

Pharmacognostic Investigation of Dried Powdered Young Leaves of Traditional Medicinal Plant *Phyllanthus Muellerianus* Used for Treating Asthma In Sierra Leone

Lahai Koroma^{a & b}, T.B.R. Yormah^b, L.M. Kamara^b and G.M.T. Robert^c

^aDepartment of Basic and Environmental Sciences, Eastern Polytechnic, Kenema, Sierra Leone

^bDepartment of Chemistry, Fourah Bay College, University of Sierra Leone, Sierra Leone

^cDepartment of Chemistry, Njala University, Njala, Bo District, Sierra Leone

^aCorresponding author: E-mail: lahaikoroma2001@gmail.com

Abstract

Pharmacognostic and mineral analysis of dried powdered young leaves of traditional medicinal plant *Phyllanthus muellerianus* used for treating asthma in Sierra Leone has been carried out. The colour of the dried powdered leaves was brown, wood odour and had bitter taste indicating that the plant organ investigated contained alkaloids. The following reagents 1M NaOH (aq), 1M NaOH (alc.), Ammonia, 50% HCl and 50% HNO₃ gave fluorescent derivatives under U/V Lamp. Phytochemical screening revealed the presence of carbohydrates, alkaloid, flavonoids, sterols/terpenes and saponins, proteins and tannins in the chloroform, ethanol, methanol and aqueous extracts support the use of the plant in traditional medicine. Elemental analysis of the sample was performed with a Niton XL3t GOLDD + Hand held X-ray Fluorescence (Thermo Fisher) indicated that the plant organ investigated contained large amounts of nutrients rich in **K, Ca, Mg, Al** and **Fe**. The other elements present in smaller quantities were **Ti, Mn, Sc, Zn, Sr, Zr, Rb, V** and **Mo**. The elements **Mn, V** and **Cu** were not within the Limits of detection of the equipment. Essential elements play important roles in asthma genesis since they take part in oxidative stress reactions as cofactors of antioxidant enzymes. Zinc is an important trace element and its concentration is frequently used to evaluate inflammatory diseases in asthma genesis. Copper concentrations in human blood serum of bronchial asthma patients have been reported to be higher than in healthy individuals. In this research work **Zinc** concentration in the plant organ investigated was found to be higher than copper. Hence the high concentration of Zinc and low concentration of copper in the plant organ investigated support the use of the plant in traditional medicine for the treatment asthma. The incidence of asthma can be reduced or prevented with the administration of cold decoction of the dried powdered Leaves of *Phyllanthus muellerianus* plant or intake of zinc supplements

Keywords —Pharmacognostic, mineral analysis, asthma genesis, Soxhlet extraction and Fluorescence analysis

I. INTRODUCTION

The cold decoction of dried powdered leaves of traditional medicinal plant *Phyllanthus muellerianus* is drunk twice a day for treatment of asthma in eastern province of Sierra Leone.

Local Vernacular Names In Sierra Leone

MENDE: ŋŋNI-WULO-JALAKŋ

KONO: KUNDUNŌMŌE

Kissi: HŌLICHUINDA

Loko: BŌLAKHŋ-SURI-SURI

Phyllanthus muellerianus (Kuntze) Exell (Euphobiaceae) is widespread in the tropical region of West Africa. It occurs from Senegal and Guinea Bissau east to Sudan and Kenya and south to northern Angola and northern Mozambique [1, 2, 3 and 4]. It is a small plant that grows throughout the season in the forest areas with canopy-forming leaves. It has fruits that are copious panicles of small red, shining berries that eventually turn black when matured. It has been reported that the leaf extract of the plant cause contraction of rat ileum with the leaves, twigs, and fruits possessing antibacterial activity [5, 6]. *P. muellerianus* has been used as an herbal remedy in many parts of the world. In Guinea, the leaves are boiled with palm fruits and administered to women undergoing labour, the root is cooked with maize meal and used for treating chronic dysentery in Ghana, hot

decoction of roots and leaves for treatment of eruptive fever in Togo, Ivory Coast, and Zambia [7]. In other parts of Sierra Leone and southern Nigeria, the fresh juice of the plant is used to treat eye infections, skin diseases [7, 8], wounds, purgative, bronchitis and for relieving urethral discharges [7, 9, and 10]. In Burkina Faso the plant is used to treat sore throat, cough, pneumonia and enlarged glands. The presence of tannins, flavonoids, saponins, alkaloids and anthraquinones in the leaves and stem bark of *Phyllanthus muellerianus* plant has been reported. Antiplasmodial activity (IC₅₀ = 9.4 µg/ml), antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* of the plant has also been investigated and results reported [11]. The aqueous extract of the plant orally administered caused significant analgesia and anti-inflammatory properties on rats, chicks, cats and rabbits [12].

In view of the significance of this plant in the local treatment of various diseases, very little work has been carried out on the pharmacognostic investigation of dried powdered young leaves of traditional medicinal plant *Phyllanthus muellerianus*.

Trace elements 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. It has been reported that changes in serum zinc levels affect the management of asthmatic patients [13, 14] and was recommended that intake of zinc supplements increase the effect of antioxidant defence system thereby reducing the incidence of asthmatic attack in asthmatic patients [13, 14]. Hence any Pharmacognostic investigation of traditional medicinal plant *Phyllanthus muellerianus* without mineral analysis cannot be completed. Excessive levels higher than that needed for biological functions of these elements can be toxic for the human body health.



FIGURE 1: Photo of the Leaves of *Phyllanthus muellerianus*

II. MATERIALS AND METHODS

Collection and preparation of dried plant materials
Fresh Leaves of *Phyllanthus muellerianus* were harvested from the Gola Forest and sun-dried for 4-7 days. The Leaves were not dried on the ground, but on a protective cloth to minimize any microbial contamination. After drying, the Leaves were then reduced in size by crushing it into smaller pieces

using a cutlass, grounded using a laboratory mill and kept in a proper container until the time of the extraction.

The plants organ investigated is the dried powdered Leaves of *Phyllanthus muellerianus* with the image of the plant shown in **Figure 1**. A voucher specimen No. 405 of *Phyllanthus muellerianus* was deposited in the Herbarium of the Botany Department, Fourah Bay College (University of Sierra Leone). The powdered plant material was used to carry out the following analyses described below:

- Organoleptic evaluation
- Fluorescence analysis
- Phytochemical screening
- Mineral analysis

III EXPERIMENTAL

Organoleptic characters

Organoleptic evaluation was carried out by means of sense organs, which provided the simplest as well as quickest means of establishing the identity and purity to ensure quality of a particular drug present in traditional medicinal plants. Organoleptic characters investigated [15] are size, colour, odour, taste and texture of the dried powdered Leaves of *Phyllanthus muellerianus* plant. The results are shown in **Table 1** and the image of the dried powdered leaves of *Phyllanthus muellerianus* shown in Figure 3

Fluorescence analysis

10 mg of dried powdered Leaves of the plant organ investigated was placed in twelve (12) different petri dishes and 2-3 drops of freshly prepared test solutions of 1 N NaOH (aq), 1 N NaOH (alc.), Ammonia, Picric acid, Petroleum ether, 50% HCl, 50% H₂SO₄, 50% HNO₃, Ethyl acetate, Ethanol, Methanol, and Bromine water added, mixed gently with a glass rod and waited for few minutes.

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 [16] in different radiations are recorded as shown in Table 2.

Phytochemical analysis

Soxhlet extraction was carried out on the dried powdered Leaves of *Phyllanthus muellerianus* 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.

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 [17, 18] to give general idea regarding the nature of constituents present in each of the solvent extracts of the plant part investigated [19, 20, 21, 22, 23 & 24]. The secondary plant metabolites tested for are carbohydrates, reducing sugar, starch, saponins, proteins, Sterols/triterpenes, tannins, alkaloids and flavonoids. The procedures used below were carried out for each of the solvent extracts stored.

Test for Carbohydrates, reducing sugar and starch.

500 mg of each of the **Solvent Extract** was dissolved in 50 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates, reducing sugar and starch.

Test for Carbohydrates

The **Molisch's test** was used to test for carbohydrates

During the test 5 ml of each of the extract filtrate was treated with 3 drops of alcoholic α -naphthol solution in a test tube and 3 ml of concentrated tetraoxosulphate (VI) acid added carefully down the sides of the test tubes. The formations of violet/purple ring at the junction between the two liquids indicate the presence of carbohydrates.

Test for reducing sugars:

The **Fehling reagent** was used to test for reducing sugar. During the experimental work 5ml of each of the extract filtrate was treated in equal volumes with 2ml Fehling A and 2ml Fehling B solutions, boiled for one minute and then 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.

Iodine Test:

2-3 drops of iodine solution was added to 5 ml of each of the extract filtrates and observed.

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

Test for Saponin:

Froth test: - Each of the **Extract filtrates** was treated with water in a tube shaken vigorously. The appearances of a persistent froth on the top of the extract filtrates indicate the presence of saponins.

Test for Proteins:

The **Biuret test** is the general test used to detect the presence of proteins. During the test 5 ml each of the **Extract filtrates** was treated with 2 ml 10% sodium hydroxide solution and heated. 3-5 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.

Test for Sterols and Triterpenoids:

Libermann-Burchard test

During the test each of the **Extract filtrate** was treated with 5-6 drops of acetic anhydride and 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

During the test each of the **Extract filtrate** was treated with 3 ml of chloroform and 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:

Ferric chloride test

5 ml of each of the **Extract filtrate** was shaken with water and warmed. 2 ml of 5% Iron III chloride solution was added and observed. The formation of green or blue colour indicates the presence of tannins

Gelatin test

3ml of 1% gelatin solution containing 10% sodium chloride was added to each of the **Extract filtrate**. The formation of white buff coloured precipitate indicates the presence of tannins

Test for alkaloids

50mls of distilled water was added to 500 mg of each of the **Solvent Extracts** stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each of the **Extract filtrate** was tested with the following reagents:

Dragendroff's test

Few drops of Dragendroff's reagent was added to each **Extract filtrate** and observed. The formation of orange yellow precipitate indicates the presence of alkaloids.

Mayer's test

Few drops of Mayer's reagent was added to each **Extract filtrate** and observed. The formation of white or cream colour precipitate indicates the presence of alkaloids.

Tests for flavonoids:

20mls of distilled water was added to 50 mg of each of the **Solvent Extracts** stirred and filtered. Each of the **Extract filtrate** was tested with the following reagents:

Shinoda's test

5ml. 95% ethanol was added separately to each of the **Extract filtrate**. 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 **Extract filtrate** and observed. The formation of yellow precipitates after few minutes indicates the presence of Flavonoids.

Results are shown in **Table 3**

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 sieved through a 250µm diameter sieve. 3.0g mass of the powdered sample was weighed using 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%.

The XRDF equipment offers a fairly uniform detection limit across a large portion of the Periodic Table and is applicable to a wide range of concentrations. 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 investigated for their presence in the dried powdered young leaves of *Phyllanthus muellerianus* plant. The mean concentrations of various metals in the plant sample are shown in **Table 4**.



FIGURE 2: EDXRF used for elemental analysis of powdered plant sample

XRDF shown in **Figure 2** is one of the sensitive, rapid and simple analytical techniques used to study the essential element content of medicinal plants [25, 26]. Many trace elements play significant roles in various physiological and biochemical reactions in humans.

III. RESULTS AND DISCUSSIONS

Organoleptic evaluation

The results of organoleptic evaluation of the dried powdered young leaves of *Phyllanthus muellerianus* plant are reported in **Table1** below with the photo of the dried powdered young leaves of *Phyllanthus muellerianus* plant shown in **Figure 3**

Table 1: Showing the results of organoleptic evaluation of the dried powdered young leaves of *Phyllanthus muellerianus* plant

Plant Organ Investigated	Property Tested				
	Colour	Odour	Taste	Texture	Particle Size
Leaves	Brown	Wood odour	bitter	Powdered	100 # wire gauge

The bitter taste indicates that the powdered plant materials contain alkaloids. The colour of the powdered plant material shown in **Figure 3** will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration.



FIGURE 3: Powdered dry Leaves of *Phyllanthus muellerianus*

Fluorescence analysis

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

TABLE 2: Results of fluorescence analysis of the dried powdered Leaves of *Phyllanthus muellerianus*

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 ₂ SO ₄	Brown	Brown
9	Powder + 50% HNO ₃	Brown	Cream white
10	Powder + ethyl acetate	Brown	Brown
11	Powder + Ethanol	Light orange	Black
12	Powder + Methanol	Light orange	Black
13	Powder + Br ₂ water	Light orange	Black

Table 2 above showed the colour change in reagents 1M NaOH (aq), 1M NaOH (alc.), Ammonia, 50% HCl and 50% HNO₃. Some constituents showed 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 the components of the powdered plant material cannot fluoresce, they can be converted into fluorescent derivatives or decomposition products by applying different reagents as illustrated Table 2 above.

Fluorescence analysis is one of the parameters for the pharmacognostic evaluation of crude drugs [17] 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 *Phyllanthus muellerianus* plant material used in traditional medicine for the treatment of asthma. Such information can act as reference for correct Identification of the dried powdered Leaves of

Phyllanthus muellerianus plant. Further, it will act as a tool to detect adulterants and substituents in the dry powdered leaves of the plant which will help in maintaining the quality, reproducibility and efficacy of natural drugs.

Phytochemical Screenings

The results of phytochemical screening of the dried powdered Leaves of *Phyllanthus muellerianus* plant using standard phytochemical methods are reported in Table 3 below;

Table 3: Results of Phytochemical Screenings of the dried powdered Leaves of *Phyllanthus muellerianus* plant

Secondary Plant Metabolites	TEST	SOLVENTS					
	Tests/Reagents	PZ	AC	CHLO	MeOH	EtOH	Water
Carbohydrates	Molisch's Test	-	-	+	++	++	+++
Reducing Sugar	Fehling's Test	-	-	+	++	++	+++
Starch	Iodine Test	-	-	-	++	++	+++
Saponins	Froth Test	-	+	+	++	+++	+++
Proteins	Biuiret Test	-	-	-	+	+	+
Sterols/Triterpenes	Liebermann-Burchard Test	-	+	+	++	++	++
	Salkowski's Test	-	+	+	++	++	++
Tannins	Iron(III)Chloride Test	-	++	++	+++	+++	+++
	Gelatin Test	-	++	-	++	+	++
Alkaloids	Mayer's Test	-	-	-	++	+	++
	Dragendoff's Test	-	+	++	+	++	++
Flavonoids	Shinoda's Test	-	+	+	++	+++	+++
	Lead acetate 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 traditional medicinal plant *Phyllanthus muellerianus* used for treatment of asthma in Eastern Province of Sierra Leone was evaluated for the presence of secondary plant metabolites.

The results of phytochemical evaluation according to Table 3, revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, sterols/terpenes

and saponins in the chloroform, ethanol, methanol and aqueous extracts.

All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins. The petroleum ether extracts gave the least concentration of the phytoconstituents investigated while proteins were only slightly present in the ethanol, methanol and aqueous extracts.

The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

Mineral Analysis

The results of mineral analysis carried out on the dried powdered Leaves of *Phyllanthus muellerianus* plant using EDXRF are reported in Table 4 below;

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

Plant Organ	K	± SD	Ca	± SD	Mg	± SD	Al	± SD
Powdered leaves	19492	132.00	10798	129	5647.00	1054	1703	153.00
Plant Organ	Ti	± SD	V	± SD	Mn	± SD	Fe	± SD
Powdered leaves	129.00	14.00	? LOD	7.73	? LOD	14.56	330.18	10.93
Plant Organ	Cu	± SD	Zn	± SD	Rb	± SD	Sr	± SD
Powdered leaves	? LOD	4.39	53.27	2.41	22.81	0.68	26.93	0.62
Plant Organ	Zr	± SD	Mo	± SD	Sc	± SD		
Powdered leaves	26.80	0.73	6.08	0.74	55.00	14.00		

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 *Phyllanthus*

muellerianus plant. The plant organ contained large amounts of nutrients and very rich in **K** (19492 ± 132.00 ppm), **Ca** (10798 ± 129 ppm), **Mg**(5647.00 ± 1054 ppm), **Al** (1703 ± 153.00ppm) and **Fe** (330.18 ± 10.93 ppm). The other elements present in smaller quantities were **Ti** (129.00 ±ppm), **Mn** (126.34 ±ppm), **Sc** (55.00 ± 14.00 ppm), **Zn** (53.27 ± 2.41ppm), **Sr** (26.93 ± 0.62 ppm), **Zr** (26.80 ± 0.73 ppm), **Rb** (22.81 ± 0.68 ppm), **V** (11.99 ± ppm) and **Mo** (6.08 ± 0.74ppm).The elements **Mn**, **V** and **Cu** were not within the Limits of detection of the equipment.

Asthma is a multifactorial disease and its severity varies with the inflammatory grade. It is a respiratory disorder characterized by wheezing and usually of allergic origin. It has been reported that essential elements may play important roles in asthma genesis since they take part in oxidative stress reactions as cofactors of antioxidant enzymes [27]. Zn is an important trace element and its concentration is frequently used to evaluate inflammatory diseases [28]. Many researchers have reported that Zn deficiency can lead to a variety of complications, including growth retardation, delayed wound healing, chronic diarrhea, and increased susceptibility to infections [29]. Cu concentrations in serum of bronchial asthma patients have been reported to be higher than in healthy individuals [27, 30]. In this research work

Zn (53.27 ± 2.41 ppm) concentration in the plant organ investigated was much higher than copper which was not within the Limits of detection of the XRDF equipment used. This makes the plant organ investigated to support the use of the plant in traditional medicine for the treatment asthma. Hence the incidence of asthma can be reduced/prevented with the administration of zinc supplements.

SUMMARY

Organoleptic Evaluation was carried out on the dried powdered Leaves of *Phyllanthus muellerianus* plant used for treating asthma in Sierra Leone. The result indicate the colour of the dried powdered leaves to be brown, wood odour and has a bitter taste indicating that the plant organ investigated contained alkaloids. This preliminary investigation can be used in the identification of the powdered plant material to prevent adulteration.

The results of fluorescent studies carried out on the dried powdered Leaves of *Phyllanthus muellerianus* using different chemical reagents gave positive test with the reagents 1M NaOH (aq), 1M NaOH (alc.), Ammonia, 50% HCl and 50% HNO₃. This indicates that Some of constituents the powdered plant material were converted into fluorescent derivatives or decomposition products by the reagents which is one of the parameters for

the pharmacognostic evaluation of active drugs in traditional medicinal plants.

Phytochemical screening was carried out on of Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered leaves of the traditional medicinal plant *Phyllanthus muellerianus* used for treatment of asthma in Eastern Province of Sierra Leone for the presence of secondary plant metabolites using standard phytochemical methods.

The results revealed from moderate to high contents of carbohydrates, alkaloid, flavonoids, sterols/terpenes and saponins in the chloroform, ethanol, methanol and aqueous extracts.

All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins. The petroleum ether extracts gave the least concentration of the phytoconstituents investigated while proteins were only slightly present in the ethanol, methanol and aqueous extracts.

The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

The EDXRF equipment was used to determine the mineral content of the dried powdered Leaves of *Phyllanthus muellerianus* plant. The results indicate that the plant organ contained large amounts of nutrients rich in **K** (19492 ± 132.00

ppm), **Ca** (10798 ± 129 ppm), **Mg** (5647.00 ± 1054 ppm), **Al** (1703 ± 153.00 ppm) and **Fe** (330.18 ± 10.93 ppm). The other elements present in smaller quantities were **Ti** ($129.00 \pm$ ppm), **Mn** ($126.34 \pm$ ppm), **Sc** (55.00 ± 14.00 ppm), **Zn** (53.27 ± 2.41 ppm), **Sr** (26.93 ± 0.62 ppm), **Zr** (26.80 ± 0.73 ppm), **Rb** (22.81 ± 0.68 ppm), **V** ($11.99 \pm$ ppm) and **Mo** (6.08 ± 0.74 ppm). The elements **Mn**, **V** and **Cu** were not within the Limits of detection of the equipment.

It has been reported that essential elements may play important roles in asthma genesis since they take part in oxidative stress reactions as cofactors of antioxidant enzymes [27]. Zn is an important trace element and its concentration is frequently used to evaluate inflammatory diseases [13, 14 & 28]. Many researchers have reported that Zn deficiency can lead to a variety of complications, including growth retardation, delayed wound healing, chronic diarrhea, and increased susceptibility to infections [13, 14 & 29]. Cu concentrations in the blood serum of bronchial asthma patients have been reported to be higher than in healthy individuals [27, 30]. In this research work **Zn** (53.27 ± 2.41 ppm) concentration in the plant organ investigated was much higher than copper which was not within the Limits of detection of the XRDF equipment used. This makes the plant organ investigated to support the use of the plant in traditional medicine for the

treatment asthma. Hence the incidence of asthma can be reduced or prevented with the administration of zinc supplements.

Conclusion

Organoleptic evaluation, fluorescence, phytochemical screening and mineral analysis was carried out on the dried powdered leaves of the traditional medicinal plant *Phyllanthus muellerianus* used for treatment of asthma in Eastern Province of Sierra Leone. The results indicate the powdered plant material to be brown, with wood odour, bitter and contained alkaloids. This was substantiated by results of the phytochemical screening of the various solvent extract which tested positive for carbohydrates, alkaloid, flavonoids, tannins, sterols/terpenes and saponins in the chloroform, ethanol, methanol and aqueous extracts, while proteins were only slightly present in the ethanol, methanol and aqueous extracts. The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

Essential elements have been reported to play important roles in asthma genesis since they take part in oxidative stress reactions as cofactors of antioxidant enzymes. Zn is an important trace element and its concentration is frequently used to evaluate inflammatory diseases. Many researchers have reported that Zn deficiency can lead to a

variety of complications, including growth retardation, delayed wound healing, chronic diarrhoea, and increased susceptibility to infections. High the concentration of zinc in the blood serum of patients lowers the incidence of asthma. Also Cu concentrations in the blood serum of bronchial asthma patients have been reported to be higher than in healthy individuals. In this research work **Zn** (53.27 ± 2.41 ppm) concentration in the plant organ investigated was much higher than copper which was not within the Limits of detection of the XRDF equipment used. This makes the plant organ investigated to support the use of the plant in traditional medicine for the treatment asthma. Hence the incidence of asthma can be reduced or prevented with the administration of daily cold decoction of dried powdered leaves of traditional medicinal plant *Phyllanthus muellerianus* or the intake of zinc supplements.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Anthony J. Kamara, Department of Physics for carrying out elemental analysis of the dried powdered leaves of traditional medicinal plant *Phyllanthus muellerianus* 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

REFERENCES

- [1] **Arbonnier, M., 2004.** Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers GmbH, MNHN, Paris, France. 573 pp
- [2] **Burkill, H.M., 1994.** The useful plants of West Tropical Africa. 2nd Edition. Volume 2, Families E–I. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 636 pp.
- [3] **Chilufya, H. & Tengnäs, B., 1996.** Agroforestry extension manual for northern Zambia Regional Soil Conservation Unit, Nairobi, Kenya. 120 + 124 pp.
- [4] **Radcliffe-Smith, A., 1987.** Euphorbiaceae (part 1). In: Polhill, R.M. (Editor). Flora of Tropical East Africa. A.A. Balkema, Rotterdam, Netherlands. 407 pp
- [5] **Neuwinger, H.D., 2000.** African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany. 589 pp.
- [6] **Breytanbach JC, Malan SF (1989):** Pharmacological properties of *Cambretumzeyhari*. *South Afr J Sci* 85: 372–374.
- [7] **Fowler DG (2006):** Traditional fever remedies: A list of Zambian plants. Available at [http://www.giftshealth.org/ritam/news/Traditinal Fever remedies1.pdf](http://www.giftshealth.org/ritam/news/Traditinal%20Fever%20remedies1.pdf)

- [8] **Dalziel JM (1937):** *The Useful Plants of West Africa*. London, Crown Oversea Agent for the Colonies, p. 158.
- [9] **Chopra RN, Nayar SL, Chopra IC (1986):** *Glossary of Indian Medicinal Plants (Including Supplement)*. New Delhi, Council of Scientific and Industrial Research, CSIR Publications, pp. 235–286.
- [10] **Siram S, Patel MA, PatelKV, Punjani NH (2004):** *Compendium on Medicinal Plants*. Gujarat SA, ed Ahmedabad, India, Agricultural University, pp. 1–54.
- [11] **Doughari, J. H. & Sunday, D. (2008)** Antibacterial Activity of *Phyllanthus muellerianus*., *Pharmaceutical Biology*, 46:6, 400-405,
- [12] **Anuka, J.A., Yaro, A.H., Wannang, N.N., Ezenwanne, E.B. & Yakasai, I.A., (2005):** Some in vivo and in vitro studies of the aqueous leaf extract of *Phyllanthus muellerianus* (Euphorbiaceae) in laboratory animals. *Journal of Pharmacy & Bioresources* 2(2): 93–99.
- [13] **Aida M. Yousef, Ekramy Elmorsy (2017)** *Serum zinc level in bronchial asthma*, *Egyptian Journal of Chest Diseases and Tuberculosis*; Vol. 88 Issue 1, January 2017, Pages 1-4
- [14] **Nazila Arieace, Reza Farid, Fahimeh Shabestari, Mohamad Shabestari, and Farahzad Jabbari, (2016)** *Trace Elements Status in Sera of Patients with Allergic Asthma*, *Rep Biochem Mol Biol.* 2016 Oct; 5(1): 20–25
- [15] **Siddiqui, Hakim MA. (1995)** Format for the pharmacopoeia analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix), 24-25 January. New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995.
- [16] **Tatiya A, Surana S, Bhavsar S, Patil D, Patil Y. (2012)** Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. Tubers (Orchidaceae). *Asian Pac J Trop Disease* 2012; 2(Suppl 1):S50-55.
- [17] **Harborne JB. (1973)** ; *Phytochemical methods* Edn 2. London: Chapman & Hall, 1973.
- [18]. **Kokate CK. (1997)** *Practical Pharmacognosy*, Edn 4, Vallabh Prakashan, Delhi, 107-111, 1997.
- [19]. **Kokoski J, Kokoski R, Salma FJ. (1958):** Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J Am Pharm Ass* 1958; 47:715-717.
- [20]. **Zhao Z, Liang Z, Guo P. (2011):** Macroscopic identification of Chinese medicinal materials: Traditional experiences

- and modern understanding. *J Ethnopharmacol* 2011; 131:556-561.
- [21] **Khandelwal KR. (1995):** Practical Pharmacognosy, NiraliPrakashan, 1995, 149-155.
- [22] **Trease E.G. and Evans W.C.(1978):** *Pharmacognosy* 1978, 11th Edition, Balliere Tindall, London 115-222.
- [23] **Sazada S, Arti V, Ayaz A, Faraha J, Maheswari MK(2009)** : Preliminary Phytochemical analysis of Some Medicinal and Aromatic Plants. *Adv. In Biological Res.*, 2009; 3(5-6): 188-5.
- [24] **Kokate C.K., Purohit A.P. and Gokhale S.B. (2006):** *Pharmacognosy*. 34th Ed. 2006 NiraliPrakashan, Pune,
- [25] **Queralt I, Ovejero M, Carvalho ML, Marques AF, Liabres JM. (2005)** Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques. *X RaySpectrom* 2005; 34: 213-217.
- [26] **Shendkar CD, Chandrachood PS, Pawar AB, Lavate SM, deshpande NR. (2011)** Quantitative estimation of macro, micronutrients and trace elements by X-ray fluorescence spectroscopy (XRF) from *Achyranthesaspera* Linn. *Int J Chem Tech Res* 2011; 3(2) : 610-613.
- [27] **Guo CH, Liu PJ, Lin KP, Chen PC. Nutritional supplement therapy improves oxidative stress, immune response, pulmonary function, and quality of life in allergic asthma patients: an open-label pilot study. Alternative medicine review. 2012;17(1):42-56.**
- [28]. Vural H, Uzun K, Uz E, Kocyigit A, Cigli A, Akyol O, et al. Concentrations of copper, zinc and various elements in serum of patients with bronchial asthma. *Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements*. 2000;14(2):88-91.
- [29] el-Kholy MS, Gas Allah MA, el-Shimi S, el-Baz F, el-Tayeb H, Abdel-Hamid MS, et al. Zinc and copper status in children with bronchial asthma and atopic dermatitis. *The Journal of the Egyptian Public Health Association*.1990;65(5-6):657-68.
- [30] **Lifschitz C. New actions for old nutrients. *TechnologiaAlimentaria*. 2012;11(2):1837-192.**