International Journal of Biology Research
ISSN: 2455-6548
www.biotechjournals.com
Volume 1; Issue 1; March 2016; Page No. 15-18

A Study on the Phytochemical, Proximate and Antimicrobial Properties of Root Extract of *Nauclea latifolia* Smith

[1] Chukwuma O Maureen, [2] Akachukwu E Esther, [3] Adimonyemma N Ruffina, [*4]Iroka F Chisom

[1, 2, 3] Department of Biology, Nwafor Orizu College of Education, Nsugbe, Anambra State Nigeria.

[4] Department of Botany, Nnamdi Azikiwe University, P. M. B 5025 Awka, Anambra State Nigeria.

Abstract
The phytochemical, proximate and antimicrobial properties of *Nauclea latifolia* were investigated using standard procedures. Fresh leaves were collected, washed, shredded, sundried before milling into powder and then subjected to qualitative phytochemical, proximate and antimicrobial analysis. The phytochemical screening recorded the presence of bioactive important substance such as tannins, flavonoids, saponins, steroids, phenols and cardiac glycoside in the leaves. The proximate analyses revealed that the dry leaves contain high amounts of ascorbic acid (66mg/100g), crude fiber (14.44%), carbohydrate (38.67%) and appreciable amounts of protein (20.05%). The powdered samples were extracted in five different solvents (hot and cold ethanol; hot and cold water and cold ether), and tested against five pathogenic organisms (*Staphylococcus aureus, Candida albicans, Escheriachia coli, Klebsiella pneumonia*, and *Proteus sp.*). The effects of the leaf extracts and a standard antibiotic (Ciprofloxacin) which served as the control were used to compare the potency of the leaf extracts. The leaf extracts recorded reasonable zones of inhibitions (5-12mm) suggesting susceptibilities of the micro-organisms to root extract in some of the solvents used.

Keywords: Phytochemical, Proximate, Antimicrobial, *Nauclea latifolia, Staphylococcus aureus, Candida albicans, Escheriachia coli, Klebsiella pneumonia*, *Proteus sp.*

1. Introduction

Nauclea latifolia Smith (family: Rubiaceae) is a straggling, evergreen, multi-stemmed shrub or small tree native to tropical Africa and Asia. It grows up to an altitude of 200 m. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. It grows rarely over 20ft high, bole crooked; or a larger tree over 100ft high and 8ft girth, in closed forest. The plant has rough bark, leaves are 7 by 4-5 inches and are glabrous obovate. Flower head is up to 2mm in diameter, sweet scented and sought by bees. Three other related species *Nauclea pobeguini, N. diderichii,* and *N. vanderguchtii* are forest trees. In folk medicine, the species *N. diderichii* and *N. orientalis* are used in the same way as *N. latifolia. Nauclea latifolia* has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces. The fruit is syncarp, up to 3 inches in diameter. The tree flowers from April to June. The fruits ripen from July to September. Baboons eat them and disperse the seeds. Livestock eat shoots and leaves. The fruits are edible too.

Nearly all plant parts are useful in treatment of diseases. Infusions and decoctions of the stem bark and leaves of *Nauclea latifolia* are used for the treatment of stomach pain, constipation, fever, and diarrhea. In kano (Nigeria) the plant is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni and Hussain 1991) [1]. In Ivory Coast infusions and decoctions from stems and roots of *N. latifolia* are used against malaria by traditional healers (Benoil-vical *et al*., 1998) [2]. In Kinshasa, extracts and preparations together with other plants are applied against diarrhoea. Abbiw (1990) [3] stated that root infusion of *N. latifolia* is used in Sudan for the treatment of gonorrhoea its roots and leaves are used in Ghana for treating sores. In Nigerian folklore medicine, the fruits are sometimes used in the treatment of piles and dysentery. Because of its reported anti-malarial activity, the plant has been known as 'African cinchona' or 'African quinine'. (Abbiw 1990; Gidado *et al*., 2004) [3, 4] demonstrated that aqueous extract of the leaves of *N. latifolia* possess hypoglycaemic activity in alloxan-induced diabetic rats. The plant is also used in hypertension (Akabue and Mittal, 1982) [5], gastrointestinal tract disorders (Maduabunyi, 1995) [6], sleeping sickness and to prolong menstrual flow (Elujoba, 1995) [7].

Taxonomy
Family: RUBIACEAE
Genus: *Nauclea*
Species: *latifolia*
Accepted name: *Nauclea latifolia* Smith.
Synonyms: *Sarcocephalus latifolius* (Smith.) E. A. Bruce
Common names
English: African peach, Pin cushion tree, Guinea peach.
Igbo: Ubulinu.
Hausa: Tafashiya, tafiyaigia.

The various usage of *N. laifolia* by local people suggests that the plant contains nutritional and bioactive substances which may be useful precursors for making drugs such as antibiotic. Hence, the focus of this study is to undertake the phytochemical/proximate screening of the plant and evaluate the antimicrobial properties of the root extract of *Nauclea latifolia* using different organic solvents.

2. Materials and Methods

The plant (*Nauclea latifolia)* was collected from the Botanical garden of Nnamdi Azikiwe University, Awka, Anambra State Nigeria. The root was cut, washed with clean water, wiped dry with paper towels and oven dried for 72 hours at room temperature. Afterwards, it was milled and sieved to obtain fine powder which was then stored in an air tight glass containers for laboratory analysis.

2.1 Phytochemicals Analysis.

The qualitative phytochemical screening of the powdered plant sample was done to determine the presence of tannins, saponins, steroids, and cardiac glycoside, alkaloids, flavonoids, phenols and reducing sugar, using standard procedures (Harborne, 1991; Sofowora 1993; Evans, 2002 and Edeoga *et al.*, 2005) [8, 9,10, 11].

2.2 Proximate Analysis

The dry powdered sample was subjected to proximate analysis following the procedure recommended by AOAC (1990) [12], to determine the contents of ascorbic acid, proteins, fiber, carbohydrates and some inorganic mineral salts.

2.3 Antimicrobial Activity

For the antibacterial sensitivity tests, 5.6g of nutrient agar was weighed and diluted with 200ml of sterile distilled water, mixed and sterilized at 21^{o}C for 15minutes, allowed to cool and then poured into different sterile Petri dishes to solidify.

2.4 Extraction in various solvents

To prepare the standard aqueous extraction of the root powder, two sets of 0.5g of root sample were weighed and placed in two separate sterile containers. 5ml of cold distilled water was added to one and hot water at 80 ^{O}C to the other. The two set-ups were properly labeled. A Petri-dish containing prepared medium was streaked with the different test organism (K. *pneumonia, C. albicans, S. aureus, E. coli, Proteus spp*.) Using a pipette, 0.1ml of the extract was inoculated using filtered paper. The medium with the streaked organisms, control antibiotic (Ciprofloxacin) as well as the inoculated medium were placed in the incubator at 20 ^{O}C and allowed to grow for 24hrs. The above procedure was repeated using cold ethanol and ether. The zones of inhibition were measured in millimeters (mm) for each extract that is cold and hot extracts.

3. Results

Table 1: Qualitative phytochemical screening of root extract of *Nauclea latifolia* constituents.

Flavonoid	Alkaloid	Saponin	Steriod	Phenol	Tannin	Cardiac glycoside	Terpene
+	+	+	+	+	+	+	+

+ = Present -= Absent

Phytochemical screening of the root of *Nauclea latifolia* revealed the presence of saponins, tannins, flavonoids, cardiac-glycoside, steroids and phenols as the major phytochemical components which occur in significant quantities.

Table 2: Proximate properties of root extract of *Nauclea latifolia*

Moisture Content (%)	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	Carbohydrate (%)	Ascorbic acid (mg/100g)
7.5	19.9	0.52	14.44	20.05	38.67	66.0

The proximate properties of the plant are summarized in Table 2. It was observed that the plant contains reasonable amount of protein (20.05%), fiber (14.44%), carbohydrates (38.67%) and ascorbic acid (66.0mg/100g) but low levels of fat (0.52%).

Table 3: Zones of inhibition (mm) of *Nauclea latifolia* root extract (0.01g/ ml) in different solvents and control antibiotic.

Test Organisms	Aqueous		Ethanol		Ether	Control Ciprofoxacin
	Hot	Cold	Hot	Cold		
Escherichia coli	9	-	10	7	-	37
K. pneumoniea	-	-	5	8	-	35
Proteus spp.	-	-	9	6	-	26
Candida albicans	-	-	10	12	-	-
S. aureus	-	-	5	7	-	18

The antimicrobial properties of the plants were also investigated and the results summarize in Table 3. The results showed that *Staphylococcus aureus* was moderately sensitive to hot and cold ethanol extract recording 5mm and 7mm inhibition zones respectively. The results showed that *S. aureus* is highly sensitive to Ciprofloxacin (18mm). However, the test organism was resistant to ether as well as hot and cold aqueous extractions of the plant under study.

E.coli was moderately sensitive to the hot and cold extract of ethanol (10mm and 7mm respectively). It was also sensitive to hot aqueous extract (9mm), but was highly sensitive to Ciprofloxacin (37mm). However, the test organism was resistance to hot aqueous and ether extracts.

Klebsiella was moderately sensitive to the hot and cold ethanol extract (5mm and 8mm respectively), but it was highly sensitive to Ciprofoaxacin (35mm). However the test organism was resistant to cold and hot as well as the ether extracts. *Proteus spp.* was moderately sensitive to hot and cold ethanol extract (9mm and 6mm respectively), but it was highly sensitive to Ciprofoaxacin (26mm). On the contrary, the test

organism was resistant to hot and cold aqueous and ether extracts.

Candida albicans was sensitive to hot and cold ethanol extract (10mm and 12mm respectively). There was no inhibitory activity observed in Ciprofoaxacin in the present study. The test organism *C. albicans* was resistant to ether and also hot and cold aqueous extractions.

4. Discussion

The phytochemical screening of the chemical constituents of *Nauclea latifolia* studied revealed the presence of flavonoids, tannins, saponins, steroids, phenols and cardiac glycoside which is in line with previous studies of Harborne, (1991) [8]; Akindehusi and Salawu (2005). The medicinal importance of tannins, saponins, flavonoid, steroids, phenols and reducing sugar which are components of traditional herbal preparation used in managing various ailments has been reported by Addae-Mensah, (1999) [14] and Mensah *et al.* (2013) [5] in other plants. Flavonoids, alkaloids, phenolics, terpenes and tannins which are known antioxidants are found to be distinctly present in the root of this plant. Tannins are known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, anti-inflammatory, diuretic effects and have been reported to hasten the healing of wounds and inflamed mucus membrane (Okwu and Okwu, 2004) [16]. Flavonoids are known for their antioxidant activity, and hence help to protect the body against cancer and other degenerative disease such as Arthritis and Type II diabetes mellitus (Lee and Shibumoto, 2002) [17]. Cardiac glycoside acts on the heart muscles and increases renal flow (dieresis). Herbal preparations containing cardiac glycoside are used for the treatment of congestive heart failure and cardiac arrhythmia. Saponins are used as mild detergent and in intracellular histochemical staining to allow antibody access to intracellular proteins. It is of great importance in medicine because it is a source of anti-oxidant, anti-cancer, anti-flammatory and body-weight loss agents. Saponins are expectorants, cough suppressors and administered for hemolytic activities (Okwu 2005) [11, 18].

Steriods increase muscles and bone synthesis (Rossier, 2006) [19] and are also associated with hormonal control in women. The most common steroid is cholesterol; steroids regulate carbohydrates and protein metabolism and possess anti-inflammatory properties. Phenolic compounds are anti-microbial agents, hence it is extensively used in disinfections and remain the standard with which other bactericides are compared (Okwu, 2005) [11, 18].

The proximate properties of the plant, it was observed that the root contained moderate amounts of ascorbic acid (66.0 mg/100g). Ascorbic acid prevents degenerative diseases and aging. The ascorbic acid content observed signifies that the plant contains antioxidant properties. According to Martinez *et al.* (1996) [21], high intake of ascorbic acid correlates with reduced gastric cancer. This vitamin plays a role as scavengers of free radicals and making this leafy vegetable effective as protector of the integrity of lipids and phospholipids. High crude fiber (14.44%) and carbohydrate (38.67.05%) were recorded in the leaves of this plant. Food fibers have been shown to aid absorption of dietary minerals as well as reduce absorption of cholesterol and aid digestion. In addition, the root contain appreciable amounts of protein (20.05%) and this compared significantly with and in most cases surpassed those reported for most medicinal plants and cereal/protein such as water spinach (6.3%), sweet potatoes(24.85%) but lower than Moringa (27.51%) (Adinortey *et al.,* 2012). This signifies that the root of the plant contain healing properties as proteins are essential for the synthesis/repair of body tissues and as enzyme. Lower levels of fat (0.52 %) were recorded when compared to water spinach (11%) and *Amaranthus* (1.6%) and hence could be used to control weight.

The antimicrobial activities observed in the various extracts of the plant *Nauclea latifolia* (cold and hot aqueous extracts, cold and hot ethanol extracts and ether extract, revealed that hot and cold ethanol extraction showed inhibitory effect against all the test organisms. The antimicrobial property of the plant was also investigated. In this report, it has been reported that *Staphylococcus aureus* was moderately sensitive to hot and cold ethanol extract recording 5mm and 7mm inhibition zones respectively. It has been observed in this study that natural and bioactive compounds in plants are best extracted using ethanol solvents. The effects could be due to the fact the active ingredients in the roots are more soluble in alcohol solvent than others as earlier reported by Martinez *et al.,* (1999). The results further showed that *S. aureus* is moderately sensitive to Ciprofloxacin (18mm). However, the test organism was resistant to hot aqueous, cold aqueous and ether extractions of *Nauclea latifolia*.

E. coli was moderately sensitive to the hot and cold extract of ethanol (10mm and 7mm). It was also sensitive to hot aqueous extract (9mm), but was highly sensitive to Ciprofloxacin (37mm). However the test organism was resistant to hot aqueous and ether extracts. Studies by Olajumole *et al.* (2012) [22] confirm that alcohol is a good solvent for the extraction of antimicrobial properties in *Nauclea latifolia*.

Klebsiella was moderately sensitive to the hot (5mm) and cold ethanol (8mm) extracts, but it was highly sensitive to Ciprofloxacin (35mm). However the test organism was resistant to cold aqueous extract, hot aqueous extract and ether extract.

Proteus spp. was moderately sensitive to hot (9mm) and cold (6mm) ethanol extract, but it was highly sensitive to Ciprofloxacin (26mm). However, the test organism was resistant to hot and cold aqueous and ether extracts.

Candida albicans was sensitive to hot and cold ethanol extracts (10mm and 12mm respectively). However, there was no inhibitory activity observed in Ciprofloxacin in the present study. The test organism *C. albicans* was resistant to hot and cold aqueous extract and ether extract. Generally the study showed that water is not a good solvent for the extraction of solutes which have inhibitory activity from this plant.

Conclusion

The phytochemical contents of the root of *Nauclea latifolia* serve as a proper supplement for nutrients due to their nutritional composition and also have the potential to improve the health status of its user. The phytochemical screening of the plant showed that it is rich in tannins, alkaloids, flavonoids, saponins, phenols, reducing sugar, steroid and cardiac glycoside which are common constituents of many traditionally prepared herbal medicines and plant extract. The presence of these compounds in plants has been attributed to their biological activities. Furthermore, it has been noted that the presence of phytochemical compounds in this plant is responsible for the observed antimicrobial activities. The

results of this study also indicated that the root extract of *Nauclea latifolia* contain essential nutrients and phytochemicals which compare favourably with other conventional plant extract which are commonly used by local indigenes.

References

1. Deeni YY, Hussain HSN. Screening for antimicrobial activity and for alkaloids of Nauclea latifolia. J Ethnopharm. 1991; 35:91-6.
2. Benoit-Vical F, Valentin A, Cournac V. In vitro antiplasmodial activity of stem and root extracts of *Nauclea latifolia* S.M. (Rubiaceae). J Ethnopharm. 1998; 61:173-8.
3. Abbiw KD. Useful Plants of Ghana, West Africa; uses of wild and cultivated plants, Intermediate Technology Publication, London. 1990, 98-212.
4. Gidado A, Ameh DA, Atawodi SE. Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr J Biotech. 2005; 4(1):91-93.
5. Akabue P, Mittal HC. Clinical Evaluation of a Traditional Herbal Practice in Nigeria: A Preliminary Rep. J Ethnopharm. 1982; 6(3):355-359.
6. Madubunyi II. Anti-Hepatotoxic and Trypanocidal Activities of the Ethanolic Extract of *Nauclea latifolia* Root Bark. J Herbs Spices Med Plants. 1995; 3(2):23-53.
7. Elujoba AAA. Female Infertility in the Hands of Traditional Birth Attendants in South-West Nigeria. Fitoterapia, 1995; 66(3):239-248.
8. Harborne JA. Phytochemical Methods, A guide to common techniques of plant analysis, 1991.
9. Sofowora AE. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993, 289.
10. Evans WC. Trease and Evans Pharmacognosy. 15th Edn. Baiuiere Tindall, London, ISBN-10.0702026174, 2002.
11. Edeoga HA, Okwu DE, Mbaebie BO. Phytochemical constituent of some Nigerian Medicinal Plants. Afr J Biotehnol. 2005; 4:685-688.
12. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis. Washington. USA. 1990.
13. Akindenusi AA, Salwu SO. Phytochemical screening and nutrient composition of selected tropical green leafy vegetable. Afr J Biotechnol. 2005; 4:497-501.
14. Addae-Mensah I. towards a rational science basis for herbal medicine. A Phytochemist's two decades contribution. Ghana University Press, Accra. 1999, 63.
15. Mensah JK, Ihenyen JO, Iyamu M. Phytochemical and antimicrobial properties of Emilia coccinea (Cass.) As J Contemp Sci, 2013; 2(2):26-31.
16. Okwu DC, Okwu ME. Chemical Composition of Spondias mombin Linn. Plant aparts. J Sustain Agric Environ, 2004; 6:30-34.
17. Lee KG, Shibumoto J. Determination of antioxidant potentials of violatile extracts isolated from various herbs and spices. J Agric Food Chem. 2002; 50:4947-4955.
18. Okwu DC. Phytochemical, Vitamin and Mineral Contents of Two Nigerian Medicinal plants. Int J Molecul Med Adv Sci, 2005; 1:372-381.
19. Rossier MF. T-channel and steroid biosynthesis: In search of a link with mitochrondria. Cell calc, 2006; 40:155-164.
20. Adinortey MB, Sarfo JK, Quayson ET, Weremfo A, Adinontey CA, Ekloh W. *et al* Phytochemical screening. Proximate and mineral composition of *Launaea taraxcifolia* leaves. Res J Med Plant. 2012; 6:171-179.
21. Martinez MJ, Betamcourt J, Alonso-Gonzalez N, Jauregai A. Screening of some Cuban Medincal plants for antimicrobial activity. J Ethnopharm, 1996; 52:171-174.
22. Olajumoke OO, Soretiwa SA, Owolabi LO. Phytochemical Screening, anti-nutrient composition, proximate analyses and antimicrobial activities of the aqueous and organic extracts of bark of Ravolfia vomitoria and leaves and Peperomia pellucida. Int Res J Biochem Bioinfor. 2012; 2:127-134.