



Phytochemical, Proximate and Antimicrobial Analysis of the Fruit of *Sarcocephalus latifolius* (Smith Bruce)

Yinusa I., Adeiza H.A. and Balkees W.A.

Department of Chemistry, Federal University, Lokoja, Kogi State, Nigeria

ABSTRACT

This work was to determine the phytochemical constituent, proximate analysis and the antimicrobial properties of various fractions of the fruit of *Sarcocephalus latifolius* (Smith Bruce) obtained from different branches of the tree growing in areas around police zone 8 in Lokoja metropolis, Kogi State, Nigeria, West Africa. The phytochemical screening revealed the presence of flavonoids, terpenoids, cardiac glycoside, tannins, saponins, phlobatannins, alkaloids, carbohydrates and glycosides. While the proximate analysis gave the following values; Moisture content (9.39%), Crude protein (7.02%), carbohydrate content (35.70%), ash content (3.85 %.), crude fibre (31.03%) and lipid composition (13.01%). The zone of inhibition was observed to be between 16 and 24mm with the ethyl acetate fraction having the highest zone of inhibition of 24mm against *Pseudomonas aureginosa* and *candida albicans*. Petroleum ether fraction had the lowest value against *Shigellia dysenteric*. The result obtained for the MIC and MBC/MFC showed that the activities of the extract were highly sensitive to the test organism and equally comparable to the activity of standard drugs.

Keywords: Proximate; Phytochemical; Antimicrobial; Sarcocephalus; Latifolius; Fruit,

INTRODUCTION

The fruits, seeds and leaves of many wild plants form many common ingredients in a variety of traditional native dishes in most developing countries (Humphrey *et al.*, 1993).

Fruits are commonly excellent sources of nutrients such as minerals and vitamins; and also contain carbohydrates in form of soluble sugars, cellulose and starch (Nahar and Mosihuzzaman, 1990).

Food and Agricultural Organization (FAO) has reported that at least one billion people use wild foods in their diets (Burhingame, 2000). In Ghana over 300 species of wild plants and fruits are consumed while about 150 wild plant species have been identified as sources of emergency food in India, Malaysia and Thailand (Umar *et al.*, 2007). Similarly, in South Africa about 1400 edible plant species are used (Hassan and Umar, 2004). It is therefore worthwhile to note that the incorporation of edible wild and semi-cultivated plants could be beneficial to nutritionally marginal populations, or to certain vulnerable groups within populations, especially in developing countries where poverty and climatic change are causing havoc

to the rural populace (Aberoumand and Deokule, 2009)

The current folk medicinal applications of *Sarcocephalus latifolius* are varied and numerous. For example, the bark and the root extracts are said to be useful in malaria treatment (Akubue and Mittal, 1982; Oye, 1990). They are used as a tonic and remedy for treating fever, toothaches, dental problems, septic mouth, and diarrhea and in dysentery treatment and also used as chewing stick (Etkin *et al.*, 1990; Lamidi *et al.*, 1995). The bark is said to be useful in the treatment of wounds, cough and gonorrhoea in Nigeria (Madubunyi, 1995).

Sarcocephalus latifolia contains diverse phytochemicals such as alkaloids, flavonoids, steroids and glycosides. Series of alkaloids were said to be isolated from this plant. For

Received 19 November, 2016

Accepted 15 December, 2016

Address Correspondence to:

instance, Naucleafoline, nauclechine and naufoline were isolated from the leaves. Other alkaloids isolated from the plant include naucletine, nauclefine, naucleidinal and epinaucleidinal, augustine and card-ambine (Hotellier *et al.*, 1975, 1979). Naucleidal and epinaucleidal have been isolated from an antiviral preparation produced by roasting *Nauclea latifolia* fruits (Morah, 1994). Furthermore, five monoterpene indole alkaloids, naucleamides were found to occur in the bark and wood of the plant. Naucleamide E was the unique monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge (Shigemori *et al.*, 2003). Recently, a pentacyclic triterpenoid Betulinic acid (Isah *et al.*, 2012) and beta-Sitosterol (Isah *et al.*, 2015), mixture of Sitosterol and sitostenone were isolated from the stem bark of this plant (Isah *et al.*, 2014)

This work is aimed at evaluating the phytochemical content, proximate analysis and antimicrobial activities of the various extract of *Sarcocephalus latifolius* fruit.

METHODOLOGY

Sample collection and preparation

The fruits of *S. latifolius* (matured) were randomly sampled from different branches of the tree growing in areas around police zone 8 in Lokoja metropolis, Kogi State, Nigeria, West Africa. The samples were transported to the laboratory in air-tight polyethylene bags to avoid any form of contamination.

The samples were air dried in the laboratory under normal laboratory conditions. The dried material was pulverized using mortar and pestle to fine particles and stored in nylon containers and kept away from moisture for further analysis.

Extraction procedure

The pulverized dried material (Fruit) was extracted using cold maceration method. Two hundred grams (200g) of the powdered fruit of the plant material were placed in a covered bottle and extracted with 500ml of the solvent (pet ether, chloroform, ethyl acetate and methanol) in the cold successively after which it was filtered using Whatman No.1 filter paper into round bottom flask. The filtrate was poured

into beakers and allowed to evaporate off the extraction solvent to obtain the crude extracts.

Phytochemical analysis

The pulverized fruit of *Sarcocephalus latifolius* was screened for plant metabolites. Standard techniques of Brain and Turner (1975) were employed in the phytochemical screening. The presence or absence of typical metabolites such as flavonoid, terpenoids, cardiac glycosides, tannins, steroids, saponins, phlobatannins, Phytosterols, alkaloids, carbohydrates and glycoside were ascertained.

Proximate analyses:

The moisture content, ash content, crude lipid were evaluated using the method described by AOAC (1990) and crude fibre was determined by the method of AOAC (1980). While the crude protein was carried out using the method adopted by Anhwange *et al.*, (2004) and the total carbohydrate content was determined by difference.

Anti-microbial activities

Diffusion method of Bauer, Barry and Thornsberry, (1985) were used in the anti-microbial screening of the extracts by the use of a standard cork borer of 6mm in diameter.

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of flavonoids, terpenoids, cardiac glycoside, tannins, saponins, phlobatannins, alkaloids, carbohydrates and glycosides. While, steroids and Phytosterols were found to be absent in the fruit. The result is as shown in Table 1. The presence of saponins, tannins and alkaloids in the fruit of this plant calls for an in-depth study on the plant. The metabolites are of various pharmacological importance. Many triterpene saponins and their aglycones have been reported by Hostettmann and Martson, (1995) and in Ndukwe *et al.*, (2005) to have varied uses as antiulcer genic, anti-inflammatory, fibrinolytic, antipyretic, analgesic and anti-edematous in action.

Table 1: Result of the preliminary phytochemical screening of the fruit of *Sarcocephalus latifolius*.

Phytochemicals	Result
Flavonoids	+
Terpenoids	+
Cardiac glycosides	+
Tannins	+
Steroid	-
Saponins	+
Phlobatannins	+
Phytosterols	-
Alkaloids	+
Carbohydrates	+
Glycosides	+

Key: + = Present - = Absent

The values obtained for the proximate analysis of this fruit are as presented in Table 2. Moisture content is generally accepted to be a measure of the water content in fruit samples, if moderate; it is an indication that it can be stored for a long time without the development of moulds. The result obtained for *Sarcocephalus* fruit was 9.39% which can be said to be within the values required as safe storage limit for plant food materials (Umar *et al.*, 2007).

Crude protein value obtained for *Sarcocephalus latifolius* fruit was 7.02%, which is lower than 8.52% as reported by Yesufu and Husaini, (2014) in a studies on dietary mineral composition of the fruit of *Sarcocephalus latifolius* (Smith) Bruce (Rubiaceae). This variation may be due to geographical location coupled with weather and atmospheric conditions.

The value obtained for the carbohydrate content was 35.70% which shows that it has moderate content and that most fruits had high carbohydrate content depending on the fruit type, maturity and environment.

Ash content is a measure of the total mineral content present in foods. The value obtained for *Sarcocephalus latifolius* fruit was 3.85%. This is an evidence that this fruit is rich in mineral composition. Ayessou *et al.* (2011) reported similar ash content result for this particular fruit in a study of Nutritional contribution of some Senegalese Forest fruits running across Soudano-Sahelian Zone.

The Crude fibre composition obtained for *Sarcocephalus latifolius* fruit was 31.03%. This

value falls within the recommended daily allowance values of 19-25%, 21-38%, 28% and 29% for children, adults, pregnant and breast feeding mothers respectively (Pearson, 1994). This result is shows that the fruit of *Sarcocephalus* could be a good source of dietary fibre. The lipid composition value was found to be 13.01%

Table 2: Proximate composition of the fruit of *Sarcocephalus latifolius* (Smith Bruce).

Parameter	Percentage Composition (% DW)
Moisture	9.39 ± 0.14
Ash	3.85 ± 0.18
Lipid	13.01 ± 0.70
Fibre	31.03 ± 0.75
Protein	7.02 ± 0.03
Carbohydrates	35.70

The data are Mean values ± Standard deviation (SD) of three replicates

* Values expressed as % of Dry weight.

The antimicrobial activities of the various extracts were carried out to ascertain which fraction of the fruit extract was more sensitive to the test organism. All the extracts were found to be sensitive to *Staphylococcus aureus*, *Escherichia coli*, *Shigellia dysenteric*, *Pseudomonas aureginosa* and *Candida Albicans*. While all the extracts were resistant to *Streptococcus pyrogens*, *Corynebacterium ulcerans*, *Klebselia pneumoniae*, *Microsperum Canis* and *Trichophyton Spp* as shown in Table 3. The zone of inhibition was observed to be between 16 and 24mm with the ethyl acetate fraction having the highest zone of inhibition of 24mm against *Pseudomonas aureginosa* and *candida albicans*. Petroleum ether fraction had the lowest value against *Shigellia dysenteric*. The values are as shown in Table 4.

The Minimum Inhibition Concentration (MIC) was determined to ascertain the lowest concentration level at which these extracts could inhibit the growth of these organisms. All the extracts were found to inhibit the growth of all the sensitive organism at 5mg/ml and 2.5mg/ml respectively as shown in Table 5. While the minimum bactericidal concentration (MBC) was carried out to determine and establish the minimum concentration at which the sensitive organism will be killed or completely exterminated. It was noted from

Table 6 that, the petroleum ether extract would exterminate the sensitive organism at 10mg/ml while, the chloroform was observed at 5mg/ml against *pseudomonas aureginosa*. The MBC/MFC for ethyl acetate fraction was observed at 10mg/ml for *staphylococcus aureus*, and *Shigellia dysenteric*. While 5mg/ml was observed for *Escherichia coli*, *pseudomonas aureginosa* and *candida albicans*. The methanol extract MBC/MFC was observed at 10mg/ml for all the sensitive organisms. The result obtained from the MBC/MFC was a little higher than that obtained from the MIC.

This result was compared to standard drugs as shown in Tables 7 and 8 and found that it is favourably comparable. It may be that these crude extracts could contain some active metabolites.

Table 3: Antimicrobial Activities of the Fruit Extracts from *Sarcocephalus Latifolius*

Test organism	Pet ether extract	Chloroform extract	Ethyl Acetate extract	Methanol Extract
<i>Staphylococcus aureus</i>	S	S	S	S
<i>Streptococcus pyrogens</i>	R	R	R	R
<i>Corynebacterium ulcerans</i>	R	R	R	R
<i>Escherichia coli</i>	S	S	S	S
<i>Klebselia pneumonia</i>	R	R	R	R
<i>Shigellia dysentriae`</i>	S	S	S	S
<i>Pseudomonas aureginosa</i>	S	S	S	S
<i>Candida albicans</i>	S	S	S	S
<i>Microsporium Canis</i>	R	R	R	R
<i>Trichophyton Spp</i>	R	R	R	R

Key: S = Sensitive R= Resistance

Table 4: Zone Of Inhibition of the Extracts against the Test Microbe (mm)

Test organism	Pet ether extract	Chloroform extract	Ethyl Acetate extract	Methanol Extract
<i>Staphylococcus aureus</i>	17	20	22	18
<i>Streptococcus pyrogens</i>	0	0	0	0
<i>Corynebacterium ulcerans</i>	0	0	0	0
<i>Escherichia coli</i>	18	21	23	20
<i>Klebselia pneumonia</i>	0	0	0	0
<i>Shigellia dysentriae`</i>	16	20	21	19
<i>Pseudomonas aureginosa</i>	18	22	24	20
<i>Candida albicans</i>	17	21	24	20
<i>Microsporium Canis</i>	0	0	0	0
<i>Trichophyton Spp</i>	0	0	0	0

Table 5: Minimum Inhibition Concentration of the Fruit Extracts against the Test Microbe

Test organism	Pet ether extract (mg/ml)					Chloroform extract (mg/ml)					Ethyl acetate extract (mg/ml)					Methanol extract (mg/ml)				
	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63
<i>Staphylococcus aureus</i>	-	O*	+	++	+++	-	-	O*	+	++	-	-	O*	+	++	-	O*	+	++	+++
<i>Streptococcus pyrogenes</i>																				
<i>Corynebacterium ulcerans</i>																				
<i>Escherichia coli</i>	-	O*	+	++	+++	-	-	O*	+	++	-	-	O*	+	++	-	-	O*	+	++
<i>Klebselia pneumonia</i>																				
<i>Shigellia dysentriae`</i>	-	-	O*	+	++	-	-	O*	+	++	-	-	O*	+	++	-	O*	+	++	+++
<i>Pseudomonas aureginosa</i>	-	O*	+	++	+++	-	-	O*	+	++	-	-	O*	+	++	-	-	O*	+	++
<i>Candida albicans</i>	-	O*	+	++	+++	-	-	O*	+	++	-	-	O*	+	++	-	-	O*	+	++
<i>Microsporium Canis</i>																				
<i>Trichophyton Spp</i>																				

Key: - = No turbidity (No growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbid, +++ = High turbidity.

Table 6: Minimum Bactericidal/Fungicidal Concentration of the Extracts against the Test Microbe

Test organism	Pet ether extract (mg/ml)					Chloroform extract (mg/ml)					Ethyl acetate extract (mg/ml)					Methanol extract (mg/ml)				
	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63
<i>Staphylococcus aureus</i>	O*	+	++	+++	++++	O*	+	++	+++	++++	O*	+	++	+++	++++	O*	+	++	+++	++++
<i>Streptococcus pyrogens</i>																				
<i>Corynebacterium ulcerans</i>																				
<i>Escherichia coli</i>	O*	+	++	+++	++++	O*	+	++	+++	++++	-	O*	+	++	+++	O*	+	++	+++	++++
<i>Klebselia pneumonia</i>																				
<i>Shigellia dysentriae`</i>	O*	+	++	+++	++++	O*	+	++	+++	++++	O*	+	++	+++	++++	O*	+	++	+++	++++
<i>Pseudomonas aureginosa</i>	O*	+	++	+++	++++	-	O*	+	++	+++	-	O*	+	++	+++	O*	+	++	+++	++++
<i>Candida albicans</i>	O*	+	++	+++	++++	O*	+	++	+++	++++	-	O*	+	++	+++	O*	+	++	+++	++++
<i>Microsporium Canis</i>																				
<i>Trichophyton Spp</i>																				

Key: - = No colony growth, o* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth

Table 7: Control against the Test Microorganism

Test organism	Ciprofloxacin	Sparfloxacin	Fulcin	Fluconazole
<i>Staphylococcus aureus</i>	S	S	R	R
<i>Streptococcus pyrogens</i>	R	S	R	R
<i>Corynebacterium ulcerans</i>	S	R	R	R
<i>Escherichia coli</i>	S	S	R	R
<i>Klebselia pneumoniae</i>	R	S	R	R
<i>Shigellia dysenteriae`</i>	S	R	R	R
<i>Pseudomonas aureginosa</i>	R	S	R	R
<i>Candida albicans</i>	R	R	R	S
<i>Microsporium Canis</i>	R	R	S	R
<i>Trichophyton Spp</i>	R	R	S	R

Key: S= sensitive R= Resistance

Table 8: Zone Of Inhibition of the Drug against the Test Microorganism

Test organism	Ciprofloxacin (5mg/ml)	Sparfloxacin (10mg/ml)	Fulcin (5mg/ml)	Fluconazole (5mg/ml)
<i>Staphylococcus aureus</i>	30	32	0	0
<i>Streptococcus pyrogens</i>	0	30	0	0
<i>Corynebacterium ulcerans</i>	29	0	0	0
<i>Escherichia coli</i>	37	34	0	0
<i>Klebselia pneumoniae</i>	0	32	0	0
<i>Shigellia dysenteriae`</i>	38	0	0	0
<i>Pseudomonas aureginosa</i>	0	30	0	0
<i>Candida albicans</i>	0	0	0	34
<i>Microsporium Canis</i>	0	0	31	0
<i>Trichophyton Spp</i>	0	0	30	0

Conclusion

This work has really shown from the phytochemical screening that the fruit of *Sarcocephalus latifolius* contains some active metabolites which were substantiated from the antimicrobial activities of the various extracts. These activities show that the active components in these extracts are favourably comparable to standard antibiotic and antifungal drugs. Likewise the proximate analysis values show that this fruit can serve as food. Despite these results, it will be of good help if further work can be done on the level of the fruit toxicity as food material.

REFERENCE

Aberoumand, A. and Deokule, S.S. (2009). Proximate and Mineral Composition of wild coco (*Eulophia Ochreata*) tubers in Iran. *Asian Journal Food Agro industry* 2: 203-209.

Akubue, P. I., & Mittal, G.E. (1982). Clinical evaluation of a traditional herbal practice in Nigeria: preliminary report. *Journal of Ethno pharmacology* 6(3):355 - 359.

Anhwange, B.A., Ajibola, V. O., Oniye, S. J. (2004). Amino Acid Composition of the Seed of *Moringa oleifera* (Lam), *Detarium microcarpum* (Guill & Sperr) and *Bauhinnia monandra* (Linn) *Chemclass Journal*. 9 – 13.

AOAC (1980). Association of Official Analytical Chemists, Official Method of Analysis 13th (ed.) Washington DC, USA 125-127.

AOAC (1990). Association of Official Analytical Chemists, Washington D.C. USA.

- Arbonnier, M. (2002). Trees, Shrubs and Lianas of West African Dry Zones (2nd edn. revised.) Neitherland: Cirad- Margraf.
- Ayessou NC, Ndiaye C, Cisse M, Sakho M (2011). Nutritional contribution of some Senegalese Forest fruits running across Soudano-Sahelian Zone. *Food and Nutrition Sciences* 2: 606-612.
- Barry, A. L. & Thornsberry C. (1985). Susceptibility tests, diffusion test procedure. *J. Chem. Pathol.* 19:492-500.
- Brain, K. R. & Turner, T. D. (1975). The practical evaluation of phytopharmaceuticals. Wright-Science Technical. Bristol Britain. pp. 56-64.
- Burhingame, B. (2000). Comparison of Total Lipids, Fatty Acids, Sugars and Non Volatile Organic Acids in Nuts from Castanea species. *J Food Comp Anal* 13: 99-100.
- Etkin, N. L., Ross, P. J., Mauzzani, U. (1990). The indigenization of Pharmaceutical Therapeutic Transitions in natural Hausa Land. *Surgical. Sciences Medicines.* 30(8):919 – 928.
- Hassan, L.G.. and Umar, K. J. (2004). Proximate and Mineral Compositions of Seed and Pulp of African Locusts Beans (*Parkia biglobosa* l.). Nigerian Journal of Basic and applied Sciences. 13: 15-17.
- Hostettmann K, Marston A (1995). Saponnins. Cambridge University Press, Cambridge, UK.
- Hotellier, F., Delaveau, P. and Pousset, J. (1975). Nauclefine et Nauclefine deux nouveaux alcaloides de type indoloquinolizidine isoles du *Nauclea latifolia*. *Phytochemistry*, Vol. 14: pp 1407-1409.
- Hotellier, F., Delaveau, P. and J.Pousset (1979). Nauclechine and Naufoline alkaloid from *Nauclea*
- Isah, Y., Ndukwe, I. G. and Amupitan, J. O. (2012). Isolation and bioactivity of pentacyclic triterpenoid (Betunilic acid) from the bark of *Sarcocephalus latifolius* (Smith Bruce). *Journal of Natural Sciences Research* Vol.2, No.4, ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online). www.iiste.org
- Isah, Y., Ndukwe, I. G., Ayo, R. G. and Rufai, Y. (2015). Vacuum Liquid Chromatographic Isolation and Characterization of Beta-Sitosterol from the Bark of *Sarcocephalus Latifolius* (Smith Bruce). *Journal of Chemistry and Chemical Sciences, Vol.5 (9), 480-488*, (An International Research Journal), www.chemistry-journal.org
- Isah, Y., Ndukwe I.G, Rufai Y. and R.G. Ayo (2014). Characterization and Microbial Activities of B-Sitosterol and B-Sitostenone Mixture Isolated From The Stem Bark of Methanol Fraction of *Sarcocephalus Latifolius* (Smith Bruce). *International Research Journal of Natural Sciences* Vol.2, No.2, pp.1-13, June 2014 Published by European Centre for Research Training and Development UK (www.ea-journals.org).
- Lamidi, M., Ollivier, E., Faure, R., Debrauwer, L., Nze-Ekekang, L., and Balansard, G. (1995). Quinovic acid glycosides from *Nauclea diderichii*. *Planta Med.*61:280–281.
- Madibunyi IT (1995). Anti-hepatotoxic of ethanolic extract of *Nauclea Latifolia* root bark. *Journal of herbs, spices and medicinal plants.* 3(2):23-25.
- Morah, F.N.I (1994). Naucleidal and Epinaucleidal from antiviral preparation from *Nauclea*.
- Nahar N, Rahman S, Mosiuhuzzaman M (1990). Analysis of carbohydrates in seven edible fruits of Bangladesh. *Journal of Sciences, Foods and Agriculture.* 51: 185-192.
- Ndukwe, I. G., Achimugu, M. O., Amako, N. F. (2005). Phytochemical and antimicrobial screening of crude extracts from the stem bark of *irvingia gabonensis*. *Journal of Pest, Diseases Vector Management.* 6: 391-397.

- Oye, G. L. (1990). Studies on anti-malarial action of *cryptolepis sanguinolenta* extract. Proceedings of International Symposium on East - West medicine. Seoul, Korea. PP 243-251.
- Pearson, A. (1994). Vitamins in Fruits. The Biochemistry of Fruit and Other Products. New York: Academic press 369-384.
- Shigemori, H., T. Kagata, T. Ishiyama, H. Morah, F. Ohsaki, A. and Kobayashi, J. (2003).
Naucleamides A-E, New monoterpene indole alkaloids from *Nauclea latifolia*. *chemical. Pharmaceutical Bulletin* Vol.51 pp 58-61.
- Umar, K. J., Hassan, L. G., Ado, Y. (2007). Mineral Composition of *Detarium microcarpum* Grown in Kwatarkwashi, Zamfara State, Nigeria. *International Journal of Pure Applied Sciences*. 1: 43-48.
- Yesufu, H. B., Hussaini, I. M. (2014). Studies on Dietary Mineral Composition of the Fruit of *Sarcocephalus latifolius* (Smith) Bruce (Rubiaceae). *Journal of Nutritional Food Sciences*. S8:006. doi:10.4172/2155-9600.S8-006