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ORGANOLEPTIC EVALUATION, FLUORESCENCE ANALYSIS, PHYTOCHEMICAL SCREENING AND MINERAL ANALYSIS OF DRIED POWDERED LEAVES OF TRADITIONAL MEDICINAL PLANT *ALTERNANTHERA SESSILIS* USED FOR THE TREATMENT OF FUNGAL INFECTION IN SIERRA LEONE

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ABSTRACT

Organoleptic evaluation, Fluorescence analysis, Phytochemical screening and Mineral analysis has been carried out on the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* used for the treatment of fungal infection in Sierra Leone. The brown colour, grass odour and bitter taste of dried powdered Leaves of the plant helps in identification and prevent adulteration of the dried powdered form of the plant organ during organoleptic evaluation. The bitter taste indicated that alkaloids are present in the leaves of the traditional medicinal plant *A. sessilis* thus supporting its use in traditional medicine in providing remedy for fungal infection. The powdered plant organ also gave fluorescent derivatives with NaOH solution, ammonia solution, 50% HCl and 50% HNO_3 when viewed under UV/Lamp Model UVGL-58 confirming the presence crude drugs in the plant organ investigated. The results of Phytochemical screening of the Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the plant organ investigated revealed high contents of carbohydrates, alkaloids, flavonoids, proteins sterols/terpenes and saponins in the ethanolic, methanol and aqueous extracts. All of the solvent extracts revealed intense concentration of Tannins and Phenolic compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents. The detection of the above secondary plant metabolites support the use of the plant as food and as traditional pharmaceutical. Elemental analysis was carried out on the plant organ investigated using with a Niton XL3t GOLD + Hand held X-ray Fluorescence (Thermo Fisher). The Niton Hand held XRF Instrument uses aAg-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% and the following results obtained; K (56714 ± 250.00 ppm), Ca (21372 ± 218.00 ppm), Mg (6333.00 ± 2084 ppm), Al (7266 ± 378.00ppm) and Fe (3655.20 ± 31.11 ppm) The other elements present in smaller quantities were Ti (592.00 ± 18.00 ppm), Zr (207.21 ± 1.65 ppm), Mn (167.42 ± 14.68 ppm), Zn (88.52 ± 3.19 ppm), Sc (79.00 ± 15.00 ppm), Sr (73.45 ± 1.02 ppm), Rb (63.53 ± 1.10 ppm), Cu (34.78 ± 4.83 ppm) V (12.90 ± 7.85 ppm)and Mo (4.92 ± 0.90 ppm). The above elements have been reported to play great role in metabolic processes in humans thus preventing various types of mineral deficiency diseases that could be associated with skin eruptions and degenerative diseases. Copper and zinc reported in this research work are the two main elements that provide curing for fungal infection in humans.

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INTRODUCTION

Alternanthera sessilis Linn is green leafy vegetable plant in Sierra Leone. The genus Alternanthera, a medicinally important member of family Amaranthaceae is reported to contain volatile constituents, essential amino acids, flavonoids, glycosides and steroids (The wealth of India: Raw Materials, 2004).

The cold decoction of dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis*is used for the treatment of fungal infection in Sierra Leone. The dried powdered leaves of *Alternanthera sessilis* is mixed with roasted and pounded Beni seeds (*Sesamum indicum)* and salt. One tea spoon of the mixture is put into a cup of tea or wine to induce or increase male virility (erection) (Lebbie Aiah, 1995).

Alternanthera sessilis has been reported to be used internally against intestinal inflammation, externally to treat wounds, hepatitis, tight chest, asthma bronchitis, lung trouble, to stop bleeding and as a hair tonic (Mrinmay Das, 2014). Young shoots and leaves are eaten as vegetable in Southeast Asia (4).

Local vernacular names in Sierra Leone

Mende: Ndatawulo

Alternanthera sessilis is a branched, glabrous, succulent herb and leaves are simple or pinnately compound. The plant is accredited with galactagogue properties, good fodder for increasing the flow of milk in the cattle and also used to treat night blindness (Bhaskar Rao *et al*., 2011). Elsewhere, in the world (i.e. Sri Lanka China, Taiwan and India) the plant is used as food, in traditional medicine (6, 7, 8, and 9).and in Ayurvedic medicine (Shyamala *et al*., 2005). The leaves and shoots of the plant are boiled and drunk as an antihypertensive remedy and for antidiabetic activity (Acharya, 2006; Erna C Arollado and Marina O Osi, 2010). *A. sessilis* is also reported to possessed anti-microbial, molluscicidal, a moderate antimutagenic, anti-diarrheal, hepatoprotective, cytotoxic, haematinic activity and antiviral activities (Devi, 2003; Hossain *et al*., 2014 14). Very little or no investigation has been reported with regard to the mineral content of *A. sessilis.*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. Excessive levels higher than that needed for biological functions of these elements can be toxic for the body health. Hence any Pharmacognostic investigation of traditional medicinal plants without mineral analysis cannot be completed.

MATERIALS AND METHODS

Collection and preparation of dried plant materials: Fresh Leaves of *Alternanthera sessilis* were harvested from the Gola Forest and sun-dried for 4-7 days. The plant organ harvested was not dried on the ground, but on a protective cloth to minimize any microbial contamination. Before drying, the Leaves were then reduced in size by crushing into smaller pieces using a knife. After the plant material had been dried, it was each grounded using a laboratory mill and kept in a proper container until the time of the extraction.

The plants organ investigated is Leaves of *Alternanthera sessilis* with the image of the plant shown in Figure 1. A voucher specimen No. 409 of *Alternanthera sessilis* 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

Experimental

Organoleptic characters: Organoleptic evaluation was carried out on dried powdered Leaves of *Alternanthera sessilis* by means of sense organs, which provided the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Organoleptic characters investigated **(**Siddiqui, 1995**)** were size, colour, odour, taste and texture of the dried powdered Leaves of *Alternanthera sessilis*.

The results are shown in Table 1 and the image of the dried powdered Leaves of *Alternanthera sessilis* shown in Figure 2. *Fluorescence analysis:* 0.5mg of dried powdered Leaves of *Alternanthera sessilis* was placed in a glass petri dish free from grease and 2-3 drops freshly prepared reagent solution added, mixed by gentle with a glass rod and waited for few minutes. The following freshly prepared reagents used are; 1 N NaOH (aq), 1 N NaOH (alc), Ammonia, Picric acid, Petroleum ether, 50% HCl, 50% H_2SO_4, 50% HNO_3, Ethyl acetate, Ethanol, Methanol, and Bromine water. The colours of each of the contents in glass Petri dish were observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations using a UV lamp Model UVGL-58. 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 (Kokoski, 1958) in Table 2.

Phytochemical analysis: Soxhlet extraction was carried out on the dried powdered Leaves of *Alternanthera sessilis* using solvents of increasing polarity (i.e. Petroleum ether (60-80 ^{o} C), Acetone, Chloroform Methanol, 95% Ethanol and Water. Each of the solvent extract was concentrated, reduced to a semisolid mass using a Rotary Evaporator at 50^{o}C and kept is special containers for phytochemical screening. During Phytochemical screening, each of the Solvent Extract was tested for the various classes of secondary plant metabolites. The methods used for detection of the various phytochemicals were followed by qualitative chemical test and by standard procedures **(**Hossain *et al*., 2014; Siddiqui, Hakim, 1995) to give general idea regarding the nature of constituents present in each of the solvent extracts of the plant organ investigated (Kokoski *et al*., 1958; Tatiya *et al*., 2012; Harborne *et al*., 1973; Kokate, 1997; Zhao, 2011; Khandelwal, 1995; Trease, 1978). 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 Extract 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 filtrate was treated with 2 drops of alcoholic α-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.

Figure 1. Photo of the plant with leaves and flowers of *Alternathera sessilis*

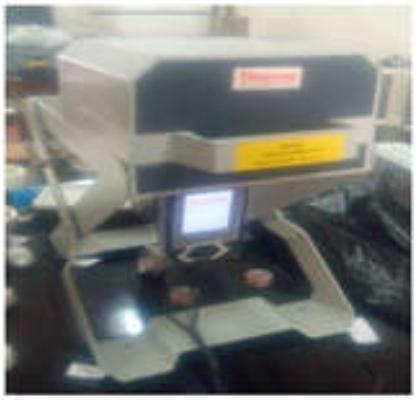

Figure 2. EDXRF used for elemental analysis of powdered plant sample

Figure 3. Dry powdered leaves of *Alternanthra sessilis*

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. The result is positive if 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 Saponins

Froth test:

Each of the **Solvent Extracts** was treated with water in a test tube and shaken vigorously. The appearance of a persistent froth on the top of the mixture indicates the presence of saponins.

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 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.

Tests for Sterols and Triterpenoids

Libermann-Burchard test: Theeach **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 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 and phenolic compounds

Ferric chloride test: Small amount each of the Solvent Extracts was shaken with water and warmed in a water bath. 2 ml of 5% ferric chloride solution was added and observed. The formation of green or blue colour indicates the presence of tannins/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.

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.

Table 1. Showing the results of organoleptic evaluation on the dried powdered Leaves of *Alternanthera sessilis*

Plant Organ Investigated	Property Tested				
	COLOUR	ODOUR	TASTE	TEXTURE	PARTICLE SIZE
Leaves	Brown	grass odour	Bitter	Powdered	100 # wire gauge

Table 2. Showing the results of fluorescence analysis on the dried powdered Leaves of *Alternanthera sessilis*

Test	Powdered plant material	Visible/day light	Ultra violet light
1	Powder	Light brown	Light brown
2	Powder + 1M NaOH(aq)	Light brown	Light orange
3	Powder + 1M NaOH(alc.)	Light brown	Bright orange
4	Powder + Ammonia	Light green	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_2SO_4	Brown	dark green
9	Powder + 50% HNO_3	Brown	Cream white
10	Powder + ethyl acetate	Brown	Brown
11	Powder + Ethanol	Light brown	Black
12	Powder + Methanol	Light brown	Black
13	Powder + Br_2 water	Light orange	Black

Table 3. Showing the results of Phytochemical Screening on the dried powdered Leaves of *Alternanthera sessilis*

Experiment		Solvents					
Secondary Plant Metabolites	Tests/Reagents	PZ	AC	CHLO	MeOH	EtOH	Water
	Molisch's Test	-	+	+	+	++	++
Carbohydrates	Fehling's Test	-	+	+	+	++	++
	Benedicts Test	-	+	+	+	++	++
	Barfoed's Test	-	+	+	+	++	++
	Iodine Test	-	-	+	+	++	++
	Mayer's Test	-	-	-	+	++	++
Alkaloids	Hager's Test	-	-	-	+	++	++
	Dragendroff's Test	-	-	-	+	++	++
Tannins and Phenolic	Iron(III)Chloride Test	-	+	+	++	++	++
Compounds	Gelatin Test	-	+	+	++	+++	+++
	Shinoda's Test	-	-	+	+	+	++
Flavonoids	Lead acetate Test	-	-	+	+	+	+++
Sterols/Triterpenes	Libermann-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

Table 4. Showing the total contents of elements (in ppm) in the dried powdered Leaves of *Alternanthera sessilis*

Plant Organ	K	± SD	Ca	± SD	Mg	± SD	Al	± SD
Powdered leaves	56714	250.00	21372	218.00	6333.00	2084	7266	378.00
Plant Organ	Ti	± SD	V	± SD	Mn	± SD	Fe	± SD
Powdered leaves	592.00	18.00	12.90	7.85	167.42	14.68	3655.20	31.11
Plant Organ	Cu	± SD	Zn	± SD	Rb	± SD	Sr	± SD
Powdered leaves	34.78	4.83	88.52	3.19	63.53	1.10	73.45	1.02
Plant Organ	Zr	± SD	Mo	± SD	Sc	± SD		
Powdered leaves	207.21	1.65	5.78	0.90	79.00	15.00		

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.

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. *Lead acetatetest:* 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 of the various tests 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 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 a 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%. X-Ray Fluorescence has long been recognized as a powerful technique for the qualitative and quantitative elemental analysis **(**Sazada, 2009; Kokate *et al*., 2006**)**. It has the advantage of being non-destructive, multi-elemental, fast and cost-effective. Furthermore, it 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 Leaves of *Alternanthera sessilis* plant using EDXRF. The mean concentrations of various metals in the plant sample are shown in Table 4. *Organoleptic evaluation* of the dried powdered Leaves of *Alternanthera sessilis.* The results of organoleptic evaluation of the dried powdered Leaves of *Alternanthera sessilis* are shown in Table1 with the photo of the dried powdered leaves shown in Figure 2. The bitter taste indicates that the dried powdered Leaves of *Alternanthera sessilis* 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 dried powdered Leaves of *Alternanthera sessilis* for medicinal purpose. It helps prevent adulteration. *Fluorescence analysis of powdered plant materials of Alternanthera sessilis*

The results of fluorescent studies carried out on the dried powdered Leaves of *Alternanthera sessilis* using different chemical reagents are given in the Table 2 below; The above Table showed colour changes in reagents, Powder + 1M NaOH(aq), Powder + 1M NaOH(alc.), Powder + Ammonia, Powder + 50% HCl, and Powder + 50% HNO_3. 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 fluorescence, they are converted into fluorescence derivatives or decomposition products by applying different reagents as reportedin Table 2 above. Fluorescence analysis is one of the parameters for pharmacognostic evaluation of crude drugs **(**Hossain *et al*., 2014**)** 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 *Alternanthera sessilis* used in traditional medicine. Such information can act as reference information for correct identification of the dried powdered Leaves of *Alternanthera* ***sessilis*** 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. *Phytochemical Screening on the dried powdered Leaves of Alternanthera sessilis.* The results of phytochemical screening using various test reagents for detecting various secondary plant metabolites on the dried powdered Leaves of *Alternanthera sessilis* are given in Table 3 below;

Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* used for the treatment of fungal infection in Sierra Leone was evaluated for the presence of secondary plant metabolites. The Phytochemical 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 intense concentration of Tannins and Phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated. The presence of carbohydrates, proteins and flavonoids support the use of the leaves of the plant as food. The detection of the other secondary plant metabolites supports the use of the plant in traditional medicine. *Mineral analyses on the dried powdered Leaves of Alternanthera sessilis.* The results of mineral analysis of the dried powdered Leaves of *Alternanthera sessilis* as shown in Table 4. 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 *Alternanthera sessilis* plant. The plant organ contained large amounts of nutrients and rich in K (56714 ± 250.00 ppm), Ca (21372 ± 218.00 ppm), Mg (6333.00 ± 2084 ppm), Al (7266 ± 378.00ppm) and Fe (3655.20 ± 31.11 ppm). The other elements present in smaller quantities areTi (592.00 ± 18.00 ppm), Zr (207.21 ± 1.65 ppm), Mn (167.42 ± 14.68 ppm), Zn (88.52 ± 3.19 ppm), Sc (79.00 ± 15.00 ppm), Sr (73.45 ± 1.02 ppm), Rb (63.53 ± 1.10 ppm), Cu (34.78 ± 4.83 ppm) V (12.90 ± 7.85 ppm) and Mo (4.92 ± 0.90 ppm).

Potassium participates actively in the maintenance of the cardiac rhythm (Nayak *et al*., 2007) and in constipation. Calcium plays a great role in the prevention or treatment of pre-eclampsia (Bucher, 1996), colon cancer (Garland *et al*., 1991), or hypertension (McCarron, 1997). Zinc has been reported to be an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It stabilizes the molecular structure of cellular components and membranes and contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in fundamental activities probably accounts for the essentiality of zinc for all life forms. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and hormonal immunity (Hambridge, 1987). It has also been reported that severe zinc deficiency in humans causes growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (Shankar, 1998; Agett, 1993; Goldenberg, 1995; Brown *et al*., 1998; Beck, 1997). Iron is reported to exhibit several vital functions in the body. It serves as a carrier

of oxygen to the tissues from the lungs by red blood cell Haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The physiology of iron has been extensively reviewed (Bothwell, 1979; Hallberg, 1982; Brock *et al*., 1994; Kühn, 1996; Mascotti *et al*., 1995). Mg has been reported to be a cofactor in formation of more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, blood pressure regulation in humans (Rude, 2010; Rude, 2012), energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione (Romani, 2013). It protects mitochondria, which is the storehouse of energy, from the dangerous oxidants (Abbott, 1992), transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm (Rude, 2012) andparticipates actively in the maintenance of the cardiac rhythm (Martin *et al*., 1985) and in constipation.

In terms of providing cure for fungal infections, copper has been reported to play important role at the host-pathogen axis during infection (Sarela Garcia-Santamarina, 2015). The effect of how the host uses either Cu compartmentalization within innate immune cells or Cu sequestration in other infected host niches such as in the brain to combat fungal infections has been investigated. It was reported that the host manipulate Cu-dependent processes at the host-pathogen axis for prevention of the growth of fungi which could lead to antifungal drug development (Sarela Garcia-Santamarina, 2015). It has also been reported that the host innate immune cells concentrate Cu in the vicinity of invading pathogens, as a means to exploit Cu toxicity and enhance microbial killing (Ladomersky, 2015; Hodgkinson, 2012). Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy. Zn is vital for the healthy working of many of the body's systems; it plays an essential role in numerous biochemical pathways. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection (Osredkar, 2011; Prasad, 2003; Plum *et al*., 2010; Burjonrappa *et al*., 2012). The lack of zinc causes postular dermatitis, diarrhea, and nail dystrophy. Irritability and emotional disturbances are due to atrophy of the brain cortex. The severity of the disease has been reported to be proportional to the zinc level. Before zinc supplementations acrodermatitis enteropathica was fatal to babies born with it (Wang, 2005; Hambidge, 1989). Zinc supplementation has been reported to provide effective treatment for acrodermatitis enteropathica, an inborn error of zinc metabolism that is inherited as an autosomal recessive disorder.

Summary

Organoleptic evaluation, Fluorescence analysis, Phytochemical screening and Mineral analysis has been carried out on the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* used for the treatment of fungal infection in Sierra Leone. Apart from the use of the plant to prevent and cure fungal infection, *A. sessilis*is also reported to induce or increase male virility (erection) (Lebbie Aiah, 1995), intestinal inflammation, externally to treat wounds, to treat hepatitis, tight chest, asthma bronchitis, lung trouble, to stop bleeding, galactagogue treat night blindness (Bhaskar Rao *et al*., 2011), antihypertensive remedy and for antidiabetic activity (Acharya, 2006; Erna C Arollado and Marina O Osi, 2010) and as a hair tonic (Mrinmay Das, 2014). *A. sessilis* possessed anti-microbial, molluscicidal, a moderate antimutagenic, anti-diarrheal, hepatoprotective, cytotoxic, haematinic activity and antiviral activities (Devi *et al*., 2003; Hossain *et al*., 2014). Young shoots and leaves are eaten as vegetable in Southeast Asia (Scher, 2004; Chandrika *et al*., 2006; Khan Tabassum *et al*., 2014; Anitha *et al*., 2012; Sheela *et al*., 2014). The results of organoleptic evaluation as shown inTable1 and Figure 3 indicate the colour of the dried powdered leaves of*A. sessilis* to be brown, grass odour and had a bitter taste. The bitter taste indicated that alkaloids are present in the leaves of the traditional medicinal plant *A. sessilis* supporting its use in traditional medicine in providing remedy for the above reported diseases. Fluorescence investigation carried out on dried powdered leaves of *A. sessilis* using different chemical reagents gave positive results withthe reagents1M NaOH(aq), 1M NaOH(alc.), Ammonia, 50% HCl, and 50% HNO_3 as shown in Table 2, being one of the parameters for pharmacognostic evaluation and detection of crude drugs (Hossain *et al*., 2014) in traditional medicinal plants. The ultra violet light produced fluorescence in the powdered plant material which was not visible in daylight when treated a particular set of reagents as shown in Table 2. Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* used for the treatment of fungal infection in Sierra Leone was evaluated for the presence of secondary plant metabolites. The results 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 intense concentration of Tannins and Phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated. The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

Elemental/mineral analysis of the dried powdered Leaves of *Alternanthera sessilis* plant was investigated for the presence of K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc. The results 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 *Alternanthera sessilis* plant. The plant organ contained large amounts of nutrients and rich in K (56714 ± 250.00 ppm), Ca (21372 ± 218.00 ppm), Mg (6333.00 ± 2084 ppm), Al (7266 ± 378.00 ppm) and Fe (3655.20 ± 31.11 ppm). The other elements present in smaller quantities are Ti (592.00 ± 18.00 ppm), Zr (207.21 ± 1.65 ppm), Mn (167.42 ± 14.68 ppm), Zn (88.52 ± 3.19 ppm), Sc (79.00 ± 15.00 ppm), Sr (73.45 ± 1.02 ppm), Rb (63.53 ± 1.10 ppm), Cu (34.78 ± 4.83 ppm) V (12.90 ± 7.85 ppm)and Mo (4.92 ± 0.90 ppm). Potassium participates actively in the maintenance of the cardiac rhythm (Siddiqui *et al*., 1995; Rude, 2012; Martin *et al*., 1985) and in constipation. Calcium plays a great role in the prevention or treatment of pre-eclampsia (Bucher, 1996), colon cancer (Garland *et al*., 1991), or hypertension (McCarron, 1997). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and hormonal immunity (Hambridge *et al*., 1987). Zinc deficiency in humans causes growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia,

impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (Shankar, 1998; Agett *et al*., 1993; Goldenberg, 1995; Brown, 1998; Beck, 1997). Iron exhibit several vital functions in the body and serves as a carrier of oxygen to the tissues from the lungs by red blood cell Haemoglobin, as a transport medium for electrons within cells (Bothwell, 1979; Hallberg, 1982; Brock *et al*., 1994; Kühn, 1996; Mascotti, 1995). Mg regulates diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, blood pressure regulation in humans (Rude, 2010; Rude, 2012), energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione (Romani, 2013; Abbott, 1992). In terms of providing cure for fungal infections, copper has been reported to play important role at the host-pathogen axis during infection (Sarela Garcia-Santamarina, 2015). The effect of how the host uses either Cu compartmentalization within innate immune cells or Cu sequestration in other infected host niches such as in the brain to combat fungal infections has been investigated.

It was reported that the host manipulate Cu-dependent processes at the host-pathogen axis for prevention of the growth of fungi which could lead to antifungal drug development (Sarela Garcia-Santamarina, 2015). It has also been reported that the host innate immune cells concentrate Cu in the vicinity of invading pathogens, as a means to exploit Cu toxicity and enhance microbial killing (Ladomersky, 2015; Osredkar, 2011). Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy, takes part in numerous biochemical pathways, important for healthy skin and is essential for a healthy immune system and resistance to infection (Osredkar, 2011; Prasad, 2003; Plum, 2010; Burjonrappa, 2012). The lack of zinc in the human body causes postular dermatitis, diarrhea, and nail dystrophy. Irritability and emotional disturbances are due to atrophy of the brain cortex. The severity of the disease is reported to be proportional to the zinc level. Before zinc supplementations acrodermatitis enteropathica was fatal to babies born with it (Wang, 2005; Hambidge, 1989). Zinc supplementation has been reported to provide effective treatment for acrodermatitis enteropathica, an inborn error of zinc metabolism that is inherited as an autosomal recessive disorder.

Conclusion

Organoleptic evaluation, Fluorescence analysis, Phytochemical screening and Mineral analysis has been carried out on the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* used for the treatment of fungal infection in Sierra Leone. The plant also has been reported to provide cure for a variety diseases (Lebbie Aiah, 1995; Mrinmay, 2014; Scher, 2004; Bhaskar Rao *et al*., 2011; Chandrika *et al*., 2006; Khan Tabassum, 2014; Anitha *et al*., 2012; Sheela *et al*., 2004; Shyamala *et al*., 2005; Acharya *et al*., 2006; Erna C Arollado and Marina O Osi, 2010; Devi *et al*., 2003; Hossain *et al*., 2014). The results of organoleptic evaluation indicate the colour of the dried powdered leaves of*A. sessilis* to be brown, grass odour and had a bitter taste. The bitter taste indicated that alkaloids are present in the leaves of the traditional medicinal plant *A. sessilis* supporting its use in traditional medicine in providing remedy for the above reported diseases. Fluorescence investigation carried out on dried powdered leaves of *A. sessilis* using different chemical reagents gave positive results withthe reagents1M NaOH(aq), 1M NaOH(alc.), Ammonia, 50% HCl, and 50% HNO_3, being one of the parameters for pharmacognostic evaluation and detection of crude drugs (Hossain *et al*., 2014) in traditional medicinal plants Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered Leaves of traditional medicinal plant *Alternanthera sessilis* was evaluated for the presence of secondary plant metabolites. The results 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 intense concentration of Tannins and Phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated. The detection of the above secondary plant metabolites support the use of the plant as food and as traditional pharmaceutical. Elemental/mineral analysis of the dried powdered Leaves of *Alternanthera sessilis* plant was investigated for the presence of K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc. The results 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 the plant.

The plant organ contained large amounts of nutrients and rich in K (56714 ± 250.00 ppm), Ca (21372 ± 218.00 ppm), Mg (6333.00 ± 2084 ppm), Al(7266 ± 378.00 ppm) and Fe (3655.20 ± 31.11 ppm). The other elements present in smaller quantities are Ti (592.00 ± 18.00 ppm), Zr (207.21 ± 1.65 ppm), Mn (167.42 ± 14.68 ppm), Zn (88.52 ± 3.19 ppm), Sc (79.00 ± 15.00 ppm), Sr (73.45 ± 1.02 ppm), Rb (63.53 ± 1.10 ppm), Cu (34.78 ± 4.83 ppm) V (12.90 ± 7.85 ppm)and Mo (4.92 ± 0.90 ppm). Potassium participates actively in the maintenance of the cardiac rhythm (Nayak *et al*., 2007; Rude, 2012; Martin *et al*., 1985) and in constipation Calcium plays a great role in the prevention or treatment of pre-eclampsia (Bucher, 1996), colon cancer (Garland *et al*., 1991), or hypertension (McCarron, 1997). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and hormonal immunity (Hambridge, 1987). Zinc deficiency in humans causes growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (Shankar, 1998; Agett, 1993; Goldenberg, 1995; Brown *et al*., 1988 and Beck, 1997). Iron exhibit several vital functions in the body and serves as a carrier of oxygen to the tissues from the lungs by red blood cell Haemoglobin, as a transport medium for electrons within cells (Bothwell, 1979; Hallberg, 1982; Brock *et al*., 1994; Kühn, 1996; Mascotti *et al*., 1995).Mg regulates diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, blood pressure regulation in humans, energy production, oxidative phosphorylation, and glycolysis (Rude, 2010; Rude, 2012; Romani, 2013; Abbott, 1992). *Role of elements present if the dried powdered leaves of Alternanthera sessilisplant to fungal infections* Copper and zinc are the two main elements that have been identified in the dried powdered leaves of *Alternanthera sessilis* plant that can provide cure for fungal infection. Copper has been reported to play important role at the host-pathogen axis during infection (Sarela Garcia-Santamarina, 2015).

The host uses either Cu compartmentalization within innate immune cells or Cu sequestration in other infected host niches such as in the brain to combat fungal infections. The host manipulates Cu-dependent processes at the host-pathogen axis for prevention of the growth of fungi which could lead to antifungal drug development (Sarela Garcia-Santamarina, 2015). The host innate immune cells concentrate Cu in the vicinity of invading pathogens, as a means to exploit Cu toxicity and enhance microbial killing (Ladomersky, 2015; Hodgkinson, 2012). Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy, takes part in numerous biochemical pathways, important for healthy skin and is essential for a healthy immune system and resistance to infection (Osredkar, 2011; Prasad, 2003; Plum *et al*., 2010; Burjonrappa, 2012). The lack of zinc in the human body causes postular dermatitis, diarrhea, and nail dystrophy. Irritability and emotional disturbances are due to atrophy of the brain cortex. The severity of the disease is reported to be proportional to the zinc level.

Before zinc supplementations acrodermatitis enteropathica was fatal to babies born with it (Wang, 2005; Hambidge, 1989). Zinc supplementation has been reported to provide effective treatment for acrodermatitis enteropathica, an inborn error of zinc metabolism that is inherited as an autosomal recessive disorder. 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. Excessive levels higher than that needed for biological functions of these elements can be toxic for the body health. Hence any Pharmacognostic investigation of traditional medicinal plants without mineral analysis cannot be completed.

Recommendations

.This research work has shown that the traditional medicinal plant *Alternanthera sessilis* contained carbohydrates, alkaloid, flavonoids, proteins sterols/terpenes and saponins and a good number of elements/minerals. We therefore recommend the plant to be used in traditional medicine and as food/mineral supplement. Further research on this plant is required in other to isolate and characterize the active compounds responsible for the medicinal properties of the plant.

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