Antimicrobial Study On Alstonia boonei, Phyllantus amarus and Nuclea latifolia, Three Ethnomedicinal Plants of Nigeria

Afolabi O[1]. Olawale, I.J.[2] & Adegbite J.A.[2]
[1]Department of Science Laboratory Technology
[2]Department of Applied Sciences
Osun State Polytechnic
Iree, Osun State, Nigeria
Correspondence: afolabioj555@gmail.com, 08038294188

ABSTRACT

Phyllanthus amarus, Alstonia boonei, Nauclea latifolia are plants which are present abundantly in Nigeria and other tropical countries. The success of chemotherapy against the challenge posed by the dynamic emergence of resistant strains lies in the continuous search for new potent drugs. Plant-derived antimicrobials have a long history of providing the much needed novel therapeutics and lead compounds. This study investigated the antimicrobial activity of methanol and n-hexane extracts of the leaves of three ethnomedicinal plants; *Alstonia boonei, Phyllanthus amarus, and Nauclea latifolia* against a panel of clinical significant microorganisms viz *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Candida albicum and Candida tropicalis*. The methanol and n-hexane extracts of the plants were subjected to microbial susceptibility assay using agar well diffusion method and The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using micro dilution assay. Methanol crude extracts of *Alstonia boonei and Phyllanthus amarus* showed a good antimicrobial activity with MIC and MBC values ranging from 1.5mg/mL to 2.5 mg mL-1. The MBC/MIC values of these extracts range from 1 to 1.67 they are thus bactericidal.

iSTEAMS Multidisciplinary Conference Proceedings Reference Format
Afolabi O., Olawale, I.J. & Adegbite J.A. (2019): Antimicrobial Study On Alstonia boonei, Phyllantus amarus and Nuclea latifolia, Three Ethnomedicinal Plants Of Nigeria. Proceedings of the 22nd iSTEAMS Multidisciplinary SPRING Conference. Aurora Conference centre, Osogbo, Nigeria. 17th – 19th December, 2019. Pp 183-190. www.isteams.net/spring2019. DOI - https://doi.org/ 10.22624/AIMS/iSTEAMS-2019/V22N1P17

1. BACKGROUND TO THE STUDY

Many cultures throughout the world still rely on indigenous medicinal plants for their primary health care needs. Farnsworth N, Akerele AO, Bingel AS, Soejarto DD, Guo Z, 1985). However, scientific proof that the active components contained in these medicinal plants are useful, safe, and effective is generally lacking and remain the main problem facing the use of herbal traditional medicines. This proof is necessary in order to eliminate the concern of their use as drugs for alternative treatment. (John Prosper Kwaku Adotey, Genevieve Etornam Adukpo, Yaw Opoku Boahen, and Frederick Ato Armah , 2012). To date, 25% of modern medicines are derived from plants that have been used by traditional medical practitioners. Cragg G. and Newman DJ, (2005)..The success of chemotherapy against the challenge posed by the dynamic emergence of resistant strains lies in the continuous search for new potent drugs. Mohamed Sham Shihabudeen. H ,Hansi Priscilla. D, Kavitha Thirumurugan, 2010).

Plant-derived antimicrobials have a long history of providing the much needed novel therapeutics. Silva MSP, Brandao DO, Chaves TP, Filho ALNF, Costa EMDB, Santos VL. (2012). There is therefore the need for screening of ethnomedicinal plants in order to validate their ethnomedicinal uses and to isolate and characterise their bioactive(s) towards the development of ethnopharmacopea, the discovery of novel active compounds and drug development. In this study methanol and n-hexane extracts of three plants (Alstonia boonei, Phyllantus amarus, Nuclea Latifolia), indigenous to Nigeria and with reported medicinal uses were screened for their antimicrobial activity.

2. MATERIALS AND METHOD

Fresh samples of leaves of three ethnomedicinal plants, namely; *Alstonia boonei* obtained from Ago owu farm settlement, Ayedaade L.G.A. Osun state, *Phyllantus amarus*, from Liverpool farm settlement, Apapa L.G.A. Lagos state, and *Nuclea latifolia* from Ogogo Oke farm in Boripe L.G.A. Osun state were screened. All samples were authenticated at the botany department Obafemi Awolowo University, Ile Ife, Nigeria.

Extract Preparation

The Air-dried and pulverised plant samples were cold extracted 7 days with methanol and hexane. The extract was filtered and allowed to evaporate in open air. The dried extract is dissolved in 10% DMSO and stored in refrigerator until used.

.

Test Microorganisms

The test organisms were collected from a stock culture maintained at 4^{o}C in the Department of Microbiology, University of Ilorin, Kwara state, Nigeria. One gram-positive bacteria: *Staphylococcus aureus*, three gram-negative bacteria: *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* and two fungi: *Candida albicans* and *Candida tropicalis* were used in the study.

Preparation of inoculum

The strains were maintained on nutrient broth. Active cultures for experiments were prepared by transferring a loopful of each organism into 50ml of the broth in 100ml conical flasks. The Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi were incubated without agitation for 24 h at 37°C and 48h at 25°C respectively.

Antimicrobial Assay

Agar well diffusion method:
200µl of bacteria and fungi were aseptically introduced and spread using cotton swab, on the surface gelled sterile Muller Hinton agar (MHA) plates and Sabouraud dextrose agar (SDA) plates respectively. A well of 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate and 50 µl of each plant methanol and n-hexane extracts were introduced into the well.

In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA). Negative control was prepared using 50ml of the respective solvent and positive control was made by placing several antibiotics disc on agar plates nd then incubated at 37^{o}C for 24h for the bacteria and 48h for the fungi. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Minimum Bactericidal Concentrations (MBC) were determined using 150mg/ml and 250mg/ml of the extracts and standardized bacterial and fungi cultures (1 x 10^{6} cfu/ml)

3. RESULTS

Table 1: Zones of inhibition (mm) of the plants extracts on selected clinical microorganisms.

Methanol extracts						
Clinical isolates	*S.aureus*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*	*C,tropicalis*
A.boonei	-	19	18	14	14	15
P.amarus	16	16	-	18	-	-
N.latifolia	-	14	-	-	-	-
n-hexane extracts						
A.boonei	20	14	-	16	17	-
P.amarus	12	15	14	-	-	-
N.latifolia	-	-	-	-	-	-

Key: -:
No zone of inhibition

Table 2: Antimicrobial activity of varying concentrations (mg/ml) of the plants extracts on selected clinical microorganisms.

Methanol extracts						
Clinical isolates	*S.aureus*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*	*C,tropicalis*
A.boonei						
150	NA	-	-	-	-	-
250	NA	-	-	-	-	-
P.amarus	12	-	NA	-	NA	NA
150	-	-	NA	-	NA	NA
250						
N.latifolia						
150	NA	-	NA	NA	NA	NA
250	NA	+	NA	NA	NA	NA
n-hexane extracts						
A.boonei						
150	-	14	NA	-	-	NA
250	-	-	NA	-	-	NA
P.amarus						
150	+	+	+	NA	NA	NA
250	-	+	+	NA	NA	NA
N.latifolia						
150	NA	NA	NA	NA	NA	NA
250	NA	NA	NA	NA	NA	NA

Key:
- : Clear (No Microbial growth)
+: Turbid (Microbial growth)
NA: Not applicable

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (mg/mL) of the plants extracts on selected clinical microorganisms.

Methanol extracts						
Clinical isolates	*S.aureus*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*	*C,tropicalis*
A.boonei						
MIC	NA	1.5	1.5	1.5	1.5	1.5
MBC	NA	1.5	1.5	1.5	2.5	1.5
MBC/MIC	-	1	1	1	1.67	1
P.amarus						
MIC	2.5	1.5	NA	1.5	NA	NA
MBC	2.5	2.5	NA	1.5	NA	NA
MBC/MIC	1	1.67	-	1	-	-
N.latifolia						
MIC	NA	2.5	NA	NA	NA	NA
MBC	NA	-	NA	NA	NA	NA
MBC/MIC	-	-	-	-	-	-
n-hexane extracts						
A.boonei						
MIC	1.5	2.5	NA	1.5	1.5	NA
MBC	1.5	2.5	NA	1.5	1.5	NA
MBC/MIC	1	1	-	1	1	-
P.amarus	+	+	+	NA	NA	NA
MIC	+	+	+	NA	NA	NA
MBC	-	-	-	-	-	-
MBC/MIC						
N.latifolia						
MIC	NA	NA	NA	NA	NA	NA
MBC	NA	NA	NA	NA	NA	NA
MBC/MIC	-	-	-	-	-	-

Key:
- : Clear (No Microbial growth)
+:: Turbid (Microbial growth)
NA: Not Applicable

Table 4: Susceptibility test (zone of inhibition [mm]) using standard antibiotics discs against selected clinical microorganisms.

Clinical isolates	*S.aureus*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*	*C,tropicalis*
AUG	-	-	-	-	-	-
GEN	16.5	-	-	13	-	-
OFL	20	-	-	-	12	-
ERY	-	NA	NA	NA	NA	NA
CRX	-	-	-	-	14	12
CAZ	9	-	-	-	-	-
NIT	-	12	-	10.5	19	10
CTR	-	NA	NA	NA	NA	NA
CXC	-	NA	NA	NA	NA	NA
AMP	12	-	-	-	-	-
CPR	-	-	-	-	-	-

Key:
- : Resistance,
NA: Not Applicable,
AUG: Amoxycillin,/Clavulatrate, GEN: Gentamycin, OFL: Ofloxacin; ERY: Erythromycin, CRX: Cefuroxime, CAZ: Ceftazidime, NIT: Nitrofuratoin, CTR: Ceftraxone, CXC: Cloxicillin, AMP: Ampicilin. CPR: Ciprofloxacin,

3. DISCUSSION

Table 1 show the zone of inhibition of the methanol and n-hexane extracts of A. boonei, P. Amarus and N.latifolia on selected clinical microorganisms. Methanol and n-hexane are common solvents used for solvent extraction of plants because they liberate greater amounts of bioactive compounds (Sasidharan et. al., 2011, Yadau and Agarivala, 2011). Zone of inhibition of methanol extracts of *A.* boonei at 19mm on E. Coli was highest, followed by *P.Aeriginosa* at 18mm and this has a correlation with *P. amarus K. pneumoniae at 18mm. N.latifolia* was not sensitive on most of the clinical microorganisms except on *E.coli at 14mm. n-hexane extracts of A.boonei* show sensitivity to most of the clinical microorganisms except for *P.aerginosa and C.tropicalis with S.aureus at 20mm, E.coli at 14mm, K.pneumoniae at 16mm, C.albicans at 17mm. same for the extract of P.amarus with S.aureus at 12mm, E.coli at 15mm, P.aerginosa at 14mm.* all the selected microorganisms show resistance to n-hexane extract of *N. latifolia. All the* extract showed no inhibition of *C. tropicalis.*

Table 2 show the antimicrobial activity of varying concentrations of the plant extracts on the selected clinical microorganism. All the extracts do not show sensitivity at the concentrations used (150mg/ml and 250mg/ml) except for the 150mg/ml methanol extract of *P.amarus* at 12mm and 150mg/ml n-hexane extract of A.boonei at 12mm. There were no microbial growth and where there were, they were turbid. Table 3 show the minimum inhibitory concentaration (MIC) and minimum bactericidal concentration of the methanol and n-hexane extracts on the selected clininical microorganisms. The MIC and MBC on methanol extracts of both A.boonei and P.amarus have identical concentrations. For example *E.Coli, P.aeriginosa, K.pneumonia and C.tropicalis* have the same MIC and MBC values, except in C.albicans where it show 1.5 mg/mL and 2.5 mg/mL respectively on the extract of A.boonei. MIC and MBC of n-hexane extracts are same for *A.boonei and P.amarus* on the microorganisms used. N. latifolia show no sensitivity on the microorganisms. This consistent with the results from Table 1 and Table 2.

Table 4 show susceptibility test using standard antibiotics discs against the selected clinical microorganisms. Susceptibility was noticed on the antibiotics GEN: Gentamycin, OFL: Ofloxacin, CAZ: Ceftazidime, and AMP: Ampicilin for *S.aureus*, NIT: Nitrofuratoin, for *E.coli*. GEN: Gentamycin and NIT: Nitrofuratoin for *K. pneumoniae*, OFL: Ofloxacin, CRX: Cefuroxime and NIT: Nitrofuratoin for *C.albicans* and CRX: Cefuroxime and NIT: Nitrofuratoin for *C. topicalis* From the above results GEN: Gentamycin, OFL: Ofloxacin, CRX: Cefuroxime and NIT: Nitrofuratoin are the most potent antibiotics while AMP: Ampicilin and CAZ: Ceftazidime are slightly sensitive. GEN, OFL and NIT are broad spectrum antibiotics having effect on both gram positive and gram negative bacteria, and compatible activity with A. boonei and P.amarus as shown in Table 1 with whose zones of inhibition met the standard antibiotics used, especially GEN and OFL antibiotics.

4. CONCLUSION

A good antimicrobial activity of methanol and n-hexane leaf extracts of A. boonei and P.amarus was observed when compared to standard antimicrobial agents. This study therefore supports the use of A. boonei and P.amarus as medicinal plant by traditional healers. Further work is needful to fractionate, isolate and characterize their specific bioactive constiyuent(s) responsible for the antimicrobial activity and reported medicinal properties towards development of our ethnopharmacopea and drug development.

REFERENCES

1. Abe F, Chen RF, Yamauchi T, Marubayashi N, Ueda I. (1989). Studies on the constituents of *Alstonia scholaris*. Part I. Alschomine and isoalschomine, new alkaloids from the leaves of *Alstonia scholaris*. *Chemical & Pharmaceutical Bulletin*. 37(4):887–890.
2. Abel C, Busia K. (2005). An exploratory ethnobotanical study of the practice of herbal medicine by the Akan peoples of Ghana. *Alternative Medicine Review*. 10(2):112–122.
3. Adegoke AA, Iberi PA, Akinpelu DA and Aiyegoro P. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. International Journal of Applied Research in Natural Products 3: 6.
4. Ademola, I.O., Fagbemi, B.O., Idowu, S.O. (2006). Anthelmintic efficacy of Nauclea latifolia extract against gastrointestinal nematodes of sheep: in vitro and in vivo studies. Afr. J. Tradit. Complement. Altern. Med 4, 148–156.
5. Abbah, J., Amos, S., Chindo, B., Ngazal, I., Vongtau, H.O., Adzu, B., Farida, T., Odutola, A.A., Wambebe, C., Gamaniel, K.S., (2010). Pharmacological evidence favouring the use of Nauclea latifolia in malaria ethnopharmacy: effects against nociception, inflammation, and pyrexia in rats and mice. J. Ethnopharmacol. 127, 85–86
6. Abd El-Ghani, M.M., (2016). Traditional medicinal plants of Nigeria: an overview. Agric.Biol. J. N. Am. 7, 220–247.
7. Abiodun, F., Igwe, A., Osahon, O., (2007). Anti-microbial evaluation of a herbal dental remedy: stem bark of Nauclea latifolia-family Rubiaceae. J. Appl. Sci. 7, 2696–2700.
8. Agomuoh, A.A., Ata, A., Udenigwe, C.C., Aluko, R.E., Irenus, I. (2013). Novel indole alkaloids from Nauclea latifolia and their renin-inhibitory activities. Chem. Biodivers.10, 401–410.
9. Alaribe, C.S., Adesegun, S., Idowu, A.O., Egere, O., Ihemedu, C., Coker, H.A.B., Smith, S., (2014). Healing, cytoprotective and anti-Helicobacter pylori activities of stem bark
10. extracts and butanol fraction of Nauclea latifolia. J. Nat. Prod. 7, 184–195.
11. Baur AW, Kirby WM, Sherris JC, Turck M.(1966). Antibiotic susceptibility testing by a standard single disk method. Am J Clin Path; 45: 493-496.
12. Cragg G. and Newman DJ. (2005) Biodiversity: a continuing source of novel drug leads. *Pure and Applied Chemistry*; 77(1):7–24.
13. Croquelois G, Kunesch N, Debray M, Poisson J. (1972). *Alstonia boonei* alkaloids. *Medicinal Plants and Phytotherapy*. 6(2):122–127.
14. Edeoga, HO, Okwu DE, Mbaebie BO. (2005) Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech; 4: 685-688
15. Farnsworth N, Akerele AO, Bingel AS, Soejarto DD, Guo Z (1985). Medicinal plants in therapy. *Bulletin of the World Health Organization*; 63(6):965–981.
16. Itoro E, Ukana D and Ekaete D. (2013). Phytochemical screening and nutrient analysis of *Phyllanthus amarus*. Asian Journal of Plant Science and Research 3: 116-122.
17. Iwu MW, Duncan AR, Okunji CO.(1999). New antimicrobials of plant origin In: Perspectives on new Crops and new Uses, eds. J. Janick, ASHS Press, Alexandria, VA; 457-/462
18. John Prosper Kwaku Adotey, Genevieve Etornam Adukpo, Yaw Opoku Boahen, and Frederick Ato Armah (2012). A Review of the Ethnobotany and Pharmacological Importance of *Alstonia boonei* De Wild (Apocynaceae). *ISRN Pharmacol*
19. Joseph B and Raj SJ. (2011). An Overview: Pharmacognostic Property of *Phyllanthus amarus* International Journal of Pharmacology 7: 40-45.

20. Kucera M, Marquis VO, Kucerova H. (1972). Nigerian medicinal plants. I. TLC thin-layer chromatographic] separation and quantitative evaluation of *Alstonia boonei* alkaloids. *Planta Medica*. 21(4):343–346.
21. Mohamed Sham Shihabudeen. H ,Hansi Priscilla. D, Kavitha Thirumurugan, (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal
22. Plants. International Journal of Pharma Sciences and Research (IJPSR): 1(10), 430-434.
23. Oigiangbe ON, Igbinosa IB, Tamo M. (2007). Insecticidal activity of the medicinal plant, *Alstonia boonei* De Wild, against *Sesamia calamistis* Hampson. *Journal of Zhejiang University Science B*. 8(10):752–755.
24. Patel JR, Tripathi P, Sharma V, Chauhan NS and Dixit VK. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phyto-chemistry and pharmacology: A review. Journal of Ethnopharmacology 138: 286-313.
25. Rukangira E. (2004). Overview on medicinal plants and traditional medicine in Africa. *Qualitative Health Research*. 14:675–690.
26. Silva MSP, Brandao DO, Chaves TP, Filho ALNF, Costa EMDB, Santos VL. (2012:). Study bioprospecting of medicinal plant extracts of the semiarid Northeast: Contribution to the control of oral microorganisms. Evi-Based Comp Alt Med 2012; 1-6.
27. Van Wyk B-E. (2008). A broad review of commercially important Southern African medicinal plants. *Journal of Ethnopharmacology*. 119(3):342–355.
28. Verma S, Sharma H and Garg M. (2014). *Phyllanthus amarus*: A review. Journal of Pharmacognosy and Phytohemistry 3: 18-22.