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Extraction and Characterization of 2-Isopropenyl-1-methoxy-7, 7-dimethyl-4, 9-imidazo-5-hydroxy pyrimidine called Alchorneinol from root bark of *Alchornea hirtella* used for the treatment toothache, and diarrhoea is Sierra Leone

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Abstract

A new compound LK001 identified as 2-Isopropenyl-1-methoxyl-7, 7-dimethyl-4, 9-imidazo-5-hydroxy pyrimidine and named Alchorneinol has been extracted from the root bark of *Alchornea hirtella* plant. It was isolated using solvent-solvent extraction method from the powdered root bark of *A. hirtella.* The white powdered compound was slightly soluble in water, ethanol chloroform and tested positive for unsaturation. The compound LK001 contained Carbon, Hydrogen, Oxygen, and Nitrogen during elemental analyses. It also tested positive for the presence Hydroxide group thus making it slightly different from the already reported drug *Alchorneine.* The fragmentation patterns proposed from the Mass spectrum for Sample LK001 and by McLafferty rule support a new compound named Alchorneinol in the root bark extract. It is therefore one of the active compounds responsible for the treatment of pain**,** particularly tooth ache, as purgative, stomach-ache and to treat tiredness after intoxication in Sierra Leone.

Keywords: McLafferty rule; Alchorneinol; Wet Chemical methods; Alchorneinol; and sedative.

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1. Introduction

This research work was geared towards investigating the active components in the traditional medicinal plant *Alchornea hirtella* (Dalziel, 1937; Agbelusi, Odukoya, & Otegbeye, 2007; Akinpelu, Aiyegoro, & Okoh, 2008) used in Sierra Leone to treat pain particularly tooth ache. The root decoction of the plant is taken in as sedative, as purgative to treat stomach-ache, sap topically applied to the affected part and the root scrapings chewed to treat tiredness after effect of intoxication.

Botanical Name of the plant: *Alchornea hirtella*
Common Names in Sierra Leone (Deighton, 1957; Burkill, 1985):

Mende: Tolo-Geŋge
Temne: Ɛ-YↃla-Ɛ-A-Nes

Alchornea hirtella is a straggly climber, a spindly shrub or a small tree that can grow to a height of about 9 meters (30 ft.) (n.n). *A. hirtella* is native to Central and Southern Africa. Its range extends from Senegal, Uganda and Kenya, southwards to Angola, Zambia, Mozambique and South Africa. It grows, sometimes in profusion, as an understorey

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shrub in tropical rain forest and in secondary forest, in riverine corridors and in the splash zone around waterfalls. It can grow at altitudes of up to 2,500 meters (8,200 ft.) (Chifundera, 2001; Burrows, Lötter, & Schmidt, 2018; Chapano, & Mamuto, 2003). Traditional uses of the plant is reported in the Democratic Republic of Congo where aqueous decoction of leaves are used for the treatment of fractures (Chifundera, 2001), and leaves and fruits extracts are used in the treatment of dysentery in Sierra Leone (Koroma & Ita, 2009; Samai & Barnish, 1992).

Fig. 1. *Alchornea hirtella plant*

1.1. Compounds reported to be present in Alchornea hirtella

The compound alchorneine isolated from the dichloromethane stem bark extract of *Alchornea hirtella* has been reported (Khuong-Huu, Le Forestier, & Goutarel, 1972). After that, the presence of alkaloidal nucleus in a whole-plant (leaves, stem and root barks) polar extracts (ethanol and water), together with flavonoids, saponins, sterols, tannins and terpenoids was confirmed and reported (Koroma & Ita, 2009). Also flavonoids and saponins were found in lesser amount in the petroleum ether extract. Additionally, low to moderate antibacterial activity was obtained from the aqueous whole-plant extract with inhibition percentages (relative to the standard drug ciprofloxacin) of 27%, 28%, 33% and 56%, against S. pyogenes, S. aureus, E. coli and P. vulgaris, respectively when evaluated through disc diffusion method at 1 mg/mL. The ethanolic leaf extract showed a MIC value of 1.9 mg/mL against S. pyogenes (Koroma & Ita, 2009).

The alkaloids below have been isolated from *Alchornea species* and reported with their corresponding structures (Khuong-Huu, Le Forestier, & Goutarel, 1972).

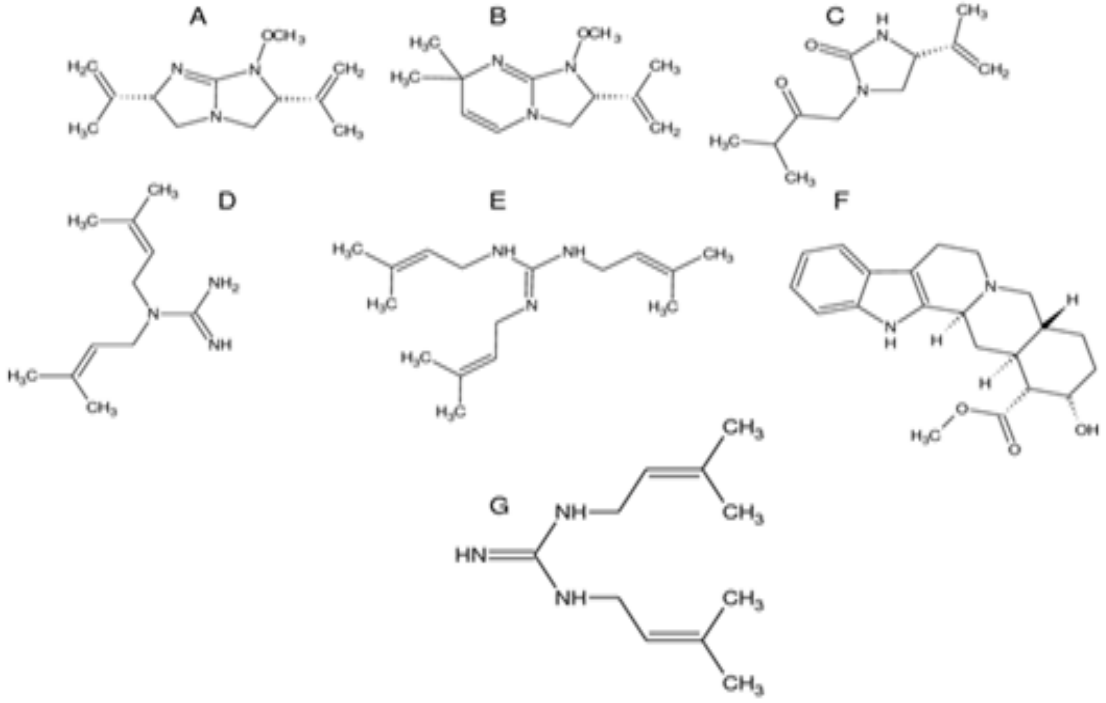

Fig. 2. Structures of known alkaloids isolated from *Alchornea Species* [12].

A. Isoalchorneine, B. Alchorneine, C. Alchorneinone, D. N1,N2- diisopentenyl guanidine, E. N1,N2,N3-triisopentenyl guanidine, F. yohimbine and G. Pterogynidine

Various Alchornea species have been reported to exhibit similar pharmacological activities including antioxidant, hepatoprotective, anti-inflammatory, antiplasmodial, antidiarrhoeal, antimicrobial and as an immunomodulatory (Bingham, Willemen, Wursten, Ballings, & Hyde, 2021; Ajali, 2000; Tor-Anyiin, Shaato, Oluma, 2003; Agbor, Talla, Ngogang, Jeanne, 2004; Urrea-Bulla, Suárez, Moreno-Murillo, 2004; Okoye, Osadebe, Nworu, Okoye, Omeje, Esimone, 2011; Adeshina, Onaolapo, Ehinmidu, Odama, 2010; Amos-Tautua, Angaye, Jonathan, 2011; Osadebe, Okoye, Uzor, Nnamani, Adiele, Obiano, 2012; Kouakou, Schepetkin, Yapi, Kirpotina, Jutila, Quinn, 2013; Lifongo, Simoben, Ntie-Kang, Babiaka, Judson, 2014).

2. Experimental

2.1. Collection of Plant Materials

Fresh root barks of the traditional medicinal plant *Alchornea hirtella* was collected in January, 2020 from the Gola Forest in the Eastern Province of Sierra Leone with permission from the Local authorities and the Forest Guards. It was identified with assistance of Mr. A. B. Feika former Chief Laboratory Technician, Department of Botany, Fourah Bay College, University of Sierra Leone now Research Coordinator at the Gola Forest National Park, Eastern Province Sierra Leone. A Voucher Specimen Number ***FBCKP001*** of the plant materials investigated was deposited in the Herbarium of the Botany Department, Fourah Bay College, Freetown (University of Sierra Leone).

2.2. Preparation of plant materials

The plant organ investigated was reduced in size by cutting it into smaller pieces using a cutlass, dried under the shade and not the sun so as to protect the thermo labile components if present from being chemically transformed. After the

plant material had been dried, it was grounded using a mortar and pestle. The powdered plant organ was kept in specially sealed containers in a refrigerator until the time of the extraction.

2.3. Extraction of alkaloids from plant sample

500g of the dried powdered root bark of *Alchornea hirtella* was moistened with water, mixed with 100ml of saturated solution of calcium (II) hydroxide [$Ca(OH)_2$] and allowed to stand for twenty-four hours. The mixture was filtered using a Buchner funnel and a vacuum pump. The filtrate was extracted with petroleum (60 – 80^{o}C) three times and concentrated. The remaining solution was then shaken with aqueous 1% Hydrochloric acid and allowed to separate. Alkaloid salt in in the aqueous layer was separated from the mixture using a separating funnel. The resulting solution was basified with $NaHCO_3$ and extracted into chloroform. The chloroform layer was allowed to evaporate at room temperature to produce 200mg of white powder and labelled LK001. Sample LK001 was found to be slightly soluble in water, ethanol and chloroform. Wet chemical methods of analysis including elemental analysis was carried out on the sample and sent to both USA and UK for Instrumental Analysis.

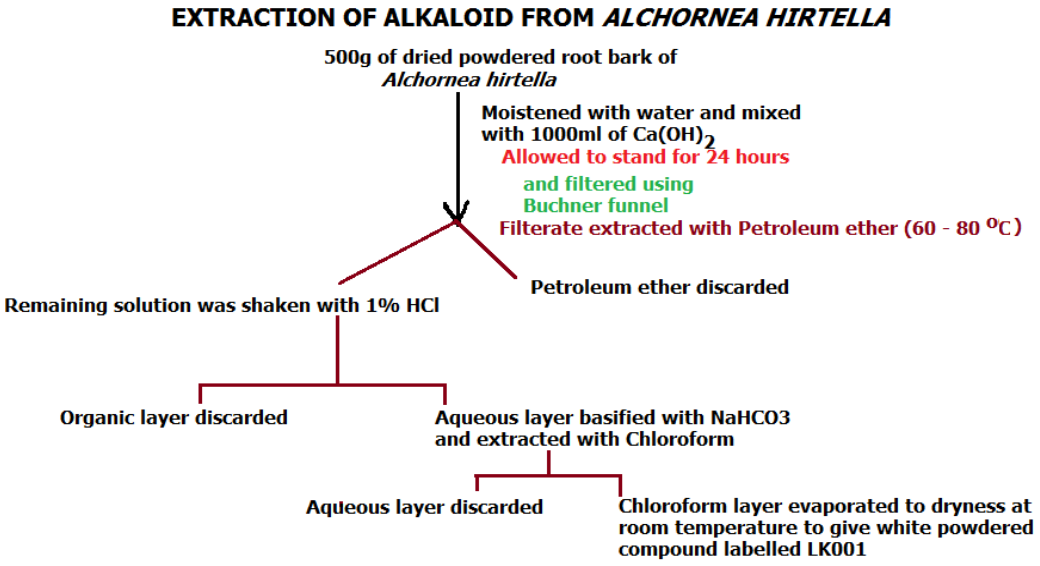

Fig. 3. An outline of extraction of Sample LK001 from the powdered root bark of *A. hirtella.*

The percentage extractive yield of the solvent extracts was calculated by using the formula below:

$$\% \text{ Extractive Yield (WW)} = \frac{\text{Weight of dried solvent extract}}{\text{Weight of dried powdered plant material}} \text{ X } 100$$

Elemental analysis was performed by wet chemical methods and confirmed by the Carlo Elba 1106 Elemental Analyser.

2.4. Instrumental analyses (USA) of Sample LK001

^{1}H and ^{13}C NMR spectra were acquired on an Agilent DirectDrive2 500 MHz NMR spectrometer equipped with a One-Probe operating at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR in $CDCl_3$, deuterated DMSO, $(CD_3)_2CO$, D_2O or toluene-d_8 and recorded at 25 °C. ^{1}H-NMR spectra were recorded with 8 scans, a relaxation delay of 1s, and a pulse angle of 45° and referenced to the various NMR solvents as necessary. ^{13}C-NMR spectra were collected with

254 scans, a relaxation delay of 0.1 s, and a pulse angle 45°. Thin-layer chromatography (TLC) was performed on plates of EMD 250-µm silica 60-F254. High-resolution mass spectroscopy was performed with APCI mass spectra recorded on Finnigan LCQ Deca (ThermoQuest) technologies with LC/MS/MS (quadrupole/time-of-flight) and Waters Xevo G2-XS UPLC/MS/MS inert XL MSD with SIS Direct Insertion Probe. Melting points for all products were measured with a Thomas HOOVER capillary uni-melt melting point apparatus and are uncorrected.

2.5. Instrumental Analyses of Sample LK001 (UK)

A set of procedures for routine characterization of **Sample LK001** that require a high level of confidence to assign purity by reverse phase Ultra high performance liquid chromatography (RP-UHPLC) under acidic mobile phase conditions were carried out. All compounds sent for analysis within and from outside the chemistry departments need to comply with an agreed analytical specification.

The **Sample LK001** was labelled as MSQ3AB_15NOV2019SLK_001 with file name EV-SLK_001 and MS file number IM-METCR-AB101-PosNeg, inlet file Number METCR-AB101 using the Open-Lynx equipment.
This specification typically comprised a test for the determination of the compound's identity and a test for the determination of the compound's purity with a high degree of confidence. Elemental analysis, UHPLC-MS, coupled with other ancillary detectors, were the predominant method of analyses used.

Common Apparatus and Reagents used were

- 0.1% Formic acid in water – Mobile phase "A"
- 0.1% Formic acid in acetonitrile – Mobile phase "B"
- Waters ACQUITY UPLC CSH C18 Column, 130Å, 1.7 µm, 2.1 mm X 100 mm column

UHPLC system that is capable of gradient elution with UV or diode array detection with other detectors as required (e.g. MS, ELS) were used in the instrumental analysis.

No test sample was used to confirm operational performance of the system during daily setup of the system as the sample was a natural product. If the test solution data does not conform to set criteria regarding peak shape, response and retention time, a trouble-shooting procedure was followed by carrying out a number of test samples.

2.6. Instrument parameters UK

Specific instrument parameters were varied depending upon the make and model of equipment used. For the LC Conditions**;** a flow rate= 0.6 ml/min**;** Column temperature = 40ºC in 5.82 minutes. UV detection was typically performed at a selected wavelength or over a scan range. MS detection was typically performed over a mass range to include target masses and other ions of interest. Additional detectors such as ELS can also be included to meet specific project requirements. Acquired data was processed automatically using Open-Lynx Software, the data is then distributed electronically and read using the Open-Lynx data browser application.

2.7. Elemental analysis and wet chemical methods of analysis of Sample LK001 isolated

Wet Chemical methods of analysis

Elemental analysis was carried out on LK001 isolated from the traditional medicinal plants investigated for the presence of carbon, hydrogen, oxygen, nitrogen, sulphur and halogens using The Middleton's Test.

a) Test for carbon, hydrogen and oxygen.
   Each of the samples initially dried in an oven were heated strongly with excess of pure copper (I) oxide in a test until no further change occurred and tests for water and carbon dioxide carried out.
b) Test for nitrogen, Sulphur and halogens
   The Middleton's Test was carried out on each of the samples isolated to ascertain the presence of nitrogen, Sulphur and halogens.

5mg of each of samples LK001, LK002 was mixed with 1g of Middleton's mixture [i.e. 2 parts of zinc powder to 1 part of anhydrous Sodium Trioxocarbonate (IV)] in a small test tube and heated strongly for 2-3 minutes in a hot Bunsen flame. The red-hot tube was the plunged into 20 ml of water in a beaker. The whole mixture for each of the isolated compound was boiled to dissolve the sodium salts formed and filtered. The filtrate was divided into three portions and tested as follows;

c) Test for cyanide ions
The first portion for each compound isolated was tested for the presence of cyanide.
To each portion was added some crystals of Iron (II) tetraoxosulphate (VI) salt and few drops of 2.0M Sodium hydroxide and boiled for one minute to form hexacyanoferrate (II) ions. 10 ml of Iron (II) chloride solution was added with sufficient concentrated hydrochloric acid to dissolve any hydroxide present and filtered.

d) Test for sulphide ions
The second portion for each compound isolated was tested for the presence of sulphide ions. To each of the second portions of the filtrate was added fresh solution of sodium nitroprusside and observed.

e) Test for halide ions
To the third portion of each of the filtrates was boiled with $2MHNO_3$ in a fume cupboard to expel hydrogen cyanide and/or hydrogen sulphide. To half of each of the portions freed from cyanide and sulphide ions, was added few drops of 2M HNO_3 and 2-3 drops of 0.05M $AgNO_3$ solution.

f) Acid test
A solution of each of the samples was tested with blue litmus paper and with Sodium hydrogen Trioxocarbonate (IV) solution.

g) Phenol test
1% iron (III) Chloride solution was added to aqueous solution of each of the compounds and the colour change observed.

h) Test for unsaturation
1% $KMnO_4$ solution was added drop wise to each of the compounds isolated in a test tube and colour change observed.

i) Test for Aromaticity – action of heat.
A portion of each of the compounds isolated was characterized using a flame test.
The results of each test are reported in the Result Chapter.

The results of **Sample LK001** isolated in this research work and sent for Instrumental Analysis abroad are Characterize by wet chemical, Elemental analysis, instrumental analytical techniques and McLafferty Rearrangement to confirm its structure.

3. Results and Discussion

3.1. Extraction of alkaloid from Alchornea hirtella plant

327 mg of white powdered compound labelled Sample LK001 was obtained from the chloroform layer. Sample LK001 was found to be slightly soluble in water, ethanol and chloroform.

100 mg of each of samples LK001 was sent to UK and the USA for instrumental analyses.

Mass of powder root bark = 500g

Mass of Compound isolated = 0.327g

$$\textbf{Percentage by mass of sample LK001} = \frac{0.327\text{g} \times 100}{500\text{g}} = 0.0654\ \%$$

Table 1. Wet Chemical Analysis on Sample LK001

Test	Observation	Inference
a. Acid Test – Solutions of sample LK001 was tested with Litmus paper	Red litmus paper turned blue	Sample LK001is basic
Sample + Ethanoic acid	Smell of ester observed	Contains OH group
Solution of Sample LK001 + $NaHCO_3$	No reaction observed	Sample LK001 is not acidic
b. Phenol Test	No reaction observed	Sample LK001 does not contain Phenolic compound
c. Test for unsaturation	The colour of 0.1M $KMnO_4$ solution changes from purple to colourless	Sample LK001 is unsaturated
d. Test for Aromaticity	smoky flame	Sample LK001 is aromatic
a) Carbohydrate		
Portion of Sample LK001 was strongly heated with in a boiling tube until no further change occurred.	Sample LK001 turned black with a colourless gas and droplets of colourless at the mouth of the test tube. Turns lime water milky.	Probably carbohydrate present
i. Gas + Lime water	Colour changes from white to blue	Presence of Carbon dioxide
ii. Liquid + $CuSO_4$		Presence of water Hence Sample LK001 contains Carbon, Hydrogen and Oxygen
b) The Middleton's test		
5mg of Sample LK001 was mixed with 1g of Middleton's mixture in small test tube and heated for two minutes in a hot Bunsen flame. The red-hot test tube was plunged into 20ml of water in a beaker. whole mixture was boiled to dissolve the sodium salts formed, filtered and the filtrate divided into three portions		
i. Test for cyanide ions	Specks of Prussian blue precipitated seen on the filter paper	Sample LK001 contained nitrogen atoms.
ii. Test for sulphide ions	No visible reaction seen	Sulphide ions are absent.
iii. Test for halides ions	No visible reaction seen	Halides ions are absent

3.2. Instrumental Methods of Analysis of Sample LK001

Table 2. Elemental Analysis of Sample LK001

#	Atom	Molar Mass (MM) ($gmol^{-1}$)	Subtotal Mass (%)	Subtotal Mass ($gmol^{-1}$)
12	C	12.01	60.74	144.13
19	H	1.01	8.07	19.15
3	N	14.01	17.71	42.02
2	O	16.00	13.48	32.00

Expected Molecular Formula $C_{12}H_{19}N_30_2$
Molar Mass = 237.3g/mol.
Expected structure

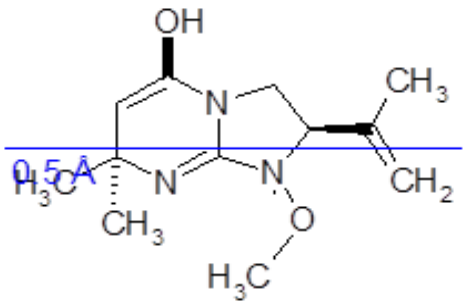

Fig. 4. Proposed structure of Sample LK001

The above Structure was confirmed from the Instrumental analysis **Sample LK001** sent to USA and The UK.

3.3. Results of Proton NMR Spectroscopy (USA)

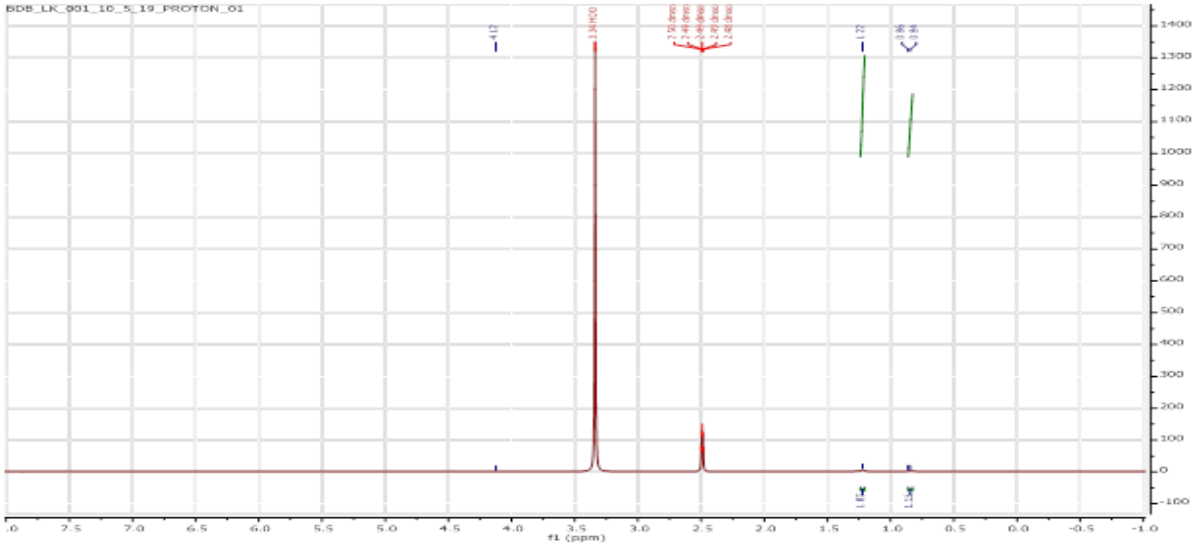

Fig. 5 1H NMR Spectrum for Sample LK001

The ^{1}H NMR Spectrum for LK001 is worthy of some comments according to Figure 6.

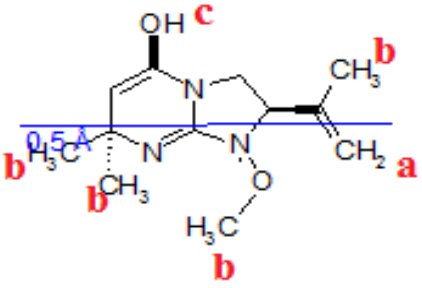

Fig. 6. Proposed proton environments around the proposed structure of Sample LK001

The interpretations of δ – values (ppm) shifts drawn above are shown below.

a. ^{1}H –C== = 1.22 ppm
b. ^{1}H –C-- = 0.84 -ppm
c. ^{1}H – O = 4.12 ppm

Solvent Peak 1 = HDO = 3.34 ppm

Solvent Peak 2 = DMSO = 2.48 – 2.5 ppm as illustrative in the figure 7.

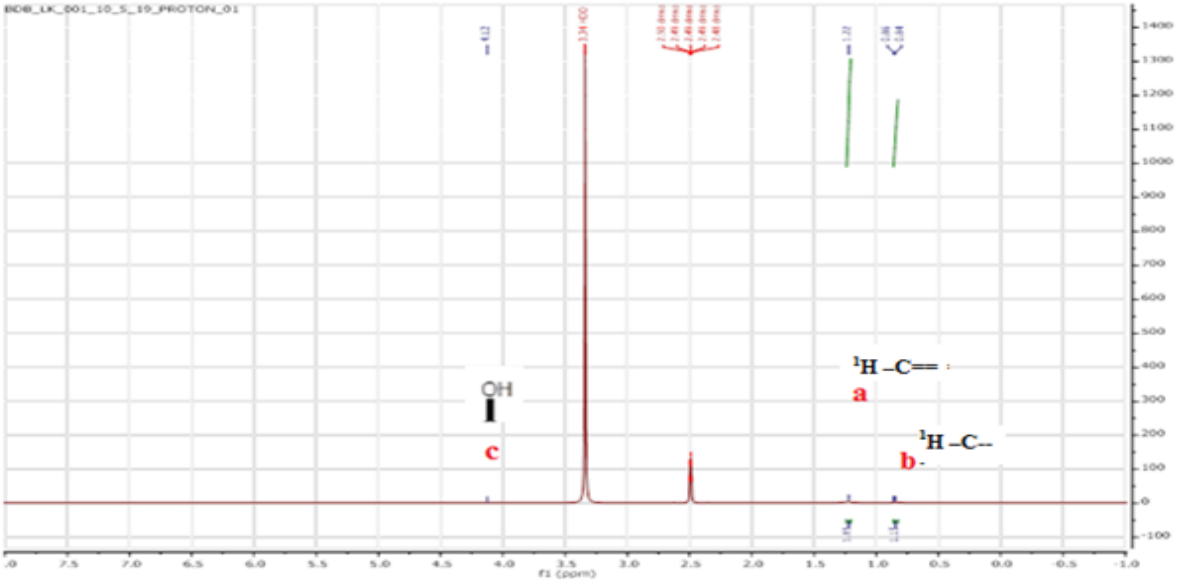

Fig. 7. Proposed location of fragments on the proton NMR for Sample LK001

The figure 7 clearly indicates the position of Hydrogen atoms in the different environments in the proposed structure of Sample LK001. The confirmation of the structure can be obtained from the mass spectrum obtained from the UK.

3.4. Results of LCMS/Mass Spectroscopy of Sample LK001

Table 3. ID and Description of mass spectrum of Sample LK001

Sample	Vial	ID	File	Date	Time	Description
2	1:10:00 AM	A2	MSQ3AB_15NOV2019SLK_001	11/15/2019	3:14:39 PM	EV-SLK_001

A total of thirteen (13) peaks were obtained from the Blind research as shown in the data (table 4).

Table 4. Number of Peaks used in obtaining Fragments of Sample LK001

Peak Number	Vial	Function	Trace	BPI	Area Abs.	Area %BP	Width	Height
1	1:10	1:MS ES+	MS ES+ :TIC	2.33E+05	5.00E+04	0.63	0.033	3008258.5
2	1:10	2:MS ES-	MS ES- :TIC	2.41E+04	6.00E+03	100	0.037	328048.938
3	1:10	1:MS ES+	MS ES+ :TIC	8.88E+06	7.00E+06	100	0.243	60490020
4	1:10	1:MS ES+	MS ES+ :TIC	4.51E+05	2.00E+06	24.78	0.25	18196318
5	1:10	2:MS ES-	MS ES- :TIC	2.89E+03	2.00E+03	36.66	0.027	144652.641

Peak Number	Vial	Function	Trace	BPI	Area Abs.	Area %BP	Width	Height
6	1:10	1:MS ES+	MS ES+ :TIC	2.48E+06	3.00E+05	4.05	0.057	16025472
7	1:10	1:MS ES+	MS ES+ :TIC	6.95E+04	2.00E+04	0.29	0.097	633263.875
8	1:10	1:MS ES+	MS ES+ :TIC	5.64E+04	1.00E+04	0.18	0.06	559444.313
9	1:10	1:MS ES+	MS ES+ :TIC	1.03E+04	2.00E+04	0.26	0.077	552473.75
10	1:10	1:MS ES+	MS ES+ :TIC	5.37E+04	2.00E+04	0.29	0.07	848647.688
11	1:10	1:MS ES+	MS ES+ :TIC	1.97E+05	3.00E+04	0.4	0.057	1632804.625
12	1:10	1:MS ES+	MS ES+ :TIC	6.83E+04	3.00E+04	0.39	0.09	541445.688
13	1:10	1:MS ES+	DAD: 215	5.10E+03	8.00E+04	100	4.528	13893.68

Fig. 8. LCMS/Mass spectroscope of Sample LK001

The following results (table 5) are obtained from thirteen peaks with respect to the fragments that could be possibly obtained from Sample LK001 and by McLafferty Rearrangement with ID. NO., obtained from the MSQ3AB_15NOV2019SLK_001.

Table 5. Fragmentation ions obtained from M^+ ion of Sample LK001

– OH = 17, – CH_3 = 15, – 2 CH_3 = 30, – CH_3O = 31, – $2CH_3O$ = 62, – $3CH_3O$ = 93, – 2OH = 34, – C_6H_6 = 78, – $C_5H_8O_2$ = 100, – $2C_6H_6$ = 156,M^+ + $C_9H_{17}N$ = 139, M^+ + C_3H_7N = 57, $C_4H_4N_2$ = 80, $C_3H_4N_2$ = 68, [CCOH] = 41, CH_2C (OH) = CH_2 =57, $NOCH_3$ = 45, 2(CH_3) = 30, C_4N_2 = 76, $C_4H_4N_2$ = 80, CHNC = 39, H_2C = CH = 27, C= CH_2 = 26, H_2C=CH_2 = 28, $C_9H_{17}N$ = 139, C_3H_7N = 57, $C_5H_8O_2$ = 84

Ion	Expected Molecular mass	Peak position	Actual Molecular Mass	Peak Intensity
M^+	236.3	2804	236.3	1016.98
M^+ - OH	219.3	937	219.48	1455.14
M^+ + OH	253.3	716	253.1	9406.32
M^+ - CH_3	221.3	938	221.87	1976.32
M^+ + CH_3	251.3	775	251.96	6611.88
M^+ – $2CH_3$	206.3	1118	206.77	1113.01
M^+ + $2CH_3$	266.3	568	266.53	3785.30
M^+ – $3CH_3$	191.3	422	191.95	2919.48
M^+ + $3CH_3$	281.3	458	281.83	3096.56

Ion	Expected Molecular mass	Peak position	Actual Molecular Mass	Peak Intensity
$M^+ - CH_3O$	205.3	427	205.37	1328.1
$M^+ + CH_3O$	267.3	569	267.24	8875.1
$M^+ - 2CH_3O$	174.3	679	174.6	2435
$M^+ + 2CH_3O$	298.3	741	298.12	1014.87
M^+ - 2OH	202.3	695	202.67	1077.91
M^+ + 2OH	270.3	1160	270.02	2043.93
$M^+ + C_6H_6$	158.3	412	158.03	1982.82
$M^+ - C_6H_6$	234.4	440	234.8	4438.63
$M^+ - 2C_6H_6$	156.4	1089	156.67	508.01
$M^+ + 2C_6H_6$	314.3	468	314.01	1393.33
$M^+ + C_9H_{17}N$	375.3	2077	375.07	354.99
$M^+ - C_4H_4N_2$	156.3	417	168.41	3865.45
$M^+ + C_4H_4N_2$	316.3	469	316.08	2334.64
$M^+ - C_4H_4N_2$- H	315.3	988	315.32	1544.78
$M^+ - C_3H_4N_2$	168.3	1497	168.14	1544.78
$M^+ - C_3H_4N_2$ - H	167.3	674	167.8	6056.65
$M^+ + C_3H_4N_2$	304.3	978	304.0	3443.97
$M^+ + C_3H_4N_2$ - H	303.3	745	303.5	1816.18

Table 6. Fragmentation ions obtained from M^+ ion of Sample LK001

[CCOH] = 41, $CH_2C(OH) = CH_2$ =57, $NOCH_3$ = 45, $2(CH_3)$ = 30, C_4N_2 = 76, $C_4H_4N_2$ = 80, CHNC = 39, $H_2C = CH$ = 27, $C= CH_2$ = 26, $H_2C=CH_2$ = 28, $C_9H_{17}N$ = 139, C_3H_7N = 57, $C_5H_8O_2$ = 84

Ion	Expected Molecular mass	Peak position	Actual Molecular Mass	Peak Intensity
M^+	236.3	2804	236.3	1016.98
M^+ - CCOH	195.3	423	195.3	3175.77
M^+ + CCOH	277.3	456	277.13	1721.86
$M^+ + CH_2C(OH)=CH_2$	293.3	738	293.8	2946.98
$M^+ - CH_2C(OH)=CH_2$	179.3	541	179.86	4142.83
$M^+ + NOCH_3$	281.3	458	281.84	3096.56
$M^+ - NOCH_3$	191.3	422	191.95	2919.48
$M^+ + 2CH_3$	266.3	568	266.53	3785.30
$M^+ - 2CH_3$	206.3	1118	206.77	1113.01
$M^+ + C_4N_2$	312.3	582	312.96	3857.81
$M^+ - C_4N_2$	160.3	413	160.1	2434.84
$M^+ + C_4H_4N_2$	316.3	469	316.08	2334.64
$M^+ - C_4H_4N_2$	156.3	1090	156.67	508.01
M^+ + CHNC	275.3	729	275.12	2387.68
M^+ - CHNC	197.3	692	197.2	1229.43
$M^+ + CH_2=CH$	263.3	724	263.25	6162.41
$M^+ - CH_2=CH$	209.3	551	209.2	5005.90
$M^+ + C=CH_2$	262.3	451	262.07	1335.08
$M^+ - C=CH_2$	210.3	429	210.36	2288.05

Ion	Expected Molecular mass	Peak position	Actual Molecular Mass	Peak Intensity
$M^+ + CH_2=CH_2$	264.3	1094	264.03	962.96
$M^+ - CH_2=CH_2$	208.3	1120	208.6	608.45
$M^+ + C_9H_{17}N$	375.3	2077	375.07	354.99
$M^+ - C_3H_7N$ 57	179.3	1102	179.9	427.98
$M^+ + C_3H_7N$	293.3	737	293.1	5744.47
$M^+ + C_5H_8O_2$84	320.3	1184	320.49	508.01

Proposed Structure of sample LK001

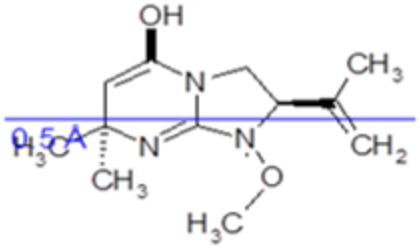

Fig. 9. Line of symmetry in the structure of sample LK001

The presence of alchorneine in dichloromethane stem bark extract of *Alchornea hirtella* was reported by Khuong-Huu *et al*, (1972) with Molecular Formula $C_{12}H_{19}N_3O$ and structure stated in figure 10.

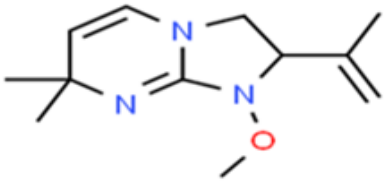

Fig. 10. Structure of alchorneine

The confirmation of this alkaloid in the stem bark extract of *Alchornea hirtella* was reported by Koroma *et.al*, 2009. Due to chemotaxonomic relationship between the various compounds extracted from the plant organs of *Alchornea hirtella*, it can be deduced that the alkaloid alchorneine is also present in the root bark of the plant. The results of wet chemical analysis indicated that the compound LK001 isolated has Hydroxyl group which makes the compound to be slightly different from the already reported structure of alchorneine. The fragmentation patterns proposed from the Mass spectrum for Sample LK001 and by McLafferty rule support a new proposed structure shown figure 11.

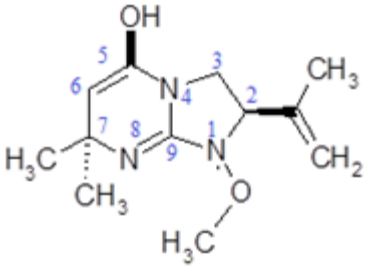

Fig. 11. 2-Isopropenyl-1-methoxyl-7, 7-dimethyl-4, 9-imidazo-5-hydroxy pyrimidine: Alchorneinol

The new compound LK001 and named as Alchorneinol (2-Isopropenyl-1-methoxyl-7, 7-dimethyl-4, 9-imidazo-5-hydroxy pyrimidine) extracted from the alkaloid extract of the root bark of Alchornea hirtella is responsible for the healing effect provided by the plant. The root extract of the plant has also been reported to have sympatholytic action and increases significantly the sensitivity of the nervous system to adrenalin. In low doses it been reported to produced slight hypotension followed by hypertension; larger doses produced an increase of blood pressure followed by a strong decrease with only slow recovery. Positive results have been reported in clinical experiments with root and leafy stem extracts in the treatment of hepatitis.

4. Conclusion and Recommendations

It can be concluded in this research work that Sample LK001 obtained from the traditional medicinal plant *Alchornea hirtella* is one of the active compounds responsible for the treatment of pain**,** particularly tooth ache, as purgative, stomach-ache and to treat tiredness after intoxication in Sierra Leone. This is the first report of the presence Alchorneinol in the root bark of *Alchornea hirtella.*

We therefore recommend that the efficacy of the plant in traditional medicine since the root bark alkaloid extract of *Alchornea hirtella* has been reported to have sympatholytic action and increases significantly the sensitivity of the nervous system to adrenalin. Positive results have been reported in clinical experiments with root and leafy stem extracts in the treatment of hepatitis. It is therefore recommended that further research on the alkaloid extracted from the plant be used on animal studies in order to determine the efficacy of the compound isolated.

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