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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Nauclea latifolia* (AFRICAN PEECH) FRUIT AND LEAF CRUDE EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

The phytochemical screening and antibacterial activity of *Nauclea latifolia* fruit and leaf extracts against some pathogenic bacteria were investigated. The phytochemical screening was conducted using standard qualitative methods. The antibacterial activity was assayed using the agar well diffusion method. The phytochemical screening of *Nauclea latifolia* leaf extracts revealed flavonoids, resins and saponins, while fruit extracts indicated the presence of alkaloids, anthraquinones, flavonoids and resins. The test isolates included *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* Typhi. The *Nauclea latifolia* methanol leaf extracts showed greater inhibitory effect against the test organisms at 10 and 20 mg/mL concentration with zones of inhibition ranging from (15.00-21.00 mm) and (28.00-31.00 mm), while methanol fruit extracts recorded zones of inhibition ranging from (11.00-18.00 mm) and (21.00- 27.00 mm) at 10 and 20 mg/mL. The results obtained show that the extracts were more active than ciprofloxacin (positive control) with zones of inhibition ranging from (3.00-6.00 mm) and (8.00-12.00 mm) at 10 and 20 mg/mL. The result has justified their utilization in the treatment of various ailments associated with the test organisms.

Keywords: Antibacterial, Extracts, Phytochemical, *Nauclea latifolia.*

INTRODUCTION

Medicinal plants are mostly herbs; shrubs, trees, and these plants are passed from generation to generation usually through some specialized people known as local Doctors or Herbalist (Lewis, 1981). However, a medicinal plant is a plant in which one or more of its organs contains bio-active ingredients that can be used for therapeutic purposes or hemi- synthesis of drugs (Sofowora, 1982).

Nauclea latifolia is a perennial plant belonging to the sub-family Rubiaceae anda large Family of *Leguminoseae*. *Nauclea latifolia* is commonly called *Gbeashi* (Nupe), *Tabasiya* (Hausa), *Mbom-mbog* (Ijaw). It was reported to be a dry savannah plant and sometimes found in undisturbed fringing forest and closed savannah woodland (Muhammad *et al*., 2016). *N. latifolia* is a deciduous plant with an open canopy and growing up to 9 meters tall. The plant has small branches that are thick and dropping. The bark is dark grey, fibrous and cracked. The leaves are shiny green, oval and rounded at the base but pointed at the top. The flowers are white-yellow and occur in a single rounded heads. The fruit is a compound fruit, red or pinkish and round, consisting of very small seeds (Mann *et al*., 2003) (Plate 1).

In Nigeria, especially Bida, Kano and Kaduna; the plant was reported to be used as a chewing stick and remedy against stomach ache and tuberculosis (Mann *et al*., 2003). In Nupe land, infusion of *N. latifolia* leaves were reported to be used to treat stomach pain, constipation, andere reported to be used to treat stomach pain, constipation, fever, and diarrhoea (Mann *et al*., 2003). The leaf was also reported to be used in Brazil to treat eczema, psoriasis, venereal diseases, impotence, bronchitis, cough and intestinal colitis and syphilis-related skin diseases (Deani and Hussain, 1991).

The stem- bark infusion and decoction of *N. latifolia* was commonly used in Ivory Coast by traditional healers to treat several diseases such as jaundice, malaria, infant gastroenteritis, and dysentery (Lamidi *at al*., 1995). Therefore, the aim of this study was to determine the antibacterial activity of *N. latifolia* fruit and leaves extracts against selected bacterial

Plate I: Photograph of *Nauclea latifolia* plant.

Materials and Methods

Collection and Identification of Plant Samples

The fresh fruits and leaves of *N. latifolia* plant were obtained around the river Musa'a water bank along Federal Girls' College road, Bida Niger State, Nigeria. The plant was harvested by Mallam Abdulateef Ahmed, identified and authenticated by a Botanist from the Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria. A voucher specimen number NL/00145 was deposited at Departmental Herbarium. The identification was done based on the literature and Atlas of medicinal plants by Mann *et al*. (2003).

Preparation of Plant Samples

The fruits and leaves of *N. latifolia* were washed with tap water, rinsed with distilled

water, cut into pieces and air dry in the laboratory for three weeks at room temperature (22^0C) in a shaded area (Muhammad *et al*., 2020). The dried plant parts were pulverized using sterile laboratory Mortar and Pestle to obtain the powder. A Mesh sieve of 0.25 mm was used to sieve the pounded powder to get a fine powder. The fine powders were stored in air-tight sterile bottles at ambient temperature (30^0C) until further analysis.

Extraction of Pulverized Plant Sample

Extraction of plant samples was carried out using Soxhlet extractor. Acetone, water, hexane and methanol were used as solvents of extraction (extractants). Methanol being polar solvent has the capacity to extract hydrophilic compound, while hexane (non-polar) can extract lipophilic compounds. However, acetone is an intermediate polar (polar aprotic) capable of extracting some hydrophilic and lipophilic compounds.

A hundred gram (100g) of the powdered fruit of *N. latifolia* was weighed and wrapped in plain paper and placed in a Soxhlet extractor and extracted with water, acetone, hexane, and methanol, respectively. The extraction was done until the solvent in the Soxhlet turned colourless. The solution (filtrate) was transferred into a Porcelain dish, then allowed to dry. The extracts obtained were labeled, weighed, and kept for further analysis (Muhammad *et al*., 2020). The same procedure was followed for leaf extraction.

Reconstitution of Plant Extracts

The dried extracts were weighed into McCartney bottles, and two hundred milligrams (200 mg). Each of the fruit and leaf extracts of *N. latifolia* was dissolved into 10mL of glycerol to make a stock solution containing 20 mg/mL concentration respectively20 mg/mL concentration. The serial dilution was performed to yield 10.0, 5.0 and 2.5mg/mL concentrations respectively (Muhammad *et al*., 2020).

Phytochemical Screening of the *N. latifolia* Extracts

The phytochemical screening of major chemical constituents was carried out using standard qualitative methods. The phytochemical constituents tested were anthraquinones, alkaloids, glycosides, flavonoids, resins, saponins, steroids and tannins.

Test for anthraquinones

To 4mL of each extract in a test tube, 4mL of 100% ammonium solution was added. A pink violet or red coloration in the ammoniac layer in each test tube indicated the presence of anthraquinones (Danlami, 2009).

Test for alkaloids

To 3mL of extract introduced into the different test tubes, 1mL of 1% of hydrochloric acid (HCL) was added. The mixture was later treated with few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids (Ogukwe *et al*., 2004).

Test for flavonoids

1mL of each extract, three drops of ammonia solution were added. Half (0.5) mL of concentrated HCL was further added to the mixture. A pale brown coloration indicated

the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for test resins

5mL of each extract, 5mL of copper acetate solution was added and shaken vigorously, and then allowed to separate. A reddish-brown precipitate indicated the presence of resins (El-mahmood and Doughari, 2008).

Test for saponins

Half milliliter (0.5mL) of each extract in a test tube, 5.0mL of distilled water was added. The mixture was then shaken vigorously for 2 minutes; the froth formation indicated the presence of saponins (Hassan *et al*., 2005).

Test for steroids

To 1mL of each extract, 1mL of concentrated tetraoxosulphate (IV) acid (H_2SO_4) was added. A reddish coloration indicated the presence of steroids (Hassan *et al*., 2005).

Test for tannins.

To 5mL of each extract, 2 drops of 5% $FeCL_3$ was added. A dirty green precipitate indicated the presence of tannins (Ogukwe *et al*., 2004).

Collection and Maintenance of Test Bacteria Species

The pure culture isolates were obtained from Department of Microbiology Laboratory of Ibrahim Badamasi Babangida University Lapai, Niger State. The four bacteria isolates were *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The bacteria isolates were sub-cultured into nutrient agar slants. Prior to inoculation, the sterility of agar slant was done after preparation by incubating them at 37°C for 24 hours.

Preparation of Inoculums

A loopful of bacteria isolates was inoculated into 4 mL of peptone water and incubated at 37°C for four hours. The turbidity standard by actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 mL of 1.75% w/v barium chloride dehydrated with 99.5 mL of 1% v/v Sulphuric acid. This turbidity was equivalent to approximately $1.2x10^8$ colony forming unit per milliliter (cfu/mL). The two hours grown suspension was used for further analysis or testing (Cheesbrough, 2006).

Determination of Antibacterial Activity of *N. latifolia* fruit and leaf extracts.

Before antibacterial susceptibility testing was carried out, the isolates were sub-cultured into fresh nutrient agar slants and incubated at 37ºC for 24 hours. Eighteen milliliters (18 mL) of molten sterile Mueller-Hilton agar was poured into a sterile Petri plate and was allowed to solidify. The standardized suspension was used to inoculate the surface of agar plates using a sterile cotton swab. A sterile standard cork borer of (6mm diameter) was used to punch five wells aseptically into the agar and each well was filled with 0.2 ml of the extract, and a diffusion time of 45 minutes was allowed. The inoculated agar plates were then incubated at 37ºC for 24 hours. Ciprofloxacin was used as a positive control for bacteria isolates. The antibacterial activities were evaluated by measuring zones

of inhibition using a transparent metric ruler (Junaid *et al*., 2006).

RESULTS

The results of phytochemical screening of *N. latifolia* extracts are presented in Table 1. The phytochemical screening of acetone leaf extract revealed anthraquinones, flavonoids, resins, and steroids, while aqueous extract contained alkaloids, flavonoids, resins, and saponins. The methanol leaf extract indicated the presence of all phytochemicals tested except steroids

Table 1: The Qualitative Phytochemical Screening of *N. latifolia* Leaf Extracts

Phytochemicals	Acetone extract	Aqueous extract	Methanol extract
Alkaloids	-	+	+
Anthraquinones	+	-	+
Flavonoids	+	+	+
Resins	+	+	+
Steroids	+	-	-
Saponins	-	+	+
Tannins	-	-	+

Key: + =Present; - =Absent

The results of phytochemical screening of *N. latifolia* fruit extract are presented in Table 2. The phytochemical screening of *N. latifolia* acetone fruit extract indicated the presence of alkaloids, anthraquinones, flavonoids, steroids and tannins , while alkaloids, anthraquinones, flavonoids, resins, saponins and tannin were found to be in aqueous extract. However, methanol fruit extract contained anthraquinones, alkaloids, flavonoids, resins, saponins, and steroids except tannins that were not detected.

Table 2: The Qualitative Phytochemical Screening of *N. latifolia* Fruit Extracts

Phytochemicals	Acetone extract	Aqueous extract	Methanol extract
Alkaloids	+	+	+
Anthraquinones	+	+	+
Flavonoids	+	+	+
Resins	-	+	+
Steroids	+	-	+
Saponins	-	+	+
Tannins	+	+	_

Key: + =Present; - =Absent

The results of the antibacterial activity of *N. latifolia* crude leaf extracts are presented in Table 3. The result of antibacterial activity of *.N latifolia* crude leaf extracts indicated that the test isolates were resistant to acetone leaf

extract at 2.5 mg/mL and susceptible at 5.0 to 20.0 mg/mL concentration, respectively. *Escherichia coli* was susceptible to acetone leaf extract with zones of inhibition which ranged between 12.00 and 24.00 mm, while at the same concentration, *Klebsiella pneumoniae* exhibited zones of inhibition ranging from 11.00-31.00 mm, *Pseudomonas aeuroginosa* (9.00-20.00 mm), *Staphylococcus aureus* (7.00-17.00 mm) and *Salmonella* Typhi (7.00-21.00 mm).

Aqueous leaf extract inhibited all tested isolates at 10.0 mg/mL and 20.0 mg/mL concentration in which *Escherichia coli* recorded zones of inhibition (14.00-18.00 mm), *Klebsiella pneumoniae* (12.00-16.00 mm), *Pseudomonas aeuroginosa* (14.00-21.00 mm), *Staphylococcus aureus* (11.00-18.00 mm) and *Salmonella* Typhi (15.00-20.00 mm) respectively. At 5.0 mg/mL concentration, *Escherichia coli* and *Pseudomonas aeuroginosa* recorded zone of inhibition (11.00 mm and 10.00 mm) respectively.

Methanol leaf extract inhibited the entire isolates at 2.5-20.0 mg/mL concentration in which *Escherichia coli* recorded zones of inhibition ranging from (9.00 mm-28.00 mm), *Klebsiella pneumoniae* (7.00-26.00 mm), *Pseudomonas aeuroginosa* (10.00-30.00 mm), *Staphylococcus aureus* (11.00-31.00 mm) and *Salmonella* Typhi (11.00-31.00 mm) respectively. In addition, ciprofloxacin which served as positive control, also inhibited the entire test isolates with zones of inhibition ranging from (8.00-12.00 mm) and (3.00-6.00 mm) at a concentration of 20.0 and 10.0 mg/mL, respectively.

Table 3: Antibacterial Activity of *N. latifolia* Leaf Extracts against Some Bacteria

Test organisms	Zone of inhibition (mm) Concentration of extracts (mg/mL)													
	Acetone extract				Aqueous extract				Methanol extract				Ciprofloxacin	
	20	10	5.0	2.5	20	10	5.0	2.5	20	10	5.0	2.5	20	10
E. coli	24	18	12	00	18	14	11	00	28	17	13	09	08	04
K. pneumoniae	31	15	11	00	16	12	00	00	26	15	13	07	08	03
P. aeuroginosa	20	13	09	00	21	14	10	00	30	21	16	10	12	05
S. aureus	17	12	07	00	18	11	00	00	31	20	16	11	10	05
S. Typhi	21	14	07	00	24	15	00	00	31	21	17	11	12	06

Key: 00 = No zone of inhibition.

The results of the antibacterial activity of *N. latifolia* crude fruit extracts are presented in Table 4. The antibacterial activity assay indicated that four of the test isolates were resistant to acetone leaf extract at concentration of 2.5 mg/mL and 5.0 mg/mL concentration except *Escherichia coli* that was susceptible with zones of inhibition of 8.00 mm and 11.00 mm respectively. At the concentration of 10.0 and 20.0 mg/mL,

Escherichia coli was susceptible to acetone leaf extract with inhibition zones ranging between 13.00 mm and 18.00 mm. In contrast, *Klebsiella pneumoniae* at the same concentrations exhibited inhibition zones ranging from 11.00-15.00 mm. On the other hand, *Pseudomonas aeuroginosa* and *Staphylococcus aureus* were susceptible to acetone fruit extract with zones of inhibition ranging from 11.00-16.00 mm and 10.00-16.00 mm respectively at 5.0-10.0 mg/mL concentration, while *Salmonella* Typhi was sensitive to acetone leaf extract with zones of inhibition (12.00 mm and 19.00 mm) respectively.

Aqueous fruit extract inhibited all test isolates at 5.0 and 10.0 mg/mL concentration in which *Escherichia coli* recorded zones of inhibition (12.00 mm and 16.00 mm), *Klebsiella pneumoniae* (8.00 and 13.00 mm), *Pseudomonas aeruginosa* (11.00 mm and 17.00 mm), and *Staphylococcus aureus* (8.0 mm and 14.00 mm) respectively. At 2.5 mg/mL concentration, *Escherichia coli* recorded zone of inhibition (8.00 mm), while *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were resistant to aqueous fruit extract at 2.5 mg/mL and 5.0 mg/mL concentration, respectively.

Methanol fruit extract inhibited all test isolates. Escherichia coli recorded zones of inhibition ranging from 12.00 mm-24.00 mm at 2.5-20.0 mg/mL concentration, while Klebsiella pneumoniae recorded zones of inhibition ranged between 8.00 mm-21.00 mm at 5.0-20.0 mg/mL concentration respectively. *Pseudomonas aeruginosa* was also susceptible to methanol fruit extract at a 5.0-20.0 mg/mL concentration with inhibition zones ranging from 11.00 mm-26.00 mm, while Staphylococcus aureus recorded zones of inhibition ranged between 9.00 mm-25.00 mm at concentration of 5.0-20.0 mg/mL. *Salmonella* Typhi was sensitive to methanol fruit extract with zones of inhibition ranging from 11.00-27.00 mm at 5.0-20.0 mg/mL concentration. The entire bacteria were resistant to methanol fruit extract at 2.5 mg/mL concentration. Positive control (Ciprofloxacin) also inhibited the entire test bacteria with zones of inhibition ranging from 8.00-12.00 mm (Table 4).

Table 4: Antibacterial Activity of *N. latifolia* Fruit Extracts against Selected Bacteria.

Test organisms	Zone of inhibition (mm) Concentration of extracts (mg/mL)													
	Acetone extract				Aqueous extract				Methanol extract				Ciprofloxacin	
	20	10	5.0	2.5	20	10	5.0	2.5	20	10	5.0	2.5	20	10
E. coli	18	13	11	8.0	16	12	8.0	00	24	15	12	00	08	04
K. pneumoniae	15	11	00	00	13	8.0	00	00	21	11	8.0	00	08	03
P. aueroginosa	16	11	00	00	17	11	00	00	26	14	11	00	12	05
S. aureus	16	10	00	00	14	8.0	00	00	25	13	9.0	00	10	05
*S.*Typhi	19	12	00	00	18	11	00	00	27	18	11	00	12	06

Key: 00 = No zone of inhibition.

DISCUSSION

The phytochemical screening of *N. latifolia* leaf extracts indicated the presence of anthraquinones, flavonoids, resins, alkaloids, and saponins as the phytochemical constituent's common to all the extracts. This could be attributed to the fact that these phytochemical constituents are mostly found in the photosynthetic part of the plants such as leaves, vegetables, fruits, grains, and seeds (Muhammad *et al*., 2020).

The result is similar to the findings of Stephen *et al*. (2017), who reported the presence of alkaloids, flavonoids, glycosides, saponins and terpenoids in their studies on the phytochemical, antimicrobial and nutritional properties of *Nauclea latifolia* and *Morinda lucida* benth leaf extract. This finding conforms to the findings of Burkill, (1985), who also reported the presence of alkaloids, flavonoids, glycosides, saponins, reducing sugar and tannins in their studies on the antimicrobial activity of *N. latifolia* leaf extracts against various human pathogenic microbes. The result is however, contrary to the findings of El-mahmood *et al*. (2008) who documented the presence of glycosides, alkaloids, saponins and tannins in the leaf extracts of *Nauclea latifolia. This was demonstrated i*n their studies conducted in Yola, Adamawa State in their studies on *in-vitro* antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri.*

The phytochemical screening of *N. latifolia* fruit extracts indicated the presence of anthraquinones, alkaloids, flavonoids, resins, saponins and tannins. This may be attributed to the nature of solvents and the method of extraction adopted. Furthermore, some phytochemical constituents are - specific. For example, anthraquinones and flavonoids are mostly found in fruits, seeds, and grains. At the same time, alkaloids, terpenoids, resins, steroids, and tannins are detected, and this could be due to the season of plant harvest and chemical shift from other organs to the fruits. The study is similar to the findings of Muhammad *et al*. (2016), who documented the presence of anthraquinones, flavonoids, glycosides, steroids, and tannins in their studies on the phytochemical and toxicological studies of *N. latifolia* extracts.

From the result of phytochemical screening; phytochemical constituents detected in various extracts contributed immensely in their antibacterial activities against the test isolates. Comparatively, the study showed variation in zones of inhibition in which leaf extracts were more active than fruit extracts. For example, acetone leaf extract was more active against the test isolates than acetone fruit extract with zones of inhibition ranging from (17.00-31.00 mm) and (15.00-19.00 mm) respectively. This might be attributed to the presence of saponins in the leaf extract, which was absent in fruit extract. However, saponin was reported to be very effective against Gram-negative than Gram-positive bacteria. According to Lacail *et al*. (2010), saponin acts by altering the permeability of the cell membrane and hence exerting toxicity on all organized tissues of bacteria. This is done by combining with the cell membrane to elicit damages in cell morphology leading to cell lysis.

Aqueous leaf extract was observed to be more active than fruit extract against the test isolates which is also as a result of phytochemical constituents present. The presence of steroids in the leaf extract lacking in fruit extract contributed to their differences in their inhibition zones. Steroids have been reported to have synergetic effect with saponins and anthraquinones, which were also reported to inhibit DNA gyrase, thereby preventing DNA synthesis (Malziga and Badar, 2010).

As for methanol extracts: all tested isolates were more susceptible to methanol leaf than fruit extracts simply because of tannin that was found to be present in the leaf extract. Tannins are polyphenol with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes that destroy bacterial ribosomes thereby prevent protein synthesis (Tapa *et al*., 2008). Furthermore, both leaf and fruit extracts were more active than ciprofloxacin. Ciprofloxacin is a nucleic acid synthesis inhibitor. Therefore, once the test bacteria undergo mutation, the ciprofloxacin would not be able to penetrate the bacteria cell wall and cell membrane before acting on nucleic acid anatomically located in the bacterial nucleoid.

This study is contrary to the findings of El-mahmood *et al*. (2008); Okiei *et al.* (2011), and Stephen *et al*. (2017), who documented the sensitivity of *Escherichia coli* to *Nauclea latifolia* methanol and aqueous leaf extracts at concentrations of 12.50 mg/mL and 50 mg/mL with zones of inhibition (10.00 and 9.00 mm) and (20.00 mm and 0.00 mm) respectively.

The susceptibility of *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* Typhi to *Nauclea latifolia* fruit extracts with variation in their zone of inhibition could be attributed to the morphology and physiology of the test bacteria. It could also be attributed to phytochemical constituents in the extracts. However, no literature was found to support our findings regarding the activity of *N. latifolia* fruit extracts against the test bacteria.

CONCLUSION

This study has indicated that the *Nauclea latifolia* crude leaf extracts commonly contained flavonoids, resins and saponins, while fruit extracts indicated the presence of alkaloids, anthroquinones, flavonoids and resin. Furthermore, the *N. latifolia* leaf extracts showed appreciably inhibitory effect against test isolates at 5.0-20.0 mg/mL concentration than fruit extract. In contrast, methanol extracts had a greater inhibitory effect on the test bacteria. The results also showed that extracts were more active than Ciprofloxacin which served as the positive control.

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