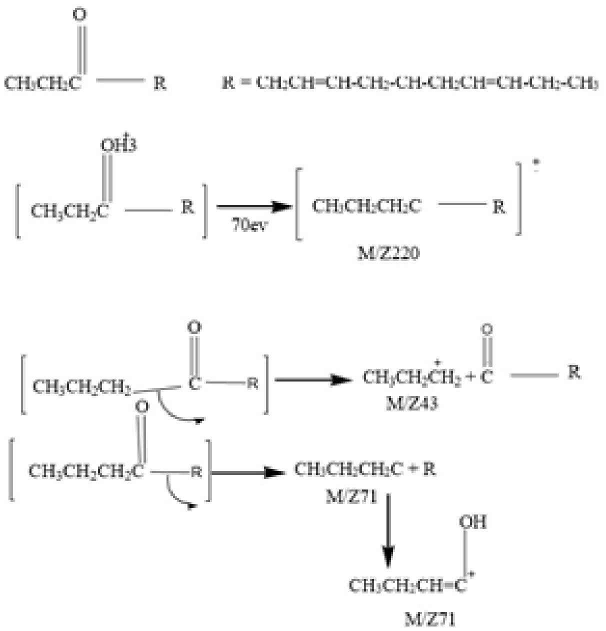


Fig 7

FRAGMENTATION OF Pentadec-3,6,9-trienone (propyl3,6,9-pentadicenone)



Conclusion

Hexane, chloroform and ethyl acetate extracts of Borreria vericillata leaves were subjected to thin layer chromatography technique for identification of the number of compounds present in each extract and to column chromatography technique for isolation and purification of the compounds. Three compounds were observed in each and collected. Infrared spectroscopic and gas chromatography/mass spectroscopic analysis of these active components (ethyl acetate fraction LEAF1) shows that LEAF1 is heptanal while hexane extract LHF1 is heptanone. Heptanal or heptanaldehyde is an alkyl aldehyde with a strong fruity odor which is used as an ingredient in cosmetics, perfumes, and flavors. Industrially, heptanal is used in the manufacture of 1-heptanol and ethyl heptanoate.

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