



# PHARMACOGNOSTIC INVESTIGATION, ISOLATION, CHARACTERIZATION AND CONFIRMATION OF THE STRUCTURE OF PHENACETINE EXTRACTED FROM THE DRIED POWDERED LEAVES OF TRADITIONAL MEDICINAL PLANT ALLOPHYLUS AFRICANUS USED FOR THE TREATMENT OF PAIN, FLU AND HEAD ACHE



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DOI: <https://doi.org/10.29121/IJOEST.v4.i6.2020.114>

**Article Type:** Research Article

**Article Citation:** Lahai Koroma, and L.M. Kamara. (2020).

PHARMACOGNOSTIC INVESTIGATION, ISOLATION, CHARACTERIZATION AND CONFIRMATION OF THE STRUCTURE OF PHENACETINE EXTRACTED FROM THE DRIED POWDERED LEAVES OF TRADITIONAL MEDICINAL PLANT ALLOPHYLUS AFRICANUS USED FOR THE TREATMENT OF PAIN, FLU AND HEAD ACHE. International Journal of Engineering Science Technologies, 4(6), 10-29. <https://doi.org/10.29121/IJOEST.v4.i6.2020.114>

**Received Date:** 04 September 2020

**Accepted Date:** 26 November 2020

**Keywords:**

McLafferty Rearrangement  
Organoleptic  
Pharmaceutical  
Anti-Inflammatory and Glycosides

## ABSTRACT

Pharmacognostic investigation involving organoleptic evaluation, fluorescent analysis, phytochemical screening and mineral analysis was carried out on the dried powdered leaves of *Allophylus africanus* used for treating pain, headaches, common cold, Fever and as antibiotics in Eastern Province of Sierra Leone. The powdered leaves were light brown in colour with woody odour, bitter taste and gave significant fluorescent derivatives with the reagents 1M NaOH (aq), 1M NaOH(alc.), Ammonia, 50% HCl, and 50% HNO<sub>3</sub> when viewed under ordinary visible light and ultraviolet light. The plant organ investigated during phytochemical screening gave positive for carbohydrates and reducing sugar, Amino acids and Proteins, Alkaloids, tannins and phenolic compounds, saponin glycosides Flavonoids, sterols/terpenes and triterpenes all of which have been reported to be pharmacologically active compounds responsible for the medicinal use of *Allophylus africanus* plant.

Elemental analysis of the plant organ was performed with a Niton XL3t GOLDD + Hand held X-ray Fluorescence (Thermo Fisher) and the results indicated that the plant organs investigated contained large amounts of **K, Ca, Mg, Al** and **Fe** whilst **Ti, Zr, Mn, Zn, Sr, Sc, Rb, Cu, V** and **Mo** were present in minute quantities. The presence of the above elements also support the use of the plant organ investigated as food and medicine.

The compounds LK003 isolated from the ethanol extract of the dried powdered leaves of the plant and structure identified using fragmentation patterns from LCMS, Proton NMR spectroscopy and by McLafferty rearrangement as N-(3-methoxyphenyl) acetamide called phenacetamide which has been reported to be a clinically tested drug for pain and fever. Hence, we concluded in this research work that the presence of N-(3-methoxyphenyl) acetamide called phenacetamide, the secondary plant metabolites and minerals present in the dried powdered leaves traditional medicinal plant *Allophylus africanus* support the use of the plant as a traditional pharmaceutical.

## 1. INTRODUCTION

This research work was geared towards investigating the therapeutic efficacy of the traditional medicinal plant *Allophylus africanus*, P. Beauv. [Belonging to Family SAPINDACEAE]; used for treating pain, headaches, common cold, Fever and as antibiotics in Eastern Province of Sierra Leone. It will basically involve pharmacognostic investigation comprising organoleptic evaluation, fluorescence analysis, phytochemical screening, mineral analysis and extraction of active compound(s) from the dried powdered leaves of the *Allophylus africanus* plant.

### Local vernacular names in Sierra Leone

Mende: KŎMI-GLUE

Temne: E-FUTETE

Kono: MUŊGŎPO

Kissi: PILŎME

Via: SANJABOE

Fula: KOLAYELE

*Allophylus africanus* is usually a shrub, but sometimes grows to a tree 10 m tall, variable with four forms known in the Region and others elsewhere in Africa. It is found in forest margins, stream banks in the savanna from Casamance, Senegal, to West Cameroons and widespread in Eastern, Central and Southern Africa [1]. It has been reported that leaves of *Allophylus africanus* are traditionally used for the treatment of various ailments such as arthritis, rheumatism, gout, hemorrhoids, dysentery, venereal diseases and malnutrition. The acute toxicity and anti-inflammatory activity of the hydro-methanol leaves extract of *A. africanus* on laboratory rats [2] has been investigated and reported. The phytochemical screening of the aqueous methanol leaves *A. africanus* extract indicated the presence of carbohydrates, tannins, steroids/triterpenes, flavonoids, alkaloids and cardiac glycosides [2].



FIGURE 1: Leaves of *Allophylus africanus*

In Senegal, maceration of the leaves is used in the treatment of conjunctivitis, while in Sierra Leone a decoction is orally taken for colic and as an antipyretic [3] Four new compounds, alloeudesmenol, hanocokinoside, allotaraxerolide and alloaminoacetaldehyde, together with two known compound, stigmastane-3 $\beta$ ,4 $\beta$ -diol and pinitol were isolated, identified and reported [4], [5] from the whole plant of *Allophylus africanus* In West Africa root and twigs of *A. africanus* are supposed to be diuretic and used for the treatment of gastritis, hookworm, venereal diseases, burns, sores and fever [6]. It has been reported that various species of *Allophylus* exhibit the presence of medicinally important phytochemicals such as 11 acetoxy-4 $\alpha$ -methoxy-eudesmane, Apigenin 8-c- $\beta$

rhamnopyranoside Carissone, Phenacetamide,  $\beta$ -Sitosterol, Rutin, Quercetin, Pinitol, Luteolin-7-O- $\beta$ -D-glucopyranoside, Apigenin-4'-O- $\beta$ -D-glucoside, and fatty acids as well as bioactivity of these compounds have been proven by clinical experiments. All the plant organs of *Allophylus species* are found to be used against wound healing, fracture healing, cuts, ulcer healing [7], [8], [9].

## 2. MATERIALS AND METHODS

### 2.1. COLLECTION OF PLANT MATERIALS

Fresh plant materials of the traditional medicinal plants *Allophylus africanus* were collected from the Gola Forest in the Eastern Province of Sierra Leone and identified with assistance of the Chief Laboratory Technician Department of Botany, Fourah Bay College, University of Sierra Leone, Freetown.

Map of Sierra Leone showing the position of Gola Forest.



**Figure 2 .:** Showing the Location of Gola Forest with respect to its position from Freetown and in West Africa

### 2.2. GOLLA FOREST

The Gola Forest is located in the South – Eastern part of Sierra Leone. It has been fragmented as a result of logging and other overexploitation resulting in 3 discrete forest blocks known as Gola East, Gola West and Gola North. Gola West and East have been renamed as Gola South and are in the vicinity of the town of Zimmi and Tiwai Island. Gola North is best reached from Kenema. In 2007, the President decreed to upgrade the status of the Gola Forest Reserves to become Sierra Leone's second National Park. For this research work, permission was obtained from the Local authorities and the Forest Guards before collection of the plant materials.

### 2.3. PREPARATION OF DRIED PLANT MATERIALS

Plant materials were dried under the shade and not the sun so as to protect the thermo labile components if present from being chemically transformed. It was then reduced in size by crushing it into smaller pieces using the hand. After the plant material had been dried, it was grounded using a mortar and pestle. The powdered plant materials of the traditional medicinal plants investigated are kept in specially sealed containers in a refrigerator until the time of the extraction.

A voucher specimen of each of the plant materials investigated was deposited in the Herbarium of the Botany Department, Fourah Bay College (University of Sierra Leone).

The plant material was used to carry out the following analyses described below:

- Organoleptic evaluation
- Fluorescence analysis

- Phytochemical screening
- Mineral analysis
- Extraction of compound from the leaves of the plant

### 3. EXPERIMENTAL

#### 3.1. ORGANOLEPTIC CHARACTERS

Organoleptic evaluation was carried out by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Organoleptic characters investigated [10] are size, colour, odour, taste and texture of the dried powdered Leaves of *Allophylus africanus*. The results are shown in Table 1 and the image of the dried powdered Leaves of *Allophylus africanus* shown in Figure 2.

#### 3.2. FLUORESCENCE ANALYSIS

0.5mg of dried powdered Leaves of *Allophylus africanus* was placed in a petri dish free from grease and 2-3 drops freshly prepared reagent solution was added, mixed by gentle with a glass rod and waited for few minutes. The following freshly prepared reagents are used;

Powder + 1 N NaOH (aq), Powder + 1 N NaOH (alc.), Powder + Ammonia, Powder + Picric acid, Powder + Petroleum ether, Powder + 50% HCl, Powder + 50% H<sub>2</sub>SO<sub>4</sub>, Powder + 50% HNO<sub>3</sub>, Powder + Ethyl acetate, Powder + Ethanol, Powder + Methanol, and Powder + Bromine water.

The colours of each of the contents in Petri dish were observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations using a U/V Lamp. A piece of white paper was dipped in each of the solutions and viewed using both visible light and under the U/V Lamp to compare the colours obtained. The colours observed by application of different reagents in different radiations are recorded [11], [12], [13] as shown in Table 2.

#### 3.3. PHYTOCHEMICAL ANALYSIS

Soxhlet extraction was carried out on 300g of dried powdered Leaves of *Allophylus africanus* using solvents of increasing polarity (i.e. Petroleum ether [60-80 °C], Acetone, Chloroform Methanol, 95% Ethanol and Water. Each of the solvent extracts was concentrated, reduced to a semisolid mass using a Rotary Evaporator at 50°C and kept in special containers for phytochemical screening and isolation of compounds.

The Phytochemical screening involved testing each of the **Solvent Extracts** for the various classes of secondary plant metabolites. The methods used for detection of various phytochemicals were followed by qualitative chemical test and by standard procedures [14], [15] to give general idea regarding the nature of constituents present in each of the solvent extracts of the plant part investigated [16], [17], [18], [19], [20], [21], [22]. They are generally tested for the presence secondary plant metabolites such as Carbohydrates, alkaloids, tannins/phenolic compounds, flavonoids, Sterols/triterpenes, Amino acids/ proteins and saponins/glycosides etc.

##### **Test for Carbohydrates**

A small quantity each of the **Solvent Extracts** was dissolved in 5 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates.

**Molisch's test:** -1ml of each of the extract filtrates was treated with 2 drops of alcoholic  $\alpha$ -naphthol solution in a test tube and 1 ml of concentrated tetraoxosulphate (VI) acid was added carefully along the sides of the test tubes. Formation of violet/purple ring at the junction may indicate the presence of carbohydrates.

##### **Test for reducing sugars:**

**Fehling's test:** - 1ml of each of the extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute. The mixtures were boiled for 5-10 minutes on water bath. The formation of Reddish brown precipitate due to formation of cuprous oxide indicates the presence of reducing sugar.

**Benedict's test:** - 1ml of each of the extract filtrates was treated with equal volumes of Benedict's reagent in test tubes. The mixtures were boiled for 5-10 minutes on water bath. A change in colour of the solution from blue to green, to yellow or brick-red precipitate depending on amount of test item present indicates the presence of reducing sugar.

#### **Barfoed's Test**

1ml of the solvent extract was placed in a boiling tube and 3ml of Barfoed's Reagent was added to it. The mixture was heated in boiling water bath for 7 minutes.

#### **Observation**

The colour of the solution changes from blue to dirty green to greenish-yellow and then to Dark yellow precipitate. Brick-red precipitates are seen on top of Dark yellow precipitate.

#### **Iodine Test**

2-3 drops of iodine solution was added to 1ml of each of the solvent extracts.

The formation blue-black colour indicates the presence of starch.

#### **Test for Glycosides:**

##### **Test for cardiac glycosides:**

**Keller Kelliani test (test for deoxysugar):** -Each of the **Solvent Extracts** was treated with chloroform and evaporated it to dryness. 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it and transferred to a small test tube. 0.5 ml of concentrated tetraoxosulphate (VI) acid was carefully added down the side of the test tube. The formation of a blue colour in the acetic acid layer indicates the presence of glycosides.

##### **Test for Anthraquinone Glycosides**

**Borntrager's test:** - Each of the **Solvent Extracts** was boiled with 1 ml of dilute tetraoxosulphate (VI) acid in a test tube for 5 min, filtered while hot. The filtrate or supernatant layer was pipette out and places into a test tube. The mixture was cooled and shaken with equal volumes of dichloromethane. The lower levels of dichloromethane was separated and shaken with half its volume with dilute ammonia. The appearance of a rose pink to red colour in the ammonical layer indicates the presence of glycosides.

##### **Test for Saponin Glycosides**

**Froth test:** - Each of the **Solvent Extracts** was treated with water in a semi-micro tube shaken well. The appearance of a persistent froth on the top of the mixture indicates the presence of glycosides.

#### **Tests for Amino acids and Proteins**

**Biuret test (General test):** - Each of the **Solvent Extracts** was treated with 1 ml 10% sodium hydroxide solution and heated. 2-3 drops of 0.7% copper (II) tetraoxosulphate (VI) solution was added to the mixture stirred and allowed to stand for few minutes. The formation of purplish violet colour may indicate the presence of proteins.

**Millions Test (for proteins):** -3 ml of each of the **Solvent Extracts** was mixed with 5 ml Million's reagent separately. The formation of white precipitate which on heating turned to brick red indicated the presence of amino acids.

**Xanthoproteic Test:** Each of the **Solvent Extracts** was placed in a test tube; 1ml of conc.  $H_2SO_4$  was added to the mixture and boiled for few minutes. 1ml of ammonia solution was added to the mixture. The formation of a white precipitate which on heating turned yellow and orange on addition of ammonia solution indicates the presence of proteins.

#### **Tests for Sterols and Triterpenoids**

##### **Libermann-Burchard test**

The each **Solvent Extracts** was treated with few drops of acetic anhydride boiled for few minutes. The mixture was cooled and concentrated tetraoxosulphate (VI) acid added down the side of the test tubes. A brown ring at the junction of two layers with the upper layer turning green indicates the presence of sterols while formation of deep red colour indicates the presence of triterpenoids.

##### **Salkowski's test**

Each of the **Solvent Extracts** was treated with chloroform with few drops of concentrated tetraoxosulphate (VI) acid, shaken well and allowed to stand for some time. The appearance of red colour in the lower layer indicates the presence of sterols while formation of yellow coloured lower layer indicates the presence of triterpenoids.

### **Tests for tannins and phenolic compounds**

#### **Ferric chloride test**

Small amount each of the **Solvent Extracts** was shaken with water and warmed. 2 ml of 5% ferric chloride solution was added and observed. The formation of green or blue colour indicates the presence of phenols.

#### **Gelatin test**

1% gelatin solution containing 10% sodium chloride was added to each of the **Solvent Extracts**. The formation of precipitate indicates the presence of tannins and phenolic compounds.

#### **Iodine test**

Each of the **Solvent Extracts** was treated with diluted iodine solution. The appearance of transient red colour indicates the presence of tannins and phenolic compounds.

#### **Nitric acid test**

Each of the **Solvent Extracts** was treated with dilute nitric acid separately. The formation of reddish to yellowish colour indicates the presence of tannins and phenolic compounds.

#### **Test for alkaloids**

About 500 mg of each of the **Solvent Extracts** was stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each filtrate was tested with the following reagents:

#### **Dragendroff's test**

Few drops of Dragendroff's reagent (solution of potassium bismuth oxonitrate iodide) was added to each filtrate and observed. The formation of orange yellow precipitate indicates the presence of alkaloids.

#### **Mayer's test**

Few drops of Mayer's reagent (Potassium mercuric iodide solution) was added to each filtrate and observed. The formation of white or cream colour precipitate indicates the presence of alkaloids.

#### **Hager's test**

Few drops of Hager's reagent (saturated aqueous solution of picric acid) was added to each filtrate and observed. The formation of yellow precipitate indicates the presence of alkaloids.

#### **Wagner's test**

Few drops of Wagner's reagent (solution of iodine in potassium iodide) was added to each filtrate and observed. The formation of reddish brown precipitate indicates the presence of alkaloids.

#### **Tests for flavonoids**

#### **Shinoda's test (Magnesium Hydrochloride reduction test)**

5ml. 95% ethanol was added separately to each of the **Solvent Extracts**. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCl. The formation of pink colour indicates the presence of Flavonoids.

#### **Alkaline reagent test**

Lead acetate solution was added a small quantity of each of the **Solvent Extracts** and observed. The appearance of yellow colour precipitates after few minutes indicates the presence of Flavonoids.

Results are shown in Table 3

### **3.4. MINERAL ANALYSIS**

#### **Sample Preparation**

Sample was thoroughly washed with pure water and rinsed with double distilled water in order to remove the sand or dust particles and all other surface contamination. The plant sample was then air dried, grounded and homogenized in an agate mortar and sieve through a 250µm diameter sieve. A quantity of 3.0g mass of the powdered sample was weighed with an analytical balance and placed in a sample cup holder.

#### **Sample Analysis**

Elemental analysis of the sample was performed with a Niton XL3t GOLDD + Hand held X-ray Fluorescence (Thermo Fisher). The Niton Hand held XRF Instrument uses Ag-anode X-ray tube with a voltage of 50kV and equipped with a Si-drift detector (SDD). Accurate energy and efficiency calibrations of the spectrometer were made using a certified reference material – SRM 1573a – Tomato Leaves supplied by the International Energy Agency

(IAEA), Vienna, Austria. The spectrum acquisition time was 480sec for the sample and the dead time was around 50%.

In this study, a total of fifteen elements (K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc) were determined in the dried powdered Leaves of *Allophylus africanus* plant by using EDXRF(Figure 3). The mean concentrations of various metals in the plant sample are shown in Table 4.



**FIGURE 3** EDXRF used for elemental analysis of powdered plant sample

### 3.5. EXTRACTION OF COMPOUNDS FROM SOLVENT EXTRACT OF THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

150 ml of distilled water was added to 18.5g of dried ethanol extract of powdered plant organs of *Allophylus africanus* and successively extracted with petroleum ether, acetone Chloroform and ethanol using a separating funnel and each of the extracts evaporated to dryness at room temperature.

Elemental analysis was carried out on Samples **LK003** for the presence of carbon, hydrogen, oxygen, nitrogen, sulphur and halogens using “The Middleton’s Test”. Acid Test, Phenol Test, Test for Aromaticity and Test for Unsaturation were carried on the sample isolated. The results are shown in Table 5

The compound isolated after characterization by wet chemical methods of analysis was sent to UK and USA for elemental analysis and Spectroscopic analysis with results shown in tables 6 - .

## 4. RESULTS AND DCUSSIONS

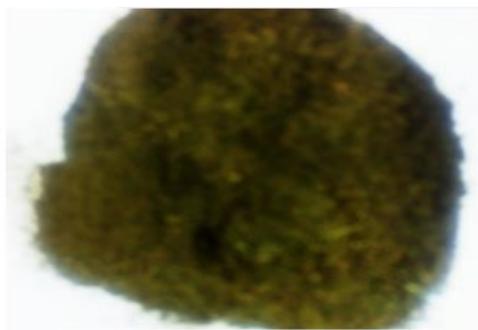
### 4.1. ORGANOLEPTIC EVALUATION OF THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

The results of organoleptic evaluation of the dried powdered Leaves of *Allophylus africanus* are reported in Table1 below with the photo of the dried powdered Leaves of *Allophylus africanus* shown in Figure 2

**Table 1:** Showing the results of organoleptic evaluation on the dried powdered Leaves of *Allophylus africanus*

PLANT ORGAN INVESTIGATED	PROPERTY TESTED				
	COLOUR	ODOUR	TASTE	TEXTURE	PARTICLE SIZE
Leaves	Light Brown	Wood odour	Slightly Bitter	Powdered	100 # wire gauge

The bitter taste indicated that the powdered plant materials contain alkaloids. The colour of the powdered plant material shown in Figure 2 will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration.



**FIGURE 2 : Powdered dry Leaves of *Allophylus africanus***

#### 4.2. RESULTS AND DISCUSSIONS OF FLUORESCENCE ANALYSIS OF CARRIED OUT ON THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

The results of fluorescent studies carried out on the dried powdered Leaves of *Allophylus africanus* using different chemical reagents are given in the Table 2 below;

**Table 2:** Results of fluorescence analysis of the dried powdered Leaves of *Allophylus africanus*

Test	Powdered plant material	Visible/day light	Ultra violet light
1	Powder	Brown	Brown
2	Powder + 1M NaOH(aq)	Brown	Light orange
3	Powder + 1M NaOH(alc.)	Brown	Bright orange
4	Powder + Ammonia	Orange	Bright orange
5	Powder + Picric acid	Light green	Yellow
6	Powder + Petroleum ether	Light Brown	Black
7	Powder + 50% HCl	Brown	Light blue
8	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Brown	Brown
9	Powder + 50% HNO <sub>3</sub>	Brown	Cream white
10	Powder + ethyl acetate	Brown	Brown
11	Powder + Ethanol	Light orange	Black
12	Powder + Methanol	Light orange	Black
13	Powder + Br <sub>2</sub> water	Light orange	Black

The above table showed a colour change in reagents like Powder + 1M NaOH(aq), Powder + 1M NaOH(alc.), Powder + Ammonia, Powder + 50% HCl, and Powder + 50% HNO<sub>3</sub>.

Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents as illustrated above.

Fluorescence analysis is one of the parameters for pharmacognostic evaluation of crude drugs [14] in traditional medicinal plants. Thus, the process of standardization can be achieved by stepwise pharmacognostic studies as stated above. This research work helps in identification and authentication of the dried powdered Leaves of *Allophylus africanus* plant material used in traditional medicine. Such information can act as reference information for correct identification of the dried powdered Leaves of *Allophylus africanus* plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituents which will help in maintaining the quality, reproducibility and efficacy of natural drugs.

### 4.3. RESULTS AND DISCUSSIONS OF PHYTOCHEMICAL SCREENINGS OF THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

The results of phytochemical screening carried out on the dried powdered Leaves of *Allophylus africanus* are shown in Table 3 below.

**Table 3:** Results of Phytochemical Screenings Results of Phytochemical Screenings

Experiment		Solvents					
Secondary Plant Metabolites	Tests/Reagents	PZ	AC	CHLO	MeOH	EtOH	Water
Carbohydrates	Molisch's Test	-	+	+	++	++	+++
	Fehling's Test	-	+	+	++	++	+++
	Benedict's Test	-	+	+	++	++	+++
	Barfoed's Test	-	+	+	++	++	+++
	Iodine Test	-	-	-	++	++	+++
Alkaloids	Mayer's Test	-	-	+++	+	+	++
	Hager's Test	-	-	+	+	+	++
	Wagner's Test	-	-	+	+	+	++
	Dragendroff's Test	-	-	++	+	+	++
Tannins and Phenolic Compounds	Iron(III)Chloride Test	-	++	++	+++	+++	+++
	Gelatin Test	-	++	++	+++	+++	+++
	Iodine Test	-	++	++	+++	+++	+++
	Dil.HNO <sub>3</sub> Test	-	++	++	+++	+++	+++
Flavonoids	Shinoda's Test	-	-	+	++	++	+++
	Lead acetate Test	-	+	+	++	++	+++
	KOH Test	-	-	+	++	++	+++
Sterols/Triterpenes	Liebermann-Burchard Test	-	-	-	+	++	++
	Salkowski's Test	-	-	-	+	++	++
Amino acids and Proteins	Biuret Test	+	-	+++	+	-	-
	Million's Test	+	-	+++	+	-	-
	Xanthoproteic test	+	-	+++	+	-	-
Glycosides and Saponins	Keller Kelliani Test	-	+	-	+	++	+++
	Borntrager's Test	-	+	-	+	++	+++
	Froth Test	-	+	-	+	+	+++

**KEY:** PZ = Petroleum ether, AC = Acetone, CHLO = Chloroform, MeOH = Methanol, EtOH = Ethanol; + + + = Intense; + + = Moderate; + = Slight; - = Absent

Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered leaves of *Allophylus africanus* plant used for treating pain, headaches, common cold, Fever and as antibiotics in Eastern Province of Sierra Leone was evaluated for the presence of secondary plant metabolites through phytochemical Screening. The results of evaluation according to Table 3, revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, proteins sterols/terpenes and saponins in the Ethanolic, methanol and aqueous extract. All of the solvent extracts revealed moderate concentration of Tannins and Phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated.

Alkaloids, Flavonoids, saponins, Sterols and Tannins are some of the identified secondary plant metabolites known to have pharmacologically active compounds [17], [18]. They play significant role in both traditional and modern medicines. Flavonoids are phenolic compounds found in plants are known to prevent the defoliation and loss of specialized function of the cells of a tissue or organ [19]. They are known to destroy fats and fibrous tissues in living organisms and prevent human degenerative diseases [20]. Flavonoids in plants are also reported as foods and are effective in reducing the changing of blood from a liquid to a solid state so as to prevent blood clot [20]. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic [21], Anti-inflammatory [21], [22], antioxidant [22], anti-microbial (antibacterial) [23], [24] antifungal [25], [26] and antiviral [25], [26], anti-cancer [22], [27] and anti-diarrhoeal activities [28].

Flavonoids have also been shown to inhibit topoisomerase enzymes [29], [30] and to induce DNA mutations in the Mixed-Linkage Leukemia (*MLL*) gene in *in vitro* studies [31].

Saponins as solution are haemolytic when injected into the blood stream of animals and therefore toxic intravenously. It has also been reported that Saponins cause abortion [32]. An example of Saponins, Yamogenin is widely used as starting material in the synthesis for birth control pills. Also, saponins are steroids and triterpene glycosides, so named because of their soap like properties [33]. Sterols are terpenes that have demonstrated medicinal property. Sterols are used for the synthesis of some birth control pills [34].

Tannins are administered internally to check diarrhoea and intestinal bleeding and also as antidotes for metabolic, alkaloids and glycoside poison by forming insoluble harmless precipitates. The detection of the above secondary plant metabolites supports the use of all the Traditional medicinal plants investigated in traditional medicine. The detection of the above secondary plant metabolites support the use of the plant in traditional medicine

#### 4.4. RESULTS AND DISCUSSIONS OF MINERAL ANALYSIS OF THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

The results of Mineral Analysis of the dried powdered leaves of *Allophylus africanus* are shown in Table 4.

**Table 4:** Showing the total contents of elements (in ppm) in the dried powdered Leaves of *Allophylus africanus*

Plant Organ	K	± SD	Ca	± SD	Mg	± SD	Al	± SD
Powdered leaves	24078	146.00	19273	168	4930	1119.00	2090	174.00
Plant Organ	Ti	± SD	V	± SD	Mn	± SD	Fe	± SD
Powdered leaves	103.00	11.00	< LOD	8.01	< LOD	16.35	371.48	11.53
Plant Organ	Cu	± SD	Zn	± SD	Rb	± SD	Sr	± SD
Powdered leaves	6.29	4.04	30.58	2.12	34.34	1.05	95.00	1.05
Plant Organ	Zr	± SD	Mo	± SD	Sc	± SD		
Powdered leaves	8.70	0.69	5.30	0.75	153.00	19.00		

The results of the current study as shown in Table 4 revealed that all the metals investigated (K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc) were accumulated in greater or lesser extent in the dried powdered Leaves of *Allophylus africanus* plant. The plant organ contained large amounts of nutrients and very rich in **K** (24078 ± 146.00 ppm), **Ca** (19273 ± 168 ppm), **Mg** (4930 ± 1119.00 ppm), **Al** (2090 ± 174.00 ppm) and **Fe** (371.48 ± 11.53 ppm). The other elements present in smaller quantities were **Sc** (153.00 ± 19.00 ppm), **Ti** (103.00 ± 11.00 ppm), **Sr** (95.00 ± 1.05 ppm), **Rb** (34.34 ± 1.05 ppm), **Zn** (30.58 ± 2.12 ppm), **Zr** (8.70 ± 0.69 ppm), **Cu** (6.29 ± 4.04 ppm) and **Mo** (5.30 ± 0.75 ppm). The other two elements **Mn** and **V** were out of limit of detection of the equipment. The presence of Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Zinc, Copper, Lead, Cadmium and Manganese in *Allophylus* has been reported [35], [36].

The above elements detected are essential components of biological structures that mediate vital effect on and play a key role in a variety of the biochemical processes necessary for life. Plant metabolites and a number of mineral

elements play important role in the metabolism [37]. They remain chelated with organic ligands and make them bioavailable to the body system [38], [39]. Excessive levels higher than that needed for biological functions of these elements can be toxic for human body health. The plant extract contained a large concentration of Potassium (24078  $\pm$  146.00 ppm) which has been reported to participate actively in the maintenance of the cardiac rhythm [37] and in constipation. Vartika and co-workers reported that the medicinal values of some plant species used in homeopathic system are due to the presence of Ca, Sr, Cu, Fe, Mg, K and Zn [40]. These elements take part in neurochemical transmission and serve as constituents of biological molecules and in a variety of different metabolic processes [41]. Determination of mineral elements in plants is very important since the quality of many foods and medicines depends upon the concentration and type of minerals present in plant organs [42]. A Zn concentration in the dried powdered seeds of *Allophylus africanus* plant was found to be 30.58  $\pm$  2.12 ppm. Zinc is the component of more than 270 enzymes [43] and its deficiency in the organism is accompanied by multisystem dysfunction. Zn is also responsible for sperm manufacture, fetus development and proper function of immune response [44]. Low levels of Zn can induce the pathogenesis of lung cancer [45], [46]. Breast cancer patients had low levels of Ca, Mg, Fe, Cu, Sr and Zn in their hair [47]. Therefore, it is of major interest to establish the levels of some metallic elements in common used plants because, at elevated levels, these metals could be dangerous and toxic [47], [48]. Iron deficiency has been reported in patients who suffer from a high burden of malaria and invasive non-typhoidal *Salmonella* (NTS) disease [49]. Iron supplementation was a common intervention until a large clinical trial in preschool children on the Tanzanian island Pemba, showed that routine iron and folic acid supplementation was associated with an increased risk for hospital admission and death [50]. In a sub-analysis of this trial in children whose baseline iron status was assessed, adverse events of iron and folic acid supplementation were more common in children without iron deficiency. In response, the World Health Organization (WHO) recommended to restrict iron supplementation to those children with proven iron deficiency [50], [51]. This indicates that the plant extract cannot be administered to children less than five years. The plant extract can only be used for adults.

#### 4.5. RESULTS OF SOXHLET EXTRACTION OF THE DRIED POWDERED LEAVES OF *ALLOPHYLUS AFRICANUS*

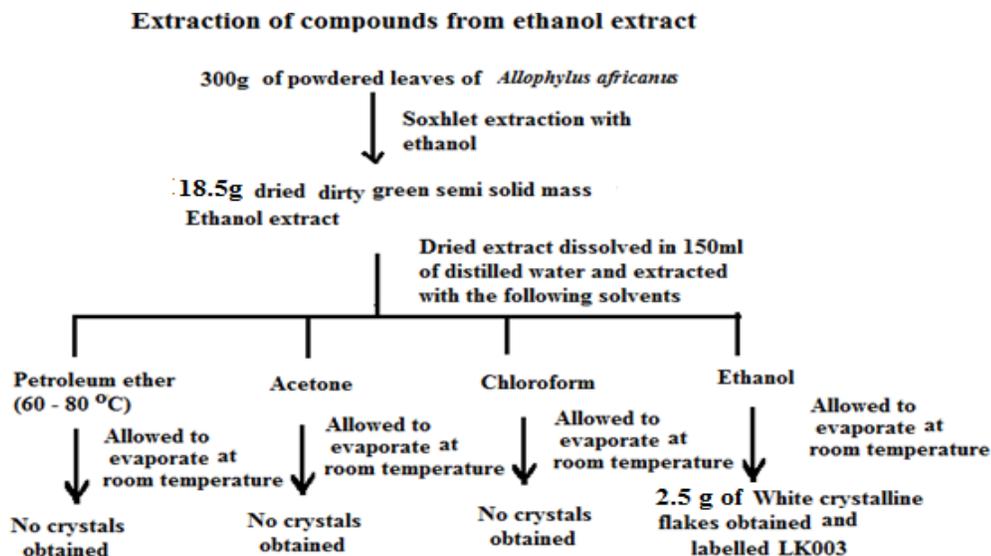
The results of the Soxhlet extraction of the dried powdered leaves of *Allophylus africanus* are reported in Table 5 and extraction of the **sample LK003** shown in figure 4.

**Table 5:** Showing the results of Soxhlet Extraction of the Dried Powdered Leaves of *Allophylus africanus*

Plant organs used	Solvent used	Mass of powdered plant material (grams)	Mass of solvent extract (grams)	Percentage of extractives (%)
Leaves	Petroleum ether	300.0	18.125	6.04
	Acetone	300.0	21.178	7.06
	Methanol	300.0	25.63	8.54
	Ethanol	300.0	29.27	9.77
	Ethanol: Water (50:50)	300.0	31.77	10.59

During the isolation of compounds, 150 ml of distilled water was added to 18.5g of dried ethanol extract of powdered plant organs of *Allophylus africanus* and successively extracted with petroleum ether, acetone Chloroform and ethanol using a separating funnel and each of the extracts evaporated to dryness at room temperature as shown in figure 4 below;

Pharmacognostic Investigation, Isolation, Characterization and Confirmation of The Structure of Phenacetine Extracted from The Dried Powdered Leaves of Traditional Medicinal Plant *Allophylus africanus* Used for The Treatment of Pain, Flu and Head Ache



**Figure 4.1:** Flow Chart for extraction of LK003 from *Allophylus africanus*

#### 4.6. RESULTS OF WET CHEMICAL METHODS OF ANALYSIS

Sample LK003 was extracted from the Ethanol extract of *Allophylus africanus* used traditionally for Headache, cold, fever and as antibiotic

Mass = 200mg

Nature = white crystalline flakes

Solubility: Slightly soluble in water, Ethanol, Chloroform

Taste = Bitter

**Table 6:** Results of Wet Chemical Analysis on Sample LK003

Test	Observation	Inference
a. Acid Test – Solutions of sample LK003 was tested with Litmus paper	Red litmus paper turned blue	Sample LK003 is basic
Sample + Ethanoic acid	Smell of ester observed	Contains OH group
Solution of Sample LK003 + NaHCO <sub>3</sub>	No reaction observed	Sample LK003 is not acidic
c. Phenol Test	No reaction observed	Sample LK003 does not contain Phenolic compound
Test for unsaturation	The colour of 0.1M KMnO <sub>4</sub> solution changes from purple to colourless	Sample LK003 is unsaturated
Test for aromaticity	smoky flame	Sample LK003 is aromatic
Carbohydrate		
Portion of Sample LK003 was strongly heated with in a boiling tube until no further change occurred. Gas + Lime water Liquid + CuSO <sub>4</sub>	Sample LK003 turned black with a colourless gas and droplets of colourless at the mouth of the test tube.	Probably carbohydrate present

	Turns lime water milky. Colour changes from white to blue	Presence of Carbon dioxide Presence of water Hence Sample LK003 contains Carbon, Hydrogen and Oxygen
The Middleton's test		
5mg of Sample LK003 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		
Test for cyanide ions	Specks of Prussian blue precipitated seen on the filter paper	Sample LK003 contained nitrogen atoms.
Test for sulphide ions	No visible reaction seen	Sulphide ions are absent.
Test for halides ions	No visible reaction seen	Halides ions are absent

Hence Sample LK003 is white powdered aromatic, slightly soluble in water, Ethanol and Chloroform. Tested positive for unsaturation contains the elements Carbon, Hydrogen, Oxygen and Nitrogen

#### 4.7. INSTRUMENTAL METHODS OF ANALYSIS OF SAMPLE LK003

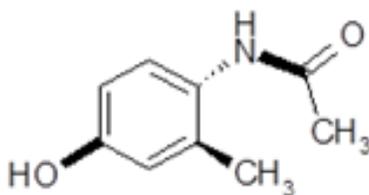
**Table 7:** Results of Elemental composition on Sample LK003

Symbol	Element	Atomic weight	Atoms	Mass percent
C	<u>Carbon</u>	12.0107	9	65.4379 %
H	<u>Hydrogen</u>	1.00794	11	6.7119 %
N	<u>Nitrogen</u>	14.0067	1	8.4792 %
O	<u>Oxygen</u>	15.9994	2	19.3710 %

**Expected Molecular Formula**  $C_9H_{11}NO_2$

**Molar Mass = 165.19 g/mol.**

**Proposed Structure of LK003**



**Figure 5** ∴ Proposed structure of Sample LK003

The above structure was characterized by its physical properties and by proton and LCMS/Mass Spectroscopic analysis shown below.

### Physical Characteristics

<b>Density:</b>	1.1±0.1 g/cm <sup>3</sup>
<b>Boiling Point:</b>	338.7±25.0 °C at 760 mmHg
<b>Vapour Pressure:</b>	0.0±0.7 mmHg at 25°C
<b>Enthalpy of Vaporization:</b>	58.2±3.0 kJ/mol.
<b>Flash Point:</b>	158.6±23.2 °C
<b>Index of Refraction:</b>	1.558
<b>Molar Refractivity:</b>	47.2±0.3 cm <sup>3</sup>
<b>Polar Surface Area:</b>	38 Å <sup>2</sup>
<b>Polarizability:</b>	18.7±0.5 10 <sup>-24</sup> cm <sup>3</sup>
<b>Surface Tension:</b>	39.7±3.0 dyne/cm
<b>Molar Volume:</b>	146.5±3.0 cm <sup>3</sup>

### 4.8. RESULTS OF PROTON NMR SPECTROSCOPY (USA) SAMPLE LK003

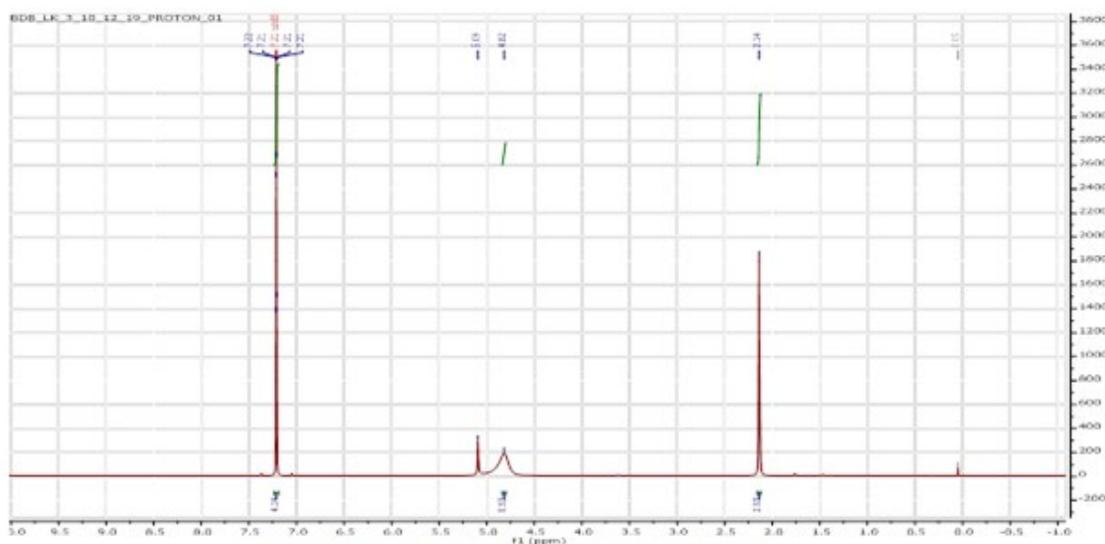


Figure 6 :: Proton NMR of Sample LK003

The <sup>1</sup>H NMR Spectrum for LK003 is worthy of some comments according to Figure 4.3.1

The interpretations of  $\delta$  - values (ppm) for <sup>a</sup>H - O, <sup>b</sup>H<sub>3</sub>C, <sup>d</sup>H -C= and <sup>c</sup>H -N shifts drawn above are shown below;

**Solvent peak = 7.21 - 7.22 ppm**

**<sup>a</sup>H - O - Ring = 5.09 ppm**

**<sup>b</sup>H<sub>3</sub> C — = 4.82 ppm**

**<sup>c</sup>H - N — = 2.14 ppm**

**<sup>d</sup>H - C<sub>6</sub>H<sub>5</sub> = 0.05 ppm**

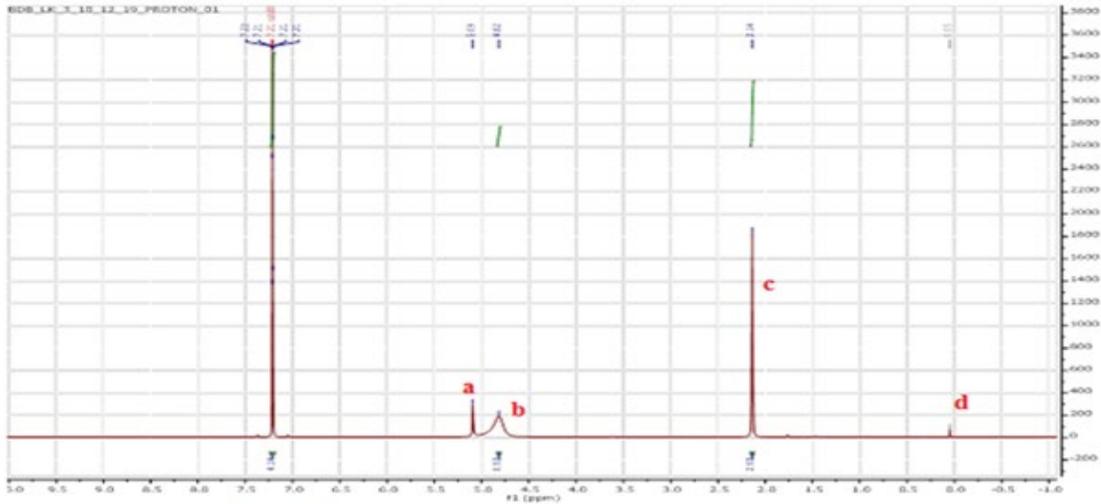


Figure 7.: Location of protons of molecular fragments on the NMR spectrum for sample LK003

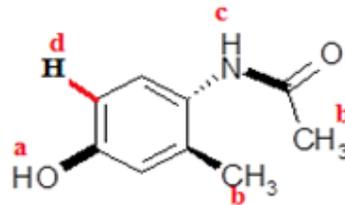


Figure 8.: Proposed structure of LK003

4.9. RESULTS OF LCMS/MASS SPECTROSCOPY OF SAMPLE LK003

Table 7: Number of Peaks used in obtaining Fragments of Sample LK003

Sample	Vial	ID	File	Date	Time	Description
4	1:12:00 AM	A4	MSQ3AB_15NOV2019SLK_003	11/15/2019	3:30:39 PM	EV-SLK_003



Figure 9.: Results of LCMS/Mass Spectroscopy of Sample LK003

**Table 8:** Number of Fragments obtained Peaks obtained from Sample LK003

Peak Number	Vial	Function	Trace	BPI	Area Abs.	Area %BP	Width	Height
1	1:12	1:MS ES+	MS ES+ :TIC	3.52E+06	2.00E+05	10.46	0.037	7494468
2	1:12	1:MS ES+	MS ES+ :TIC	3.94E+06	1.00E+06	65.37	0.06	38766248
3	1:12	1:MS ES+	MS ES+ :TIC	1.27E+06	8.00E+04	4.55	0.037	3533426.25
4	1:12	1:MS ES+	MS ES+ :TIC	1.09E+05	1.00E+04	0.66	0.03	697732.25
5	1:12	2:MS ES-	MS ES- :TIC	1.15E+05	3.00E+04	24.36	0.07	635660.313
6	1:12	2:MS ES-	MS ES- :TIC	9.67E+04	9.00E+03	6.38	0.037	507313.906
7	1:12	1:MS ES+	MS ES+ :TIC	1.88E+05	3.00E+04	1.72	0.047	1269256.5
8	1:12	1:MS ES+	DAD: 215	2.39E+04	4.00E+02	34.49	0.07	16308
9	1:12	1:MS ES+	MS ES+ :TIC	3.06E+06	4.00E+05	21.19	0.067	18695750
10	1:12	1:MS ES+	DAD: 215	1.26E+07	1.00E+03	100	0.053	60822
11	1:12	1:MS ES+	DAD: 215	1.28E+07	9.00E+01	7.74	0.033	5347.24
12	1:12	2:MS ES-	MS ES- :TIC	5.38E+03	2.00E+03	1.32	0.023	156521.281
13	1:12	1:MS ES+	DAD: 215	4.47E+04	1.00E+02	9.72	0.033	7093.2
14	1:12	1:MS ES+	DAD: 215	1.96E+04	9.00E+01	8.47	0.07	4025.9
15	1:12	1:MS ES+	DAD: 215	9.41E+03	6.00E+01	5.1	0.04	3152
16	1:12	1:MS ES+	DAD: 215	5.83E+04	1.00E+02	13.08	0.055	7886.54
17	1:12	1:MS ES+	MS ES+ :TIC	1.14E+05	3.00E+04	1.81	0.05	1495491.5
18	1:12	1:MS ES+	DAD: 215	7.76E+03	1.00E+02	10.91	0.053	6393
19	1:12	1:MS ES+	MS ES+ :TIC	1.07E+05	3.00E+04	1.73	0.053	1496777.625

The following results are obtained from nineteen peaks of Mass Spectroscopy with respect to the fragments that could be possibly obtained from Sample LK003 and by McLafferty Rearrangement with ID. NO of the MSQ3AB\_15NOV2019SLK\_003 are interpreted as shown below

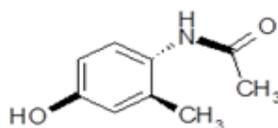
- OH = 17, - 2OH = 34, --3OH = 51, - CH<sub>3</sub> = 15, - 2CH<sub>3</sub> = 30, - 3CH<sub>3</sub> = 45, - 4 CH<sub>3</sub> = 60, - NH = 15, - 2NH = 30, - 3NH = 45, - C<sub>6</sub>H<sub>6</sub> = 78, - 2C<sub>6</sub>H<sub>6</sub> = 156, - 3C<sub>6</sub>H<sub>6</sub> = 234, - CH<sub>3</sub>CO = 43, - 2 CH<sub>3</sub>CO = 86, - 3 CH<sub>3</sub>CO = 129, M<sup>+</sup> + C<sub>6</sub>H<sub>5</sub>OH = 94, M<sup>+</sup> + C<sub>6</sub>H<sub>5</sub>OH = 188, M<sup>+</sup> + C<sub>6</sub>H<sub>5</sub>OH = 282, M<sup>+</sup> + HNC<sub>2</sub>H<sub>3</sub>O = 58, M<sup>+</sup> + HNC<sub>2</sub>H<sub>3</sub>O = 116, M<sup>+</sup> + HNC<sub>2</sub>H<sub>3</sub>O = 174

**Table 9:** Usual fragmentation pattern and by Maclerfty Rule corresponds to molecular ions in the various peak spectrums

Ion	Expected Molecular mass	Peak position	Actual Molecular Mass	Intensity
M <sup>+</sup>	164.19	666	164.69	18300
M <sup>+</sup> + OH	181.19	517	181.02	106537
M <sup>+</sup> + 2OH	198.19	434	198.3	9634.44
M <sup>+</sup> + 3OH	215.19	533	215.13	1952669
M <sup>+</sup> + - NH	181.19	517	181.02	106537
M <sup>+</sup> + - 2NH	198.19	434	198.3	9634.44
M <sup>+</sup> + - 3NH	215.19	533	215.13	1952669
M <sup>+</sup> + CH <sub>3</sub>	179.19	515	179.01	10359990.54
M <sup>+</sup> + 2CH <sub>3</sub>	194.19	942	194.93	3993.02
M <sup>+</sup> + 3CH <sub>3</sub>	209.19	441	209.17	18271.34
M <sup>+</sup> + 4CH <sub>3</sub>	224.19	677	224.59	276.89
M <sup>+</sup> + C <sub>6</sub> H <sub>6</sub>	242.19	969	242.9	5305.90
M <sup>+</sup> + 2C <sub>6</sub> H <sub>6</sub>	320.19	567	320.09	43017.29

M <sup>+</sup> + 3C <sub>6</sub> H <sub>6</sub>	398.19	1540	398.24	1333.69
M <sup>+</sup> + CH <sub>3</sub> CO	207.19	528	207.17	10271.04
M <sup>+</sup> + 2CH <sub>3</sub> CO	250.19	547	250.03	10368.67
M <sup>+</sup> + 3CH <sub>3</sub> CO	293.19	561	293.97	1558.2
M <sup>+</sup> + C <sub>6</sub> H <sub>5</sub> OH	258.19	552	258.16	37100
M <sup>+</sup> + 3C <sub>6</sub> H <sub>5</sub> OH	446.19	774	446.02	1190.90
M <sup>+</sup> + HNC <sub>2</sub> H <sub>3</sub> O	222.19	539	222.9	1048583
M <sup>+</sup> + 2HNC <sub>2</sub> H <sub>3</sub> O	280.19	463	280.19	3086.05
M <sup>+</sup> + 3HNC <sub>2</sub> H <sub>3</sub> O	338.19	1517	338.15	3360.39
M <sup>+</sup> + C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub>	256.19	552	258.16	37100
M <sup>+</sup> + 2C <sub>6</sub> H <sub>5</sub> OH	348.19	774	446.02	1190.90

The fragmentation patterns detected from the mass spectrometer corresponds to the fragments of the proposed structure of sample LK003 thus confirming the structure below.



**Figure 10.: Confirmed Structure of Sample LK003**

**IUPAC Name = N-(3-Methoxyphenyl) acetamide**

The structure of Sample LK003 is now identified as N-(3-Methoxyphenyl) acetamide commonly known as phenacetamide; a clinically tested drug. It has been reported that various species of *Allophylus* exhibited medicinally important phytochemicals such as 11 acetoxy-4 $\alpha$ -methoxy-eudesmane, Apigenin 8-c- $\beta$  rhamnopyranoside Carissone, Phenacetamide,  $\beta$ -Sitosterol, Rutin, Quercetin, Pinitol, Luteolin-7-O- $\beta$ -D-glucopyranoside, Apigenin-4'-O- $\beta$ -D-glucoside, sesquiterpenes, *phenacetamide*, L-quebrachitol and fatty acids as well as bioactivity of these compounds is proven by clinical experiments. All the plant organs of *Allophylus species* are used against wound healing fracture healing, cuts, ulcer healing [1, 2 and 7]. Thus the findings in this research work therefore supports the efficacy of the plant in providing cure for pain and fever in Sierra Leone.

## 5. CONCLUSION AND RECOMMENDATIONS

### 5.1. CONCLUSION

Pharmacognostic investigation involving organoleptic evaluation, fluorescent analysis, phytochemical screening and mineral analysis was carried out on the dried powdered leaves of *Allophylus africanus* used for treating pain, headaches, common cold, Fever and as antibiotics in Eastern Province of Sierra Leone. The powdered leaves were light brown in colour with woody odour and bitter taste. The bitter taste indicated that the powdered plant materials contain alkaloids. The colour of the powdered plant material also helps who so ever wish to buy and use the plant material for medicinal purpose to prevent adulteration

The powdered plant organ gave significant fluorescent derivatives with the reagents 1M NaOH (aq), 1M NaOH(alc.), Ammonia, 50% HCl, and 50% HNO<sub>3</sub> when viewed under ordinary visible light and ultraviolet light. The plant organ investigated during phytochemical screening gave positive for carbohydrates and reducing sugar, Amino acids and Proteins, Alkaloids, tannins and phenolic compounds, saponins glycosides Flavonoids, sterols/terpenes and triterpenes all of which have been reported to be pharmacologically active compounds responsible for the medicinal use of *Allophylus africanus* plant.

Elemental analysis of the plant organ was performed with a Niton XL3t GOLDD + Hand held X-ray Fluorescence (Thermo Fisher) and the results indicated that the plant organs investigated contained large amounts of **K, Ca, Mg**,

**Al** and **Fe** whilst **Ti, Zr, Mn, Zn, Sr, Sc, Rb, Cu, V** and **Mo** were present in minute quantities. The presence of the above elements also support the use of the plant organ investigated as food and medicine.

The compounds LK003 isolated from the dried powdered leaves of *Allophylus africanus* was characterized using both wet chemical and instrumental methods of analysis. The structure identified using fragmentation patterns from LCMS, Proton NMR spectroscopy and by McLafferty rearrangement as N-(3-methoxyphenyl) acetamide called phenacetamide. It has been reported to be a clinically tested drug for pain and fever. All the plant organs of *Allophylus species* have been reported to be used for pain, fever, wound healing, healing of fractures, cuts and ulcers. Hence, we concluded in this work that the presence of N-(3-methoxyphenyl) acetamide called phenacetamide in the dried powdered leaves traditional medicinal plant *Allophylus africanus* to be one active compound that is responsible for its use as a traditional pharmaceutical.

## 5.2. RECOMMENDATIONS

This research work has shown that the traditional medicinal plant *Allophylus africanus* contained carbohydrates, alkaloid, flavonoids, proteins sterols/terpenes and saponins and a good number of elements/minerals and N-(3-methoxyphenyl) acetamide called phenacetamide that is responsible for the analgesic effect of the plant. We therefore recommend the efficacy of the plant to be used in traditional medicine and as food/mineral supplement. Further research on this plant is required through antimicrobial sensitivity testing in order isolate the active compounds responsible for the antibacterial properties of the plant.

## SOURCES OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## CONFLICT OF INTEREST

The author have declared that no competing interests exist.

## ACKNOWLEDGMENT

The authors are grateful to Dr. Lukulay Sombay (UK) and Mr. Mohamed Barrie (USA) for LCMS/GCMS, elemental analysis and spectral analysis of Sample sent to them, Mr. Anthony F. Kamara, Department of Physics for carrying out elemental analysis of the dried powdered leaves of *Allophylus africanus* plant using EDXRF, Laboratory technicians of the Department of Chemistry, Fourah Bay College, University of Sierra Leone and the Principal Eastern Polytechnic, Kenema for providing financial assistance.

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