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ANTI-SALMONELLA ACTIVITY OF ETHYL ACETATE FRACTION OF *Nauclea latifolia* LEAF USED FOR TYPHOID FEVER TREATMENT IN MINNA, NIGER STATE

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Abstract

Nauclea latifolia is used in ethnomedicine for the treatment of typhoid fever and other infections. This study was aimed at investigating the anti-salmonella activity of the ethyl acetate leaf fraction of *N. latifolia* against clinical isolates of *Salmonella enterica* Typhi. Cold maceration method was used to obtain ethanol extract from 200 g of *N. latifolia* fine powdered leaf sample using 1000 mL sterile conical flask for 72 hours. Fractionation of the ethanolic crude extract (19.8g) of *N. latifolia* leaf was performed with ethyl acetate (1500ml) and n-hexane using a Soxhlet apparatus. Agar well diffusion method was used to determine the anti-salmonella activity of the fraction on the isolates with the following concentrations: 40 mg/ml, 80 mg/ml, 120 mg/ml, 160 mg/ml, and 200 mg/ml. The result of the qualitative

Introduction

Typhoid fever is an infection that affects people of all ages, mostly in low-income and middle-income countries of the world (Basnyat *et al.*, 2021). This is because of factors such as poverty, insufficient access to clean water, and unhygienic practices (Ohanu *et al.*, 2019).

Typhoid fever can be an acute infection but can be severe occasionally. It has an incidence rate of 12.5 million globally with children mostly affected. *Salmonella enterica* serovar Typhi, a gram-negative bacterium, is the

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phytochemical analysis showed that ethyl acetate fraction of *N. latifolia* leaf contains saponins, tannins, flavonoids, alkaloids, terpenoids, steroids, and phenols while glycosides were absent. The ethyl acetate leaf fraction of *N. latifolia* was active on *Salmonella enterica* Typhi in all the concentrations used. The diameter of the zones of inhibition ranged from 8.00±0.67 mm to 11.00±0.53 mm. The Minimum Inhibitory Concentration (MIC) of ethyl acetate fraction of *N. latifolia* against *Salmonella* Typh*i* was 6.25±0.18 mg/ml while the Minimum Bactericidal Concentration (MBC) was 12.50±0.11 mg/ml. It could be concluded that *N. latifolia* leaf has antibacterial activity on *Salmonella enterica* Typhi and may be used in the production of drugs against infections associated with the pathogen. Further tests should be carried out to determine the toxicological profile of the leaf.

Keywords: Typhoid fever, *Salmonella enterica* Typhi, *Nauclea latifolia*, Ethyl acetate fraction, Anti-salmonella activity..

Causative agent of typhoid fever and it spreads through faecal contamination of food and water supplies (Adeyi *et al*., 2023). A persistent fever, frontal lobe headache, weakness, and appetite loss are the symptoms of typhoid fever. There are also occasional reports of abdominal pain, which in extreme cases can lead to intestinal perforation and neurological problems (Dougan and Baker, 2014).

Nauclea latifolia is an important medicinal plant which belongs to Rubiaceae family. It is found in tropical Africa and Asia. It is grown in different parts of Nigeria (Oke *et al*., 2020), where its different parts are used locally in the treatment of diseases. It is an evergreen, multi-stemmed shrub or small tree, growing up the height of 200 metres. The leaves, roots, and the stem-bark of *N. latifolia* are used for the treatment of diseases such as typhoid fever, malaria, hypertension, diarrhoea, tuberculosis, dysentery and constipation (Olusola, 2020).

Treatment of some infectious diseases including typhoid fever with antibiotics is severely hampered by the rise of multidrug-resistant (MDR) species of bacterial pathogens (Arshad *et al*., 2017). Typhoid is usually treated with antibiotics, but the emergence of multi-drug resistant *S. enterica* Typhi has limited the effectiveness of these antibiotics, thereby prompting the search for other treatment options (Nsofor *et al*., 2015). Therefore, this study was to evaluate the

anti-salmonella activity of *N. latifolia* leaf ethyl acetate fraction used in treatment of typhoid fever in Minna, Nigeria. *Nauclea latifolia* leaves, stems, and flowers is shown is plate I.

Plate I: *Nauclea latifolia* tree showing leaves, stems, and flowers.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Leaves of *Nauclea latifolia* were identified by an herbalist, harvested from Maikunkele, Bosso Local Government Area, Niger State, Nigeria, and then authenticated by a plant biologist from the Department of Plant Biology, Federal University of Technology, Minna. A voucher number (FUT/PLB/RUB/002) was assigned to the sample**.** The leaves were washed with water, drained, air drying under laboratory condition (25 -30°C) for one week. Then, the leaves were cut into smaller pieces and ground mechanically into powder using a laboratory blender (Iheagwam, *et al*. 2020), then stored in a labelled air-tight container for future use.

Extraction Procedure

Extraction of *Nauclea latifolia* leaves was carried out using cold maceration method of Ahoyo *et al.* (2019) with some modifications. Powdered leaf sample (200 g) was extracted by successive soaking for 72 hours using 1000 ml of ethanol in a 1000ml conical flask. The extract was filtered using Whatman No.1 filter paper and heated in water bath at 40°C to dryness until a solid residue was obtained. The solid concentrated filtrate, now the extract was then stored in universal bottles in the refrigerator at 4°C until when it would be used.

Fractionation of *Nauclea latifolia* Ethanolic Extract

Ethanol crude extract (19.8 g) was dissolved in ethanol and then sequentially extracted with n-hexane and ethyl acetate (1500ml) using a Soxhlet apparatus. The ethyl acetate solution was evaporated to dryness with a water bath (40°C) and weighed to determine their yield (Ahoyo *et al,* 2019; Okwute and Ohiakwu, 2021). The fraction recovered was stored in universal bottle and put in the refrigerator at 4°C until when it would be used.

Bacterial Sample Collection

Clinical isolates of *Salmonella enterica* Typhi were obtained from the Microbiology Laboratory, General Hospital, Minna, and transported to the Microbiology Laboratory of Microbiology Department, Federal University of Technology Minna for analysis.

Bacterial Strain Identification and Storage

The isolates were identified through standard bacteriological methods such as gram staining and biochemical tests (Cheesebrough, 2010). Molecular identification was conducted on the isolates to confirm their identity. They were incubated at 37°C for 48 hours before storing in a refrigerator at 4°C until required for future use.

Gram Staining and Microscopy

A thin smear of each of the pure 24 hours old culture was prepared on clean grease-free slides, fixed by passing over gentle flame two times. Each heat-fixed smear was stained by addition of 2 drops of crystal violet solution for 60 seconds and rinsed with tap water. The smears were again flooded with Gram's iodine for 30 seconds and rinsed with tap water, decolourized with 70% alcohol for 30

seconds and were rinsed with tap water. They were then counterstained with 2 drops of Safranin for 60 seconds and finally rinsed with tap water, then allowed to air dry. The smears were mounted on a microscope stage and observed under oil immersion objective lens (X 100 magnification). Gram negative cells appeared pink or red while gram positive organisms appeared purple (Abdallah *et al.*, 2016; Kuta *et al.*, 2011).

Biochemical Tests for the Identification of *Salmonella enterica* Typhi

Citrate utilization test

Approximately 2.4 g of Simon citrate agar was measured and dissolved in 100 mL of distilled water. About ten millilitres (10 mL) of Simon citrate medium was dispensed into each test tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the test isolates once across the surface and incubated at 37°C for 24 – 96 hours. Green is the original colour of the medium. A change from green to blue indicated utilization of the citrate, that is, it is positive. No colour change is a negative result (Mahe *et al.*, 2021).

Voges Proskrauer (VP) test

Twenty-four (24) hours broth culture of bacteria was mixed with 2.0 mL of potassium hydroxide (40% KOH) and 1.0 mL of 5% alcoholic alpha napthol. Formation of purple colour after 5 minutes of mixing is positive result, the formation of any other colour is a negative result (Mahe *et al.*, 2021).

Methyl Red (MR) test

Methyl red screening is used to differentiate *Enterobacteriaceace*. The bacteria to be tested was cultured in broth for 24 hours, and 0.1 millilitre of methyl red reagent was added. The development of a deep red colour is a positive (acid) reaction (Shoaib *et al.*, 2020).

Indole test

Indole test is used to ascertain the ability of some microorganisms to produce the enzyme tryptophan which breakdowns the amino acid tryptophan into indole, pyruvic acid and ammonia. The method of Mahe *et al.* (2021) was used. Tryptone broth (5 mL) was placed into different test tubes, then a loopful of the bacterial isolate was inoculated into the test tubes, one of the test tubes was not inoculated

to serve as control. The test tubes were then incubated at 37°C for 48 hours. After incubation, 0.5 mL of Kovac's reagent was introduced and shaken gently; it was allowed to stand for 20 minutes to allow the reagent to rise. A red or red-violet colour (red ring) at the top surface of the tube indicated a positive result while yellow coloration indicated a negative result.

Urease test

Urease which is a common source of nitrogen for many microorganisms can be hydrolysed by specific numbers of microorganisms to ammonia and carbon dioxides. The reason is that they have the capability to produce Urease. This test is used to differentiate *Enterobacteriaceae* family. The accumulation of ammonia makes the pH of the medium alkaline (pH 8.4). This then changes the colour of pH indicator phenol to red-pink. Agar slants containing Urease agar were prepared, and then inoculated with a 24 hours bacterial culture and incubated at 37°C for 2 hours (Shoaib *et al.*, 2020).

Motility test

A straight wire was used to stab inoculate motility medium with test organisms, and then incubated under appropriate growth conditions. It was examined for motility at intervals of 6 hours up to 48 hours. A diffuse hazy growth that spreads out of the medium indicates motility. Growth confined to the line of inoculation indicates non-motility.

Hydrogen sulphide test

This test is used in the identification of *Enterobacteriaceace* and other bacteria. The organism was inoculated in peptone water in tubes. A lead acetate paper strip was inserted in the neck of the tube above the medium and well stopped, and then incubated at 37°C for 24 hours. It was then examined for blackening of the lower part of the strip of the paper. Blackening indicates H_2S production.

Triple sugar iron agar test

Triple sugar iron agar (TSI) is a differential medium containing trace amounts of glucose, sucrose, lactose, ferrous sulphate, and the pH indicator phenol red. The medium changes to yellow if the organism ferments any of the sugars. Iron sulphide, which appears as a black precipitate, is generated when a species that can reduce sulphur interacts with the hydrogen sulphide gas that is produced.

Signs that the fermentation generated gas include fissures in the medium or the entire slant rising above the bottom of the test tube. The bacterial colony was introduced into TSI Agar by cutting through the medium's centre to the test tube's base, streaking over the agar slant's surface, and then incubating the tube for 24 hours at 35°C (Kuta *et al*., 2011).

Standardization of Organisms

Sterile nutrient broth (5 ml) was inoculated with test organism and incubated at 37°C for 24 hours. Exactly 0.2 ml of the 24h culture was sub-cultured into 5 ml sterile nutrient broth and incubated at 37°C till the turbidity of 0.5 McFarland's Standard with approximate cell density of 1.5 x 10^6 CFU/ml was achieved (Osuagwu *et al*., 2021). A loop-full of the standardized culture was then used for the determination of the antibacterial activity of the plant extract.

Antibacterial Susceptibility Assay of *Nauclea latifolia* Fraction

The antibacterial susceptibility assay of *N. latifolia* ethyl acetate fraction was determined using agar well diffusion method as described by Osuagwu, *et al*. (2021), with modifications. The plates were prepared by pouring nutrient agar media into sterile Petri dishes and left to set. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (1.5 x 10^6 CFU/ml) was inoculated on three (3) plates (replicates) into sterile Nutrient Agar medium using a swab stick. A 6mm diameter sterile cork borer was used to make six (6) wells into each 20 ml solidified agar medium on Petri dishes. The following concentrations of the fraction: 40 mg/ml, 80 mg/ml, 120 mg/ml, 160 mg/ml, and 200 mg/ml were prepared. Each of the wells was filled with 0.2mL of the different specific concentration of the fraction. The inoculated plates were allowed to stand on the laboratory bench for a period of one hour in order to allow for proper diffusion of the fraction into the agar medium. An antibiotics (ciprofloxacin) was used as positive control. The plates were incubated at 37°C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured.

Determination of the Minimum Inhibitory Concentration (MIC) of the Fraction

The determination of the MIC of *N. latifolia* ethyl acetate leaf fraction was performed using broth dilution method described by Oikeh *et al*. (2020). A double fold serial dilution of the fraction was prepared to obtain the following seven concentrations (100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg/ml). The 8th

test tube did not contain any fraction, but a solution and served as negative control. Then 0.1 ml of an 18 hours old culture of the bacteria earlier adjusted at 1.5 x 10^6 CFU/ml was inoculated into each tube and thoroughly mixed. The tubes were incubated at 37°C for 18-24 hours and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of ethyl acetate leaf fraction of *N. latifolia* was determined by taking a loopful from each tube of bacterial suspension from the MIC tubes without any growth and streaked into nutrient agar plates and incubated at 37°C for 24 hours (Oikeh *et al*., 2020). After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

Qualitative Phytochemical Screening of *N. latifolia* Fraction

Qualitative phytochemical screening was performed on the ethyl acetate fraction of *N. latifolia* to investigate the presence or absence of phytochemical compounds in the fraction, using procedure described by Abdallah and Mohammad (2018) and Osuagwu *et al*. (2021).

Data Analysis

Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA) at 95% confidence interval was used on the data obtained. Data obtained from this study were expressed as mean $\pm$ SEM. One-way ANOVA was used to test the means. Values were considered statistically significant at $P < 0.05$. All results were represented as mean $\pm$ SEM ($n = 3$). Values with different superscripts were significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Phytochemical Constituents of *Nauclea latifolia* Leaf Ethyl Acetate Fraction

The phytochemical constituents of ethyl acetate fractions of *N. latifolia* leaf shows that saponins, tannins, flavonoids, alkaloids, terpenoids, steroids, and phenols were present while glycosides were absent (Table 1).

Antibacterial Susceptibility Assay of *Nauclea latifolia* Leaf Ethyl Acetate Fraction

The antibacterial susceptibility assay of ethyl acetate fraction of *N. latifolia* leaf against clinical isolates of *Salmonella enterica* Typhi is summarized in Table 2.

The fraction was active on the test organism in all the concentrations used, with the diameter of the zone of inhibition ranging from 8.00±0.67 mm to 11.00±0.53 mm. Ciprofloxacin, the standard drug for treating this pathogen inhibited the growth of the test organism more than the leaf fraction

.

Table 1: Phytochemical constituents of *Nauclea latifolia* leaf ethyl acetate fraction

Parameter/Solvents	Ethyl acetate Fraction
Saponins	+
Tannins	+
Flavonoids	+
Glycosides	-
Alkaloids	+
Terpenoids	+
Steroids	+
Phenols	++

Keys: -: Not detected, +: Present, ++: Highly Present

Table 2: Zones of inhibition in mm of different concentrations of *Nauclea latifolia* leaf fractions against test isolate

Isolates	Conc. (mg/mL)	Mean Zone of Inhibition (mm)	
		Ethyl acetate fraction	Ciprofloxacin (10µg/mL)
Salmonella enterica Typhi	200	11.00±0.53^{b}	42.00±0.44
	160	10.00±0.38^{a}	
	120	10.00±0.38^{a}	
	80	8.00±0.67^{a}	
	40	8.00±0.67^{a}	

Values are expressed in mean ± standard error of mean. Values with different superscript on the same column have a significant difference at $p<0.05$.

Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Nauclea latifolia* Ethyl Acetate Fraction

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ethyl acetate fraction of *N. latifolia* leaf against

Salmonella enterica Typhi are shown on Table 3. The minimum inhibitory concentration (MIC) was 6.25±0.18 mg/ml while the minimum bactericidal concentration (MBC) was 12.50±0.11 mg/ml.

Table 3: Minimum inhibitory concentration of *Nauclea latifolia* leaf ethyl acetate fraction

	Concentration mg/ml								
Test Organism	100	50	25	12.5	6.25	3.125	1.5625	MIC	MBC
S. Typhi	-	-	-	-	+	+	+	6.25	12.5

Keys: + = Growth, - = No Growth

Discussion

The results of phytochemical screening of *Nauclea latifolia* leaf revealed the presence of saponins, tannins, flavonoids, alkaloids, terpenoids, steroids, and phenols but glycosides were absent. These phytochemicals are found to be responsible for the medicinal property of some plants. The results of this study were in accord with Eze and Obinwa (2014) who reported the presence of these phytochemicals including glycosides. The absence of glycosides in the leaves of *N. latifolia* in this study could be due to geographical location, variation in the season cycle, the age of plants, and the variety of plant sample used.

Nauclea latifolia leaves ethyl acetate fraction showed antibacterial activity against *Salmonella enterica* Typhi with the diameter of zones of inhibition ranging from 8.00±0.67 mm to 11.00±0.53 mm. It exhibited concentration-dependent activity against the isolate with zones of inhibition increasing with increasing concentrations. This shows that a higher concentration is required for better result. This result agrees with the report of Nyong *et al*. (2021); Okwute and Ohiakwu (2021), and Ugye *et al*. (2018), who all reported that extracts and fractions of *N. latifolia* were active against *Staphylococcus aureus, Escherichia coli, Salmonella* Typhi*, Bacilus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyogenes*.

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of ethyl acetate fraction of *N. latifolia* leaf revealed that the fraction had the ability to inhibit the growth and/or kill all the isolate. The test organism exhibited higher MBC than the MIC (Tables 3). This demonstrated that

higher concentrations of fraction were needed to kill the bacteria than to inhibit their growth. This result agrees with the findings of Bolaji *et al*. (2018) and Okunye *et al*. (2020) who got similar MIC values (0.782 – 50 mg/mL) against *Salmonella* Typhi and *E. coli*. This supports the claim by traditional medicine practitioners and vendors that *N. latifolia* leaf extracts could serve as antimicrobial agents for the treatment of many bacterial infections.

CONCLUSION AND RECOMMENDATIONS

The results of this study showed that the *N. latifolia* leaf ethyl acetate fraction contains important phytochemicals and has antibacterial activity on *Salmonella enterica* Typhi. Therefore, it may be used for the treatment of typhoid fever and in the production of drugs against infections associated with the pathogen. It is recommended that further tests should be carried out to determine the toxicological profile of the leaf.

REFERENCES

Abdallah, M. S., & Muhammad, A. (2018). Antibacterial Activity of Leaf and Stem Bark Extracts of *Adansonia Digitata* Against *Escherichia coli* and *Salmonella typhi* Grown in Potiskum, Yobe State, Nigeria. *Annals of Microbiology and Infectious Diseases*, 1(4), 1-7.

Abdallah, M. S., Gambo, A., Ishaq, S., Mustapha, T. & Nasir, M. (2016). Effect of Aqueous and Ethanolic Leaf Extract of *Khaya Senegalensis* against *E. coli,* Shigella and Salmonella Species. *Imperial Journal of Interdisciplinary Research,* 2(6), 680-684.

Adeyi, A. J., Adeyi, O., Oke, O. E., Ajayi, O. K., Otolorin, J. A., Akinyemi, O., ... & Gbadamosi, B. (2023). *Nauclea latifolia* herb root waste reinforced epoxy polymer composite: The study of effects, modelling, certainty and sensitivity analysis. *Cleaner Materials*, *7*, 100167.

Ahoyo, C. C., Deguenon, P. M., Dah-Nouvlessounon, D., Haziz Sina, H., Houehanou, H. D., Alain S. & Houinato, M. R. B. (2019). Comparative *in vitro* antimicrobial effect of *Sarcocephalus latifolius* (Sm.) E. A. Bruce leaves and roots on foodborne pathogens. *African Journal of Microbiology Research,*13(22), 357-368. DOI:10.5897/AJMR2019.9062.

Arshad, N., Mehreen, A., Liaqat, I., Arshad, M., & Afrasiab, H. (2017). In vivo screening and evaluation of four herbs against MRSA infections. *BMC Complement Alternative Medicine*, 17(1), 498-504.

Basnyat, B., Qamar, F. N., Rupali, P. Ahmed, T., & Parry, C. M. (2021). Enteric fever. *British Medical Journal*, 372.n437. http://dx.doi.org/10.1136/bmj.n437.

Bolaji, O., John, O.J., Fasusi, Toluwani, F.E. & Adisa, L.U. (2018). Evaluation of Phytochemical Constituents and Antimicrobial Activity of Leaves and Stem Bark Extracts of *Sarcocephalus latifolius*. *Microbiology Research Journal International* 24(2), 1-10.

Cheesebrough, M. (2010). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, UK, pp, 35-38.

Dougan, G., & Baker, S. (2014). Salmonella enterica serovar Typhi and the pathogenesis of typhoid fever. *Annual Review of Microbiology*, 68, 317-336.

Eze, O., & Obinwa, E. (2014). Phytochemical and nutritional evaluation of *Psidium guajava*. *Journal of Biochemistry*, *2*(1), 8-24.

Iheagwam, F. N., Israel, E. N., Kayode, K. O., DeCampos, O. P., Ogunlana, O. O., & Chinedu. S. N. (2020). *Nauclea latifolia* Sm. Leaf Extracts Extenuates Free Radicals, Inflammation, and Diabetes-Linked Enzymes. *Oxidative Medicine and Cellular Longevity*, 20(20), 13-25.

Kuta, F. A., Damisa, D., Adabara, N. U., Bello, I. M., & Samuel, S. (2011). Antibacterial Activity of *Aloe Vera* Plant Extract Against *Salmonella* Typhi. *Techno Science Africana Journal,* 6(1), 83 – 86.

Mahe, A., Sabiu, B., Adam, A. A., Abdullahi, U. Z. & Ado, B. A. (2021): EFFECT OF CITRIC ACID AT DIFFERENT pH ON THE SURVIVAL OF *Escherichia coli. Bayero Journal of Pure and Applied Sciences,* 14(1), 79 - 84.

Nsofor, C. A., Okaro, C. C., Nsofor, C. M. (2015). Antibiotic susceptibility pattern of clinical Salmonella isolates from patients attending hospitals in Owerri, Metropolis Nigeria. *International Journal of Microbiology and Immunology Research*, 4(3), 21-25.

Nyong, B. E., Etim, A. N., Ita, P. O., & Ucheokoro, A. S**.** **(**2021). Antimicrobial properties of alcoholic extract of African peach (*Nauclea latifolia*) leaves. *International Journal of Current Research in Medical Sciences*, 7(9), 1-7.

Ohanu, M. E., Iroezindu, M. O., Maduakor, U., Onodugo, O. D., & Gugnani, H. C. (2019**)**. Typhoid fever among febrile Nigerian patients: Prevalence, diagnostic performance of the Widal test and antibiotic multi-drug resistance. *Malawi Medical Journal,* 31(3), 184-192.

Oikeh, E. I., Oviasogie, F. E., & Ehimwenma, S. & Omoregie, E. S. (2020). Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of *Citrus sinensis* (L.) Osbeck (sweet Orange) peels. *Clinical Phytoscience,* 6(1), 46-52.

Oke, E. O., Okolo, B. I., Adeyi, O., Agbede, O. O., Nnaji, P. C., Adeyi, J. A., ... & Ude, C. J. (2021). Black-box modelling, bi-objective optimization and ASPEN batch simulation of phenolic compound extraction from Nauclea latifolia root. *Heliyon*, *7*(1).

Okunye, O. L., Idowu, P. A., & Kolade, T. T. (2020). Antibacterial activity of crude extracts of the leaves of *Nauclea latifolia* Smith (Rubiaceae) and some selected conventional antibiotics on clinical isolates of *Salmonella* Typhi. *Annals of Health Research,* 6(3), 258-264. doi:10.30442/ahr.0603-02-88.

Okwute, S. K., & Ohiakwu, C. S. (2021). Phytochemical and GCMS analysis of the leaves extracts of *Nauclea latifolia*. *Journal of Pharmacognosy and Phytochemistry*, 10(1), 116-120.

Olusola, S. E. (2020). Phytochemical Screening and Antibacterial Potential of African Peach *Nauclea Latifolia* Leaves Extracts against some Clinical Isolates of Pathogenic Bacteria from *Clarias Gariepinus***.** *Coast Journal of the Faculty of Science*, 1(2), 164 – 171.

Osuagwu, O, S., Ichor T. S., Oluma, H. O. A., & Onyemaechi, A. (2021). Antibacterial Activity and Phytochemical Properties of Selected Medicinal Plants Against *Salmonella Typhi*, *Salmonella Paratyphi* A, B and C, Clinical Isolates in Lafia, Nigeria. *American Academic Scientific Research Journal for Engineering, Technology, and Sciences,* 81(1), 203-221.

Shoaib, M., Iqra, M. I., Hammad, M., Ahmad, Z., Bhutta, Z. A., & Yaseen, I. (2020). A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria. *International Journal of Research Publications*, 54(1), 8-8.

Ugye, J. T., Egwaikhide, P. A., Ama, S. O., Imarenezor, K. E., & Abhulimen, W. I. (2018). Phytoconstituents Screening and Bioactivity of *Jatropha curcas* Linn (physic leaf) and S*sarcocephalus latifolius* (African peach) Leaves Extracts. *Trends in Science & Technology Journal*, 3(2B), 1024 – 1028.