

Antimicrobial Study on *Alstonia Boonei*, *Phyllanthus Amarus* and *Nuclea Latifolia*, Three Ethnomedicinal Plants of Nigeria

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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.

Index Terms- antimicrobial activity; ethnomedicinal plants; methanol extract; n-hexane extract, antibiotics

I. INTRODUCTION

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 Etonam 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*, *Phyllanthus amarus*, *Nuclea Latifolia*), indigenous to Nigeria and with reported medicinal uses were screened for their antimicrobial activity.

II. 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, *Phyllanthus 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°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 and then incubated at 37°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 10⁶ cfu/ml)

III. RESULTS

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

Methanol extracts

Clinical isolates	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>	<i>C.albicans</i>	<i>C.tropicalis</i>
<i>A.boonei</i>	-	19	18	14	14	15
<i>P.amarus</i>	16	16	-	18	-	-
<i>N.latifolia</i>	-	14	-	-	-	-

n-hexane extracts

<i>A.boonei</i>	20	14	-	16	17	-
<i>P.amarus</i>	12	15	14	-	-	-
<i>N.latifolia</i>	-	-	-	-	-	-

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	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>	<i>C.albicans</i>	<i>C.tropicalis</i>
<i>A.boonei</i>						
150	NA	-	-	-	-	-
250	NA	-	-	-	-	-
<i>P.amarus</i>	12	-	NA	-	NA	NA
150	-	-	NA	-	NA	NA
250						
<i>N.latifolia</i>						
150	NA	-	NA	NA	NA	NA
250	NA	+	NA	NA	NA	NA

n-hexane extracts

<i>A.boonei</i>						
150	-	14	NA	-	-	NA
250	-	-	NA	-	-	NA
<i>P.amarus</i>						
150	+	+	+	NA	NA	NA
250	-	+	+	NA	NA	NA
<i>N.latifolia</i>						
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	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>	<i>C.albicans</i>	<i>C.tropicalis</i>
<i>A.boonei</i>						
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
<i>P.amarus</i>						
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	-	-
<i>N.latifolia</i>						
MIC	NA	2.5	NA	NA	NA	NA
MBC	NA	-	NA	NA	NA	NA
MBC/MIC	-	-	-	-	-	-
n-hexane extracts						
<i>A.boonei</i>						
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	-
<i>P.amarus</i>						
MIC	+	+	+	NA	NA	NA
MBC	-	-	-	-	-	-
MBC/MIC	-	-	-	-	-	-
<i>N.latifolia</i>						
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	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>	<i>C.albicans</i>	<i>C.tropicalis</i>
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: Amoxicillin./Clavulatrane, GEN: Gentamycin, OFL: Ofloxacin; ERY: Erythromycin, CRX: Cefuroxime, CAZ: Ceftazidime, NIT: Nitrofurantoin, CTR: Ceftriaxone, CXC: Cloxacillin, AMP: Ampicillin. CPR: Ciprofloxacin,

IV. 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.aeriginosa* 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.aeriginosa* 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 concentration (MIC) and minimum bactericidal concentration of the methanol and n-hexane extracts on the selected clinical 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: Ampicillin for *S.aureus*, NIT: Nitrofurantoin, for *E.coli*. GEN: Gentamycin and NIT: Nitrofurantoin for *K. pneumoniae*, OFL: Ofloxacin, CRX: Cefuroxime and NIT: Nitrofurantoin for *C.albicans* and CRX: Cefuroxime and NIT: Nitrofurantoin for *C. topicalis* From the above results GEN: Gentamycin, OFL: Ofloxacin, CRX: Cefuroxime and NIT: Nitrofurantoin are the most potent antibiotics while AMP: Ampicillin 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.

V. 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.

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