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Proximate Composition, Acute Toxicity and Antimicrobial Activity of Methanol Extract of *Picralima nitida* Stem Bark

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ABSTRACT

The existence of essential bioactive compounds in the *Picralima nitida* stem bark has demonstrated useful advantages in terms of antioxidant, antibacterial, and antifungal activities. In this work, concentrated methanol extract of the *Picralima nitida* stem bark was investigated for its proximate composition, phytochemical constituents, acute toxicity and antimicrobial activity. The results revealed the presence of saponins, glycosides, tannins, alkaloids, eugenols, flavonoids, proteins, reducing sugars and terpenoids. From the proximate analysis of *P. nitida* stem bark, the moisture content, ash value, acid insoluble ash, water soluble ash, water extractive value and alcohol soluble extractive values were found to be 4.00%, 7.00 %, 6.50 %, 4.00 %, 3.20 %, and 0.60 % respectively. Investigation of the acute toxicity effect revealed that the lethal dose (LD_{50}) of the extract was ≥ 5000 mg/kg body weight which was considered practically non toxic conforming to the toxicity standards. The outcome of the antimicrobial study revealed that the methanol extract of *P. nitida* stem bark has higher inhibitory effect on the bacterial isolates (*S. aureus* and *E. coli*) with improved activity against *E. coli* at higher concentrations. But the extract was inactive to the fungi isolates (*A. flavus* and *C. Albicans)* even at a higher dosage (100mg/ml). The minimum inhibitory concentration revealed that the extract was capable of inhibiting the growth of both *S. aureus* and *E. coli* at 100 mg/ml, and the minimum bactericidal concentration of the extract against the tested isolates was 150mg/ml and 125 mg/ml for *S. aureus* and *E. coli* respectively.

Keywords: Acute toxicity, Antimicrobial activity, *Picralima nitida*, Phytochemicals, Proximate composition

INTRODUCTION

The use of natural bioactive compounds from the medicinal plant in the treatment of diseases has been dated back to man's origin. So many diseases and infections have been treated using herbal medicine by herbalists without the full knowledge of the major ingredients in the plant that brought about the cure of the disease. Plants of medicinal values are known to contain bioactive chemicals such as essential oils, saponins, flavonoids, tannins, alkaloids, and terpenoids with healing properties (Ogbeide and Akhigbe, 2019). Consequently, there is a great interest on these floras to explore their bioactive chemical constituents aimed at potential drug discovery and developments (Okenwa and Mgbemena, 2014). One of these floras used in herbal medication in Nigeria is *Picralima nitida*. *Picralima nitida* belongs to *Apocynaceae* family and is mostly found in West Africa. It is a flowering small shrub with a height of 35 meters high with silvery sap present in the whole parts. The plant has leaves with knife-edge elongated to rectangle in form, 10 to 25cm by 2-14 cm. The pods are made up of 11-20 cm long obovoid to ellipsoid follicles, apex rounded, smooth, yellow to orange, with 2-valved and numerous seeds (Okenwa and Mgbemena, 2014). The seed remain diagonally ovate, firmed, 2.5-4.0 cm long, plane, browns to orange, fixed in spineless white to orange pulps (Okenwa and Mgbemena, 2014).

Several compound which includes, saponins, tannins, glycosides, terpenoids, steroids, flavonoids and alkaloids have been reported to exist in the plant which has demonstrated promising antioxidant, antibacterial, and antifungal activities (Nkere and Iroegbu, 2005, Mabeku *et al.*, 2008, Kouitcheu *et al.*, 2013). They can reestablish the medical application of long existed antibiotics by improving on their potency and as a result, avoid the development of resistance (Barbieri *et al.*, 2017; Iyokowa *et al.*, 2019). However, toxicity study of the plant's constituents becomes very essential to guarantee safety while being used for treatment. The *P. nitida* stem bark has been reported to demonstrate trypanocidal, antimicrobial, antimalarial and antioxidant properties (Mabeku *et al.*, 2008 and Erharuyi *et al*, 2014). The roots, seed, and stem bark extracts of *Picralima nitida* have been reported to exhibit a wide spectrum of activities against bacteria strains such as *Salmonella kintambo*, *Bacillus subtilis*,

Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa because of the bioactive ingredients present in the plant (Nkere and Iroegbu, 2005). *Picralima nitida* seed aqueous and ethanol extracts have been shown to have a high antibacterial activity against bacteria such as *Candida albicans, Aspergillus flavus*, and *Microsporum canis* (Ubulom *et al.,* 2011), with the ethanol extract exhibiting an improved antifungal activity more than the aqueous extract. The aim of this study therefore was to explore the proximate composition, acute toxicity study, and antimicrobial effect of the methanol extract of *Picralima nitida* stem bark.

MATERIALS AND METHODS

Sample Collection and Authentification

The sample (*P.nitida* plants) was purchased from Ikpoba Hill market located in Ikpoba Okah LGA, Edo State Nigeria.The sample was identified and given a voucher number UBH-P424 by a botanist at the University of Benin's Plant Biology and Biotechnology Department, Benin City, Edo State, Nigeria.

Sample Preparation

The *P. nitida* stem bark were chopped into pieces and placed air-dried within the period of one month. The sample was crushed to powder using a Lexus mixer grinder. 500g of the powdered sample was weighed into a container containing two liters (2L) of methanol, maceration was carried out with random shaking for 3 days (72hrs). The mixture was filtered using vacuum filtration and allowed to air dry. Afterwards, the extract yield was calculated in percentage.

Phytochemical Screening

Screening of the bioactive constituents of the methanol extracts of the *P. nitida* was carried out using an already known procedure as reported by Amita and Shalini, (2014) and Trease and Evans (2002).

Proximate Analysis

To determine the alcohol soluble extract value, water soluble extract content, acid insoluble ash content, water-soluble ash, ash content and moisture content of *P. nitida* stem bark was done using a standard method as described by Odoh and Ulasi (2020).

Determination of Acute toxicity (LD_{50})

Animal

Swiss mice (20-35 g) were purchased from Animal House, University of Ambrose Alli (College of Medicine) Ekpoma, Edo State, Nigeria. They were kept under standard laboratory environments with light and dark cycle of 12 hrs. at the animal facility, Animal and Environmental Biology Department, University of Benin, BeninCity. Standard rodent pellet and water *ad libitum* was freely given to the mice. The mice were accommodated for 2 weeks preceding the start of experiment.

Acute Toxicity Studies

The method described by Lorke, (1983) was used to determine acute toxicity effect. Nine (9) mice were categorized into three category containing three mice each. Different dosages (10, 100 and 1000 mg/kg) of methanol extract of the bark of *P. nitida* stem were administered to each group of the animals respectively. Mice were carefully monitored for 24 hours for mortality and changes in behaviour. The remaining three mice were shared into three groups of one mouse each the next day and each set of the animals administered with different quantities (1600, 2900 and 5000 mg/kg) of *P. nitida* respectively. Mice were also observed for 24 hours, 72 hours and two weeks for mortality and behavioural changes.

Antimicrobial study

Source of Microorganisms

The microorganisms used were obtained from the University of Benin Teaching Hospital (UBTH), Department of Medical Microbiology, Benin-City, Edo State and they include "*Candida albicans, Staphylococcus aureus, Escherichia coli* and *Aspergillus flavus*".

Antimicrobial Activity

The agar well diffusion technique was applied in investigating the antibacterial activities of the extracts. 5 x 10^5 inoculum of individual bacteria was standardized and spread across on the Sterile Muller Hinton (MHA) plate in order to deposit a continuous growth. Additionally, the plate were kept to dry and a diameter of 5.00mm cork borer (sterile) was applied to bore holes like well in the agar plate. The plant extract was first dissolve in dimethylsulphoxide (DMSO) 20%, thereafter, it was diluted to a concentration of 100mg/ml with sterile water. Subsequently, in the MHA plates, wells made in triplicate were introduced and 100µl each of the extracts was pour gradually into the triplicate well. Thereafter, the plate was kept for 1hr to stand, before incubated at 24hrs on ambient temperature. The minimum inhibition zone (in mm) was recorded and further evaluations were performed on extract with clearer inhibition zone.

Evaluation of Minimum Inhibitory Concentration and Bactericidal Concentration

The extracts of *P. nitida* stem bark (methanol extract) was examined for its MIC value using an improved agar well diffusion techniques (Okeke *et al*., 2001). The extracts were prepared using a two-fold serial dilution method, which was done by first dissolving the extract in 20% DMSO, then diluted in distilled sterile water in the direction

of decreasing concentrations ranging from 100 to 6.25 mg/ml.100µl was measured from each of the dilution and included into the triplicate wells bored in the MHA plates which were already containing the uniform inoculums (5×10^5) of the investigated bacterial cells. Incubation of all test plate was done at a temperature of 37°C for 24 hrs. A wide inhibition zone of the extract with lowest concentration was chosen as the control for MIC value and MBC which was also assessed for the two isolates.

RESULTS AND DISCUSSION

The methanol extract of the *P. nitida* stem bark gave a percentage yield of 5.8%. The qualitative phytochemical screening (Table 1) reveal the presence of alkaloids, saponins, phenols, tannins, flavonoids, terpenoids, reducing sugars, proteins, glycosides, and eugenol. A previous study reported the activity of saponin against *Escherichia coli*, *Staphylococcus aureus*, *Neisseria gonorrhea*, and *Streptococcus pneumonia* (Ifijen *et al.*, 2020). Ojokuku *et al*. (2010) reported that flavonoids, Alkaloids, phenols and tannins also have been reported to exhibit anti-inflammatory, antibacterial, antioxidant antithrombotic, antiallergic, antimutagenic, antineoplastic, and antiviral activities.

Table1: Phytochemical screening of methanol extracts of *P. nitida* the stem bark

Phytochemicals	Inference
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	+
Reducing sugar	+
Proteins	+
Glycosides	+
Eugenols	+

+ = indicate the presence of the compound

Table 2: Proximate Analysis of *P. nitida* Stem bark

Quantitative Parameters	Value (%)
Moisture content	4.00
Total ash	7.00
Acid insoluble ash	6.50
Water soluble ash	4.00
Water soluble extractive content	3.20
Alcohol soluble extractive content	0.60

The proximate composition of *P. nitida* stem bark as presented in Table 2, gave 4.00%, 7.00 %,6.50 %,4.00 %, 3.20 %, and 0.60% for moisture content, total ash, acid-insoluble ash, water-soluble ash content, water-soluble extractive value, and alcohol soluble extractive value respectively. Moisture content is among the most important and frequently used measurements in the processing, preservation, and storage of food. Hence, low moisture content tends to hinder or prevent microbial infection and chemical dilapidation (Hussain *et al*., 2009). Crude drug containing excess moisture content may as well undergo rapid breakdown of important ingredients and increased growth of microorganisms especially during the period of stowage of the drug (Adesina *et al*., 2008). Ash value is useful in defining the sample's purity and authenticity and these values are significant qualitative standards (Vidita *et al*., 2013). A substantial roles are being played by the water-soluble extractive value in the assessment of crude drugs. The less extractive value indicates contamination or incorrect processing during drying or storage (Vidita *et al*., 2013). The alcohol-soluble extractive value was seen to also play the same role as that of the water-soluble extractive value. The water-soluble extractive value which was 3.20% has been found to be higher than the alcohol-soluble extractive value which was 0.60%. This implies that the components of the drug dissolve more in water as compared to alcohol. These results have shown that alternatively, the stem bark of *P. nitida* could serve as a source of drugs and could equally be consumed by man, after all the toxic components have been removed by undergoing quality processing.

Acute Toxicity effect of methanol extracts of the Bark *P. nitida* Stem

The acute toxicity of the methanol extracts of *P. nitida* stem bark is given in Table 3. The toxicity of the methanol extracts of the barks of *P.*

nitida stem as obtainable in Table 3, shows that no mortality was recorded for all doses administered to mice in any of the first three groups (10, 100, 1000mg/kg body weight) in 24 hours and also show no mortality after an increase in the dose for the remaining three groups of mice (1600, 2900, 5000 mg/kg body weight) after being monitored for 24 h and up to two weeks (14 days). The dose of 10 and 100 mg/kg showed no sign or symptoms of toxicity. However, at higher dose of 1000, 1600, 2900, and 5000 mg/kg of *P. nitida* stem bark, there were some behavioral changes such as hyperactivity, piloerection, fast breathing and calmness. Hodge and Sterner (2005) and Lorke (1983) reported a toxicity standard stating that any compound having an oral intake LD_{50} of 5000mg/kg or more should be deliberated as virtually non-toxic. Compared with the findings of Nwankwo *et al.* (2019), the ethanol extract of *P. nitida* seed at a dosage ≥ 5000 mg/kg was also found to be safe when orally administered, thus confirming the safety margin as detected in the stem bark extract. Hence, oral administration of *P. nitida* extract at a prescribed amount ≥ 5000 mg per kg body weight is practically safe. Table 3 showed that the LD_{50} of methanol extracts of *P. nitida* stem bark was greater than 5000 mg/kg since there was no death recorded at dose 5000 mg/kg which was the highest dose administered. This, therefore, suggested that the plant extract could be recommended as safe for consumption at lower concentration and should be well monitored at higher concentrations.

Table 3: Acute toxicity effect of methanol extracts of the bark of *P. nitida* stem

Dose (mg/kg)	Number of Deaths/ Number of Mice	Mortality (%)	Symptoms
10	0/3	0	None
100	0/3	0	None
1000	0/3	0	Hyperactivity and calmness
1600	0/1	0	Hyperactivity, piloerection, fast breathing, and calmness
2900	0/1	0	Hyperactivity, piloerection, fast breathing, and calmness
5000	0/1	0	Hyperactivity, piloerection, fast breathing, and calmness

Antimicrobial activity of the bark of *P. nitida* stem

The antimicrobial activities then inhibitory zone (MIC and MBC values) of methanol extracts from the stems of *P. nitida* are given in Tables 4 and 5, while the susceptibility pattern of the test isolates to the normally used antibiotics is shown in Table 6 using Hudzicki, 2009.

Table 4: Inhibition zones of various concentrations of methanol extracts from *P. nitida* stem bark in (mm) diameter

Test isolates	**Concentrations of extract in mg/ml**				
	100	50	25	12.50	6.25
S. aureus	14.17± 0.17	12.17± 0.17	8.83± 0.17	5.17± 0.17	0.00± 0.00
E. coli	20.83± 0.17	15.67±0.17	10.83± 0.17	6.67± 0.17	4.83± 0.83
A. flavus	4.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
C. albicans	5.83± 0.17	3.67± 0.17	0.00± 0.00	0.00± 0.00	0.00± 0.00

Table 5: Minimum Inhibitory Concentration (MIC) and bactericidal concentration (MBC) of the methanol extracts of *P. nitida* stem bark in mg/ml

Test isolates	**(MIC)**	**(MBC)**
***S. aureus**	100	150
***E. coli**	100	125
***A. flavus**	ND	ND
***C. albicans**	ND	ND

- ***=bacteria,** + = ***Fungi***

Table 6: Antibiotic susceptivity test on isolates:

Antibiotic	**Bacteria/fungi**			
Disc	**Potency**	**Staph**	**E. coli**	**A. flavus**	**C. Albicans**
Septrin	30 µg	R	R	R	R
Amoxicillin (AM)	30µg	R	R	R	R
Chloramphenicol (CH)	30µg	R	I	R	R
Sparfloxacin (SPX)	10µg	R	R	R	R
Ciprofloxacin (CPX)	10µg	I	R	R	R
Augumentin (AU)	25µg	R	R	R	R
Pefloxacin (PEF)	10µg	R	R	R	R
Streptomycin (S)	30µg	R	R	R	R
Zinnacef (Z)	20µg	R	I	R	R
Gentamycin	10µg	R	I	R	R

- Compared with Clinical lab. Standard Institute, Key: R:- Resistant, S:- Sensitive. I:-Intermediate

Table 7: CLSI recommended standard for antibiotics

Antibiotics	Susceptible	Intermediate	Resistant
Septrin (30 µg)	≥18	14 – 17	≤13
Amoxicillin (30 µg)	≥18	14 – 17	≤13
Chloramphenicol(30µg)	≥18	13 – 17	≤12
Sparfloxacin (10µg)	≥21	16 – 20	≤15
Ciprofloxacin(10 µg)	≥21	16 – 20	≤15
Pefloxacin(10 µg)	≥21	16 – 20	≤15
Streptomycin (30µg)	≥15	12 – 14	≤11
Zinnacef (20 µg)	≥18	14 – 17	≤13
Gentamycin (10 µg)	≥15	13 – 14	≤12

SOURCE: CLSI= Clinical Laboratory Institute, 2015

According to the Clinical and Laboratory Standard Institute, 2015; which states that inhibitory zone diameter of antimicrobial agents such as Ciprofloxacin to the nearest whole mm, ≥ 21 is sensitive, 16-20 is intermediate and ≤ 15 is resistant (Table 7). Staphylococcus aureus was resistant to the stem bark extract (at 100 mg/ml) and E. coli was found to be sensitive. The antimicrobial agent most commonly used was intermediately sensitive to the tested isolates *(S. aureus*) and resistant to *E. coli* with a region of reticence of 16.00 also 0.00mm respectively (Table 6). While comparing with Zinnacef, with a standard zone of inhibition in mm, ≥ 18 is sensitive, 14 -17 is intermediary, and ≤ 13 is Resistant (Table 7), Staphylococcus aureus was shown to be intermediately sensitive to the extract (at 100 mg/ml) with a zone of inhibition of 14.17± 0.17mm and *E. coli* was shown to be sensitive to the extract at the same concentration with a zone of inhibition of 20.83± 0.17mm, Were as the commonly used antibiotics (zinnacef) was shown to be Resistant to tested isolates (*S. aureus*) with 0.00mm zone of inhibition and intermediately sensitive to E. coli with 15.00mm zone of inhibition (Table 6).The test fungi, *Aspergillus flavus* and *Candida albican* were resistant to the extract at a concentration of 100 mg/ml (Table 4). Hence, The result in Table 4revealed that the methanol extract usually exhibit the higher inhibitory activities on the bacterial isolates (*S. aureus* and *E. coli*), through enhanced activities compared to *E. coli* (Gram-negative bacteria) at a higher level of concentrations but was resistant to the fungi (*A. flavus* and *C. Albicans*) even at a higher dose (100mg/ml). However, as the concentration decreased, the anti-bacterial activities of the extract decreased, this shows that the effect of antibacterial produced by the extract was dose-dependent. The evaluation of the MIC and MBC were also carried out (Table 5). From the result, it was observed that methanol extracts hindered both *S. aureus* and *E. coli* growth at 100mg/ml. MIC of the two fungi isolates was not determined because at 100 – 150 mg/ml the two strains of fungi were still able to grow when subjected to the extract. The MBC of the extract on the tested isolate revealed that methanol was 125 and 150 mg/ml respectively for *E. coli* and *S. aureus*. This result has shown that the MICs and MBCs values of the methanol extract displayed a better variety of inhibition of the bacterial isolates compared to the controlled drug which is normally used clinically for the treatments of a numbers of bacterial infections. The research of Nkere *et al.* (2005) reported that the *P. nitida* stem bark ethanol extract using a cold maceration method had a wider range of inhibition against *E. coli* than *S. aureus* which is in alignment with the result of this present study. Eze *et al*. (2013) also testified that *P nitida* was the maximum active extracts contrary to *E. coli* when compared with some other plant extract which also conforms with this present finding. The microbes which the

extracts were active against are pathogens already involved in the severity of human diseases.

CONCLUSION

In this work it was concluded that the low moisture content of *P. nitida* stem bark suggests that the plant sample can be kept for an extended time deprived of spoilage before use if processed and preserved properly. The proximate and phytochemical analysis has revealed that the *P. nitida* stem bark is composed of essential nutrients and rich phytochemicals, safe and active against microorganisms capable of causing different ailments. *P. nitida* stem bark has shown no toxicity effect at a dose of 5000mg/kg, which indicates that the plant extracts is almost nontoxic and relatively safe for use at a moderate dose. The microbial activity of the methanol extract of *P. nitida* stem bark has further revealed that the plant extract had a dose-dependent activity and compete favorably well with the positive control drugs used for this study.

RECOMMENDATIONS

1. Since the *Picralima nitida* stem bark is simple to find, prepare, active against some bacterial and fungi and practically non-toxic at a high dose, it should be used to manage traditionally, the diseases caused by bacteria such as *S. areus* and *E. coli* and also developed into effective antimicrobial agents.
2. Its toxicity should be investigated at a much higher dose and the antifungal effects should also be evaluated at a much higher concentration.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCE

Adesina G. O., Onaolapo. J. A., Ehinmidu, J. O. and Odama, L. E. (2008). Antimicrobia Extracts (Unpublished). *International Conference on Research and Development, Ghana. Of Anogeissuslati folia. Journal of Pharmacognosy and Phytoche*1 (6): 61- 65.

Amita, P. and Shalin, I. T. (2014). Concept of Standardization, Extraction and Pre Phytochemical Screening Strategies for Herbal Drug. *Journal of Pharmacology and Phytochemistry*, 2(5):115-11.

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sanchez, E., Nabavi, S. F., and Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol Res.*196: 44–68.

Clinical and Laboratory Standards Institute (2015), Performance Standards for Antimicrobial.

Erharuyi Osayemwenre, Falodun Abiodun and Langer Peter (2014). Medicinal uses, phytochemistry, and pharmacology of *Picralima nitida* (Apocynaceae) in tropical diseases: A review. *Asian Pacific Journal of Tropical Medicine*. 1-8.

Eze, E. A, Oruche N. E, Onuora V. C and Eze C. N (2013). Antibacterial screening of crude ethanolic leaf extracts of four medicinal plants. *Journal of Asian Scientific Research*, 3(5):431-439.

Hodge, A. and Sterner, B. (2005).Toxicity classes. In: Canadian center for occupational Health and safety.

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. ASM Microbe Library. American Society for Microbiology. http://www.microbelibrary.org/component /resource/laboratory-test/3189-kirbybauer-disk-diffusion susceptibility-test protocol.

Hussain, K.; Ismail, Z.; Sadikun, A.; Ibrahim, P. (2009).Proximate and qualitative analysis of different parts of Piper sarmentosum, and quantification of total amides in various extracts. Phcog. Res. 1, 113–119.

Ifijen, I. H, Aghedo, O. N., Odiachi, I. J., Maliki, M., Okereke, O. C. (2020). Investigation of the Anti-malaria Potency and Chemical Constituents of the Bark Extracts of *Ficuselastica* in *Plasmodium berghei* Infected Mice. *Chemistry Africa*.3 (4): 1045-1051.

Iyokowa O., Aghedo, O. N., and Bassey P.(2019).synthesis, ft-ir characterization, and antibacterial activity of 5-Bromo-8-quinolinoxyacetic acid. *Journal of Science and Technology Research* 1(1):246-253.

Kouitcheu LBM, Tamesse JL, Kouam J (2013). The anti-shigellosis activity of the methanol extract of *Picralima nitida* on *Shigella dysenteriae* type I induced diarrhoea in rats. BMC; 13: 211.

Lorke, D. (1983). A new approach to tropical acute toxicity testing. *Arch. Toxicol*. 53: 275-287.

Mabeku LBK, Kouam J, Paul A, Etoa FX (2008). Phytochemical screening and toxicological profile of methanolic extract of *Picralima nitida* fruit rind (Apocynaceae). *Toxicol Environ chem.* 90(4): 815-828.

Nkere, C. K. and Iroegbu, C. U. (2005). Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida*. African Journal of Biotechnology. 4 (6): 522-526.

Nwankwo, N. E., Egbuonu, A. C. C., Joshua, P. E., Okeke, E. S., Cosmas, S., Arazu, A. V. and Nwodo, O. F. C. (2019*). Inhibitory Effect of Ethanol Picralima nitida Seed Extract On Malaria Parasite, Indo Am. J. P. Sci*, 06(07).

Nwankwo, N.E., Egbuonu, A.C.C., Joshua, P.E., Okeke, E.S., Cosmas S., Arazu A. V and Nwodo, O.F.C.(2019) inhibitory effect of ethanol *Picralima nitida* seed extract on the malaria parasite. *Indo American Journal of pharmaceutical sciences*. 06 (07): 13491-13496.

Odoh, U. E. and Ulasi, I. N.(2020).Establishment of Quality Parameters and Pharmacognostical Profiling of Dialium Guinness L. (Caesalpiniodeae*). World Journal of Innovative Research (WJIR).* 8 (5):37-46.

Ogbeide, O. K. and Akhigbe, I. U. (2019). anti-haemolytic, anti-anaemic and biosafety examination of combined *telfairia occidentalis* and *ipomoea batatas* leaves extract. *Journal of Pharmaceutical and Allied Sciences.* 16 (4) (2019) 3106 – 3113.

Okeke, M. I, Iroegbu, C. U, Eze, E. N., Okoli, A. S, Esimone, C. O. (2001). Evaluation of extracts of the root of *Landolphia owerriensis* for antibacterial activity. *J. Ethnopharmacol. 78: 119-127.*

Okenwa UchennaIgwe and Mary-Ann Nkoli Mgbemena (2014). Chemical profiling and antibacterial activity screening of the leaves of *Picralima nitida* (Apocynaceae). *International Journal of Medicinal Chemistry & Analysis.* 4 (3):155-161

Trease, G. E., and Evans, W. C. (2002). *Phytochemicals. In: Pharmacognosy*, Saunders Publishers, London, p214.

Ubulom, P, Akpabio, E., Udobi, C. E. and Mbon, R. (2011). Antifungal activity of aqueous and ethanolic extracts of *Picralima nitida* seeds on *Aspergillus flavus*, *Candida albicans* and *Microsporum canis*. Res. Pharm. Biotech. 3 (5): 57-60.

Vidita V. Bhargava1, Ajay K Saluja and Kishor K. Dholwani (2013). Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia*. *Journal of Pharmacognosy and Phytochemistry*. 1(6):61