VOLUME 1, 2018
DOI: 10.31058/j.ac.2018.11002

Phytochemical, Antimicrobial and Heavy Metals Analyses of *Sarcocephalus Latifolius* Leave Extract

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Received: 24 February 2018**; Accepted:** 10 March 2018**; Published:** 3 April 2018

Abstract:

Extraction of the leaves of *Sarcocephalus latifolius* was done using acetone, methanol, diethylether, petroleum ether, water and ethanol as solvent to analyze its phytochemical constituents followed by the quantitative analysis and antimicrobial property. Phytochemical analysis indicated the presence of alkaloids (5.22%) in diethylether, petroleum ether and water extracts, saponins (18.2%) in methanol and water extracts, but trace amount in acetone, petroleum ether and ethanol extracts, tannins (0.63%) in water and ethanol extracts, glycosides (0.72%) in acetone, diethyl ether, ethanol, water and methanol extracts while steroids and flavonoids were absent. Antimicrobial susceptibility test of water and methanol extract was done using the agar well diffusion method against clinical isolates of gram positive bacteria (and *staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The result of water extract showed higher inhibition potency than the methanol extract when compared with the positive control Ciprofloxacin. Heavy metal analysis was also conducted, the result indicated absence of chromium, lead, and arsenic, presence of zinc(0.18ppm), nickel(7.69ppm), manganese(2.21ppm), iron(1.36ppm),) and copper(0.1ppm). All were within W.H.O acceptable limit except nickel. This result concludes that the leaves of *Sarcocephalus latifolius* is medicinal as speculated by the orthodox medicine, but the heavy metals constituents of the plant should also be considered to avoid taking contaminated extract as medicine.

Keywords:

Phytochemicals, *Sarcocephalus Latifolius*, Heavy Metals, Anti-Microbial

1. Introduction

Healing with medicinal plants which is called Herb is as old as mankind itself, the connection between man and medicine in nature dates from the ancient days. This is

as a result of one ailment or the other that always befall man. The only option man had at that time was the use of certain plant's parts as drugs for healing and this has been effective; only it does not have scientific prove of the active ingredients that are doing the work of healing and the dosage to be taken. The World Health Organization estimated that 80% of the people in developing countries of the world rely on traditional medicine for their primary healthcare, and about 85% of traditional medicine involves the use of plant extracts [1, 2, and 26]. This means that about 3.5 to 4billion people in the world rely on plants as sources of drugs. [1,2]. Despite the enormous breakthrough in science on health care in the twentieth century's, many of the world's population in developing countries lack regular access to afford able essential drugs. For these people, modern medicine is never likely to be a realistic treatment option. Again traditional medicine is widely available, affordable and cheaper than modern medicine [3, 4]. But caution should be put in place while administering these medicinal plants because many of them are contaminated with heavy metals as a result of industrial effluent, emission, acid rain, addition of fertilizers, pesticide and herbicide, to the farmland and mining processes. These heavy metals can also occur as natural constituents of the earth crust, to a small extent, they enter the body system through food, air, and water and bio-accumulate over a period of time [5,6]. In rocks, they exist as their ores in different chemical forms, from which they are recovered as minerals. Heavy metal ores include sulphides, such as iron, arsenic, lead, zinc, cobalt, gold-silver and nickel sulphides; oxides such as aluminium, manganese, gold, selenium and antimony. Some can be recovered as both sulphide and oxide ores such as iron, copper and cobalt [7]. One of the greatest problems that the world is facing today is that of environmental pollution, which is increasing yearly and causing grave and irreparable damage to the earth and one of these is soil pollution.

Sarcocephalus latifolius (SL) formerly known as *Nauclea latifolia* is an evergreen multi-stemmed shrub or a tree; it grows up to an altitude of 200m [8]. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. *Sarcocephalus latifolius* is known as Pin cushion tree, African peach, and Guinea peach in English. In Nigeria it is called the following by the three major ethnic group; Ibos (ubuluinu), Hausas (Tafashiya, tashiyaigia) while Yorubas "Egbesi". It is a multi-stemmed tree or shrub up to 12m with an open canopy, flowers with terminal spherical head-like cymes of small whitish flowers. It belongs to tribe called Naucleae and the fruit is a syncarp. It is a hermaphroditic tree flowering from April-June. Fruits ripen from July- September [9]. This plant is believed to cure fever, pain, dental caries, septic mouth, malaria, hypertension, dysentery, diarrhea and diseases of the central nervous system such as epilepsy and treatment of wound infection. [9, 10-16]. The aim of this work is to determine the Phytochemical, Anti-Microbial and heavy metals analyses of the leave extract *of Sarcocephalus latifolia.*

2. Materials and Methods

The chemicals used in this work were of analytical grade bought from local market in Nigeria. The preparations of the reagents were done according to the specified standard.

2.1. Preparation of Plant Materials

Figure 1 is the picture of the *Sarcocephalus latifolia*. The leaves of *Sarcocephalus latifolius* (SL) were collected from Ugbolu town in Delta state, Nigeria. Mr P.O.

Ugwuozor of Botany Department of Nnamdi University Awka, confirmed it as *Sarcocephalus latifolia*. It was shade dried, and pulverized in an electric blender.10g of pulverized sample was weighed into different plastic containers. 50ml of different solvents (methanol, water, diethyl ether, petroleum ether (p/ether), ethanol and acetone) were added and shaken for 3 hours with an adjustable vibrator shaker at room temperature. The mixtures were then allowed to stand at room temperature overnight, it were filtered, and the filtrates/extracts were concentrated with a rotary evaporator and preserved for further qualitative and quantitative analysis.

Figure 1. *The Sarcocephalus latifolius plant.*

2.2. Phytochemical Screening

The extracts of *Sarcocephalus latifolius* was analysed for the presence of flavonoids, alkaloids, steroids, saponins, tannins and glycosides according to standard methods[17].

2.2.1. Test for Alkaloids

1ml of 1% HCl was added to 3ml of acetone leaf extract into a test tube. The resulting mixture in the test tube was put in a 100ml beaker and heated mildly on a heating mantle for about 5-10 minutes and the test tube shaken at intervals. After heating, the sample was filtered to remove the residue. 0.5ml of Wagner's reagent was added to the sample and a reddish brown colour indicated the presence of alkaloids. This procedure was repeated for every other solvent extract in different test tubes.

2.2.2. Test for Saponin

Two tests were used; Emulsion test and frothing test

Frothing Test:

3ml of acetone extract was put into test tube and 2ml of distilled water was added to it. A persistent frothing movement was observed. This indicated the presence of saponin.The procedure was repeated for other solvent extracts.

Emulsion Test:

3ml of acetone extract was pipetted into test tube. 5 drops of olive oil was added to the content of the test tube and shaked vigorously. The formation of persistent foams for at least 15 minutes indicated the presence of saponins. The experiment was repeated for other solvent extract.

2.2.3. Test for Flavonoids

3ml of acetone leaf extract was pipette into a test tube.10ml of distilled water was added to the test tube and the solution was shaken.1ml of 10% NaOH (solution) was added to the mixture. The presence of a yellow colouration indicated the presence of flavonoids. The procedure was repeated with other solvent extracts.

2.2.4. Test for Steroids

The method used is called Salkowski's test. 1ml of the each sample extract was pipetted into various test tubes. 5 drops of concentrated H_2SO_4 was added to each test tube. A red colour indicated the presence of steroids.

2.2.5. Test for Glycosides

1ml of each aqueous extract was pipetted into different test tubes. 1ml of 2% solution of 3,5-dinitro salicyclic acid in methanol and 1ml of 5% aqueous NaOH was added into the test tubes. The presence of bright orange colouration indicated the presence of glycoside. The content of test tubes was heated in boiling water to obtain a brick red colouration.

2.2.6. Test for Tannins

2ml of each extract were pipetted into test tubes and allowed to boil for 2minutes, then cooled. 3 drops of ferric chloride solution was added after cooling. A bluish green colour or green precipitate or brownish green precipitate indicated the presence of tannin.

2.2.7. Quantitative Determination of Alkaloids

5g of the sample was weighed into separate 250ml conical flask. 100ml of 10% acetic acid in ethanol was added to the conical flask containing the sample and covered with a cotton wool. The conical flask was then agitated for 4 hours using a vibrator/shaker. The content was allowed to settle overnight, filtered, concentrated on a water bath or heating mantle. 20ml of concentrated NH_4OH was added drop wise to form precipitate of alkaloid in the filtrate. Then an empty filter paper was weighed before being used for the filtration, this was followed by oven drying of paper and residue at 40^oC. It was later cooled in a desiccator and reweighed. Alkaloid content was calculated and expressed as a percentage of the sample analyzed.

2.2.8. Quantitative Determination of Glycosides

5g of the sample was weighed into a 250ml conical flask. 100ml of distilled water was added into the sample in the conical flask. The sample was then closed and put in the vibrator/shaker to be agitated for 3 hours. After agitating for 3 hours, the sample was filteredusing a muslin cloth. The volume was measured and recorded as 54ml (total volume of extract). 2ml of 10% DNS solution was added.The mixture was boiled in a thermostatically controlled heating mantle for 20 minutes at room temperature. Then, it was cooled and 10ml of distilled water was introduced for dilution to take place. This solution of the extract was analyzed with a UV/Visible spectrophotometer at 540nm and the absorbance expressed in percentage.

2.2.9. Quantitative Determination of Saponin

5g of the sample was weighed into a 250ml conical flask. The mixture was soaked in 100ml of 20% ethanol for few minutes, and then it was heated for a period of 3

hours at mild temperature of 55^0C and was filtered. 100ml of another 20% ethanol was used again to re-extract, the extract were mixed together and concentrated to 40ml at 90^0C. The concentrate was then transferred into a 500ml separating funnel, 20ml of diethyl ether was added and shaken vigorously. Two layers were formed, and the aqueous layer (upper layer) was discarded. The purification process was repeated. 60ml of n-butanol was added, two layers was formed. The bottom layer was discarded and the upper layer recovered. Then the combined n-butanol extract was washed with 10ml of 5% aqueous NaCl twice. The lower layer was discarded and upper layer recovered. The remaining solution was heated in a water bath to dryness and the weight recorded. Saponin content was calculated and expressed as a percentage of the sample analysed.

2.2.10. Quantitative Determination of Tannins

0.5g of the sample was weighed into a plastic bottle. 50ml of distilled water was added and shaken for 3 hours in an electrical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then, 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M ammonium chloride and 0.008M potassium ferrocyanide. The absorbance was measured at 202nm within 10mins of preparation and expressed in percentage.

2.3. Antimicrobial Screening

2.3.1. Test Organism

The Gram-positive bacteria (Streptococcus pyogenes, Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Salmonella typhi) used in this investigation were obtained from the stocks in National Agency for Food and Drugs Administration and Control, Agulu, Anambra State.

2.3.2. Antimicrobial Sensitivity Test

The antimicrobial activities of the water and methanol extracts were determined using agar well diffusion technique [18]. Mueller Hinton agar was used which was prepared by dissolving 7.6g of the powdered agar in 200ml of water. 20ml of the prepared agar was poured into 8 petri dishes each and 0.1ml of standardized broth cultures of the bacteria was used to seed molten Mueller Hinton agar. The seeded plates were allowed to set and then solidify. A sterile cork borer of 8mm diameter was used to bore 2 uniform wells on the surface of the agar in each petri dish. Exactly 1ml of each extract and 1ml of Ciprofloxacin (50mg/ml) to serve as a positive control was placed in the wells respectively. The plates were kept on the bench and allowed for pre-diffusion for 40minutes followed by an overnight incubation at 37^0C for bacterial isolates. The degree of antimicrobial activity of each extract was measured as the inhibition zone diameter in millimetres.

2.4. Heavy Metal Analysis

Heavy metal analysis was conducted using Bulk scientific VGP210 Atomic Absorption spectrophotometer according to the method of APHA 1995 (American Public Health Association). 2g of pulverized/powdered was electronically weighed into a 50ml beaker. 10ml of nitric acid and 10ml of perchloric acid was also added to the sample. This was immediately transferred into the fume cupboard and heated until a clear solution was obtained. Then the digested sample was filtered into a 250ml

volumetric flask and made up to the mark with distilled water. This dilute solution was then atomized using VGP210Atomic absorption spectrophotometer for the analyses of some heavy metals.

3. Results and Discussion

3.1. Phytochemical Analysis

The presence of secondary metabolites in *Sarcocephalus latifolius* may be responsible for its potential use as drug [1,19]. The result of qualitative phytochemical analysis to determine these secondary metabolites is summarized in Table 1.The Table shows the presence of alkaloids, saponins, tannins and glycosides in the leave of SL. The water extract had high content of saponins, glycosides and tannins while flavonoids and steroids were absent. Diethyl ether and petroleum ether had the highest content of alkaloids. This report correlates the findings of other authors as phytochemical constituents being responsible for medicinal aspect of a plant [20-23]. The result of quantitative analysis of the Phytochemical screening is recorded in Table 2. From that Table it could be observed that saponins has the highest percentage, followed by alkaloids, tannins and glycosides.This shows that the leaf of SL contained these secondary metabolites in this order saponins>alkaloids>tannins>glycosides.

Table I. *Qualitative Report on Phytochemical Analysis.*

S/N	**Test**	**Solvents**					
		Acetone	Methanol	Diethylether	P/ether	Water	Ethanol
1.	Alkaloids	-	-	++	+++	+	-
2.	Saponins	+	+++	-	+	+++	++
3.	Flavonoids	-	-	-	-	-	-
4.	Steroids	-	-	-	-	-	-
5.	Tannins	-	-	-	-	++	+
6.	Glycosides	+	++	+	-	++	+

Keys: *+ = Insignificantly Present, ++ = Moderately Present, +++ = Abundantly Present, - = Not Present*

Table 2. *Quantitative result of phytochemical analysis.*

S/N	**Test**	**Percentage Yield**
1.	Alkaloids	5.22%
2.	Saponins	18.1%
3.	Glycosides	0.72%
4.	Tannins	0.93%

3.2. Antimicrobial Analysis

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infectious diseases and in food spoilage. The active components of the medicinal plant usually interfere with growth and metabolism of microorganisms in a negative manner [24, 25, and 26]. Table 3 is the sensitivity of water extract against test organisms while Table 4 is sensitivity of methanol extract against test organisms. It can be inferred from both Tables that the water extracts of the SLare active against the entire tested bacteria and methanol extract was active against two out of the four tested bacteria. This indicated that water extract showed remarkable activity against all the microorganisms used in this work and can be used as antibiotics as speculated.

Table 3. *Sensitivity of water extract against test organisms.*

S/N	Organism	Zone diameter for water extract	Zone diameter for Ciprofloxacin (standard)
1.	Escherichia coli	20mm	50mm
2.	Streptococcus pyogenes	15mm	50mm
3.	Staphylococcus aureus	10mm	20mm
4.	Salmonella typhi	15mm	65mm

Table 4. *Sensitivity of Methanol extract against test organisms.*

S/N	**Organism**	**Zone diameter for Methanol extract**	**Zone diameter for Ciprofloxaxin (standard)**
1.	Escherichia coli	10mm	60mm
2.	Streptococcus pyogenes	0.2mm	60mm
3.	Staphylococcus aureus	14mm	25mm
4.	Salmonella typhi	0.2mm	20mm

3.3. Heavy Metals Analysis

Table 5 shows the result of heavy metals analyses. Visual inspection of Table 5 indicated that iron, manganese, zinc and copper were all within W.H.O permissible limit of medicinal plant, chromium, lead and arsenic were absent while the concentration of nickel is higher than the WHO permissible limit. Nickel is an essential element for plants and animals. In small quantity, nickel is necessary for the regulation of lipid contents in tissues and for the formation of red blood cells. But at high level, it becomes toxic and causes severe diseases like loss of body weight, loss of vision, and heart and liver failures, as well as skin irritation. The concentration of nickel in this plant was 7.69ppm while maximum permissible limit of W.H.O for nickel in herbs is 1.5ppm, this high in nickel concentration may be attributed to the environment where this plant was harvested [27]. The environment which *Sarcocephalus latifolius* was harvested is farmland settlement, there is no Industrial activity going on there, the possibility of the high concentration of nickel in the plant may be as a result of natural sources and anthropogenic sources [28, 29]. Natural source may be due to nature since nickel occur naturally in the soil and anthropogenic sources may be due to the type of water farmers used to irrigate their farm ie contaminated irrigation water, the use of fertilizers and organic manure. All of the above mentioned can contribute to the high level of nickel in this plant [30-32].

Table 5. *Heavy metals Analyses of Sarcocephalus latifolius and their concentration.*

S/N	**Element**	**Concentration in ppm**	**Permissible limit by W.H.O (ppm)**
1.	Zinc	0.18	50
2.	Lead	0.00	10
3.	Nickel	7.69	1.5
4.	Manganese	2.21	200
5.	Iron	1.36	20
6.	Arsenic	0.00	0.01
7.	Copper	0.10	2.3
8.	Chromium	0.00	1.5

4. Conclusions

Sarcocephalus latifolius extract contained Phytochemical constituents which are responsible for the medicinal aspect of the plant. Quantitative analysis of the Phytochemical screening demonstrated that the leave of SL contained the following

secondary metabolism in the increasing order glycosides<tannins<alkaloids< saponins. The inhibition of micro-organism by water extract is more efficient than the methanol extract. *Sarcocephalus latifolius* harvested in Ugbolu community, Delta State of Nigeria contains heavy metal called nickel which is not healthy to be taken as medicine. Although *Sarcocephalus latifolius* leaves extract is medicinal but one should not overlook the aspect of contamination while harvesting this plant. Therefore, before any plant's parts can be eaten as herbs, it is advisable to conduct heavy metals analyses of that plant to determine its safety, since pollution is within us but is beyond our control.

Conflicts of Interest

The author hereby declares that there is no conflict of interest regarding the publication of this article.

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