

## Antimalarial, Non Alkaloidal Molecules from Preliminary Elucidation of *Nauclea Diderechi* Extract

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### Abstract

The work investigated pharmacologically useful classes of compounds, from the petroleum ether fraction of the stem bark of *Nauclea diderichii*. From the bioassay, most of the microbes showed sensitivity except for *Streptococcus pyrogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and the fungus *Candida stellatoidea*, where the extracts were non-active. Petroleum ether extract was below detectable limits (BDL) in methicillin resistant *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Corynebacterium ulcerans*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and the fungi, *Candida albicans*, *Candida stellatoidea* and *Candida tropicalis*. Chloroform extract had the highest activity, followed by the ethyl acetate, then the methanol and lastly the petroleum ether. Minimum inhibitory concentration (MIC) was 1.25 mg/mL on the average. MBC was 2.25 mg/mL and MFC 5.00 mg/ml. Anti-malaria activity showed the LD<sub>50</sub> of the acute toxicity as  $\geq 3800 \text{ mg kg}^{-1}$  body weight. The suppressive test, curative effect, prophylactic effect and the survival periods were highly encouraging. Three compounds were isolated from the preliminary pet. Ether fraction of *Nauclea diderichii* and identified by infrared (IR), ultraviolet (UV) and gas/liquid chromatography–mass spectroscopy (GC/LC-MS), analyses as Hexadecanoic acid, 15-methyl-, methyl-ester, 7-Tetradecenal and 9- and 10- octadecenoic acid, methyl ester. These findings were in consonance with literature of plants and lent credence for the use of the stem bark of *Nauclea diderichii* as a potential natural drug source for treatment of some ailments in Adamawa State of northeastern Nigeria and beyond.

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**Keywords:** *Nauclea diderichii*; Bioassay; Pharmacology; Preliminary-elucidation, Microbes, Anti- Malaria; Chromatography

### Introduction

Phytomedicine has been found to be active against a wide variety of micro-organisms and has shown great promise in the treatment of many intractable diseases including opportunistic AIDS infections. Most of them have been investigated for their chemical components and some of the isolated compounds have been shown to possess diverse and interesting biological activities<sup>[1,2]</sup>.

In Mubi north and south LGAs of Adamawa State Northeastern Nigeria, malaria, diarrhea and dysentery are health challenges<sup>[3,4]</sup> and is believed *Nauclea diderichii* possesses medicinal properties effective in their management since decoctions of this plant are used for their cure like the rinds of *Punica granatum*<sup>[5,6]</sup>.

*Nauclea diderichii* (De Wild & Dur) Meril (Rubiaceae). (Commercial name: *bilinga*) Common name; African peach, is reputed for its numerous medicinal uses as a tonic and fever medicine, chewing stick, toothaches; dental caries, septic mouth, malaria,



diarrhea and dysentery cure<sup>[7]</sup>.

Scientists are still to justify the claim that children suffer from severe dysentery/diarrhea during teething. But it could be inferred that this is a time when the children are exposed to lots of contaminated things as they crawl around and imbibe virtually everything. In Mubi LGAs, North Eastern Nigeria, the stem bark of *bilinga* are used (oral decoction), for the treatment of malaria and dysentery /diarrhea both in children and adults (Per comm.).

### Subject

This paper makes an attempt at the preliminary elucidation of some of the structures found in this indigenous medicinal plant, their potentials against some disease causative micro-organisms, plasmodium *berghei berghei* Nk65, bacteria and fungi, due to antibiotic resistance in nosocomial and community acquired infections, thus viewing *Nauclea diderichii* as a potential source for phytomedicine development.

### Materials and Methods

#### Collection of plant materials:

Fresh samples of the stem bark of the *African peach* were collected in Michika Adamawa State and were identified in the Biological Sciences Department of Adamawa State University Mubi. The FHI number is 0194 and a specimen of the plant was deposited in the herbarium. The sample (1.4 kg) was air dried in the laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh sizes and then stored in a dry container.

#### Extraction

400 g of the powdered stem bark was accurately weighed and percolated with 10L of distilled ethanol for 72 hrs to get the crude extract. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 35°C. It was later macerated with different solvents of increasing polarities starting with petroleum ether, chloroform, ethylacetate and methanol using serial extraction to have their corresponding fractions.

#### Anti-bacterial Profile (Susceptibility Test)

The antibacterial screening was carried out using the agar diffusion method, (Lino and Deogracios, 2006) with slight modifications. Overnight culture of the various bacteria in blood agar and the fungi in Sabouraud dextrose agar slant media were sterilized to produce inoculums size of 10<sup>6</sup> cfu/mL. The medium was seeded with standard inoculums (0.1 mL) of the micro-organism (mc-for land 0.5). The inoculums were sprayed evenly by the use of sterile swab over the surface of the medium; the seeded plates were allowed to dry at 37°C for 30 mins inside incubator. A standard cork borer (6 mm) was used to cut a well at the centre of each seeded medium used and the solution of the crude extracts (10mg/mL) was then introduced into each hole on the surface of the medium. In one medium was introduced dimethylsulphoxide (DMSO) (0.1 mL) to serve as negative control and sparfloxacin (20 µg/mL) for the bacteria and fluconazole (50 µg/mL) for the fungi to serve as positive controls respectively. The medium was incubated at 37°C for 24 hours for 2 - 7 days for the bacteria and fungi respectively. The plates were then observed for zones of inhibitions which were measured with a transparent ruler and results recorded.

#### Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using broth dilution method<sup>[8]</sup>, modified by<sup>[9]</sup> Usman, Abdulrahman, and Ladan, 2007). Two fold serial dilutions of the crude extract were made to obtain concentrations of 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) suspension of standard inoculum of each organism was inoculated to the different concentrations. The test tubes were then incubated at 37°C for 24 h after which they were observed for growth. Inhibition of growth was indicated by a clear solution. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism.

#### Determination of Minimum Bactericidal Concentration / Minimum Fungicidal Concentration (MBC / MFC)

The contents of the above MIC tubes and the preceding tubes in the serial dilution were sub-cultured into appropriately labeled nutrient agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the labeled nutrient agar plates. The plates were then incubated at 37°C for 24 hrs after which they were observed for growth of colony. The lowest concentration of the subculture with no growth for both bacteria and fungi were considered as minimum bactericidal/fungicidal concentrations<sup>[8,9]</sup>.

#### Anti-Malarial Studies

An antimicrobial study was carried out in two phases using modified<sup>[10]</sup> Lorke (1983). The oral median lethal dose (LD<sub>50</sub>) was calculated using Plasmodium *berghei berghei* NK 65 parasites<sup>[11-13]</sup>.

The extracts caused a significant (P < 0.05) chemo-suppression dose dependent reduction in parasitaemia levels in the extracts treated groups of *Plasmodium berghei berghei* NK 65 malaria parasite with a similar reduction in the chloroquine treated group (positive control). The results indicated that the stem bark possessed blood schizonticidal activity as evident from the chemo-suppression obtained during the four day early infection test and the 30 days curative / established infection comparable to the standard drug chloroquine, 5 mg / kg / day<sup>[14,15,16]</sup>.

### Spectroscopic Measurement and component identification

Continuous purification of the petroleum ether fraction led to the isolation of samples (H-2, structure 4), (H-3 structure 7), & (H-1 structure 6), colourless and oily in nature. These were chromatographed several times and sent for GC-MS analysis using Agilent 1100 Series Liquid Chromatography (LC/MSD Trap - VL Model) coupled to 3207 Mass Selective Detector (MSD), in DMSO as solvent in Natural Product Research Laboratory School of Chemistry University of Kwazulu – Natal West-Ville Campus, Durban, 4000, South Africa and the most prominent were:-

- structure 4, intensity of 5.500, column oven temperature of 60°C injection temperature 250°C at retention time of 27.7 minutes,
- structure 7, intensity of 6.500, column temperature of 60°C injection temperature 250°C at retention time of 29.2 minutes, and,
- structure 6 with intensities of 7.552, with column oven temperature of 60°C, injection temperature of 250°C at the retention time of 28.9 minutes, respectively.

### Discussion

Pharmacologically useful classes of compounds, from the ethanol extract of the stem bark of *Nauclea diderichii* included, tannins, saponins, terpenoids, glycosides, sterols, alkaloids, carbohydrates, flavonoids and phenols. Resins and phlobatannins were below detectable levels. From (Table 1), the study also confirmed that the stem bark had antibacterial activity against gram positive and negative bacteria and antifungal activity (Iwu 1993). It was most effective against *Corynebacterium ulcereas*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *klebsiella pneumonia* and the fungus *Candida tropicalis*<sup>[17]</sup>.

**Table 1:** Zone of inhibitions; Petroleum ether, Chloroform, ethyl acetate and methanol extracts of the stem bark of *N. diderichii* on some fourteen micro-organisms.

Micro-organisms	Zones of inhibition (mm)						
	Pet. Ether	Chloro.	Ethyl Act.	Methanol	Sparflo	.Flocona	DMSO
<i>Methicillin R.S.A</i>	00	25	00	21	33	00	00
<i>Staphylococcus aureus</i>	15	27	21	22	35	00	00
<i>Streptococcus pyrogenes</i>	00	00	00	00	32	00	00
<i>Bacillus subtilis</i>	14	26	20	23	40	00	00
<i>Corynebacterium ulcereas</i>	00	24	20	21	28	00	00
<i>Escherichia coli</i>	17	27	21	22	30	00	00
<i>Proteus mirabilis</i>	00	22	00	00	28	00	00
<i>Proteus vulgaris</i>	00	00	00	00	28	00	00
<i>Pseudomonas aeruginosa</i>	00	00	00	00	28	00	00
<i>Salmonella typhi</i>	17	27	20	21	32	00	00
<i>Klebsiella pneumoniae</i>	19	25	21	25	40	00	00
<i>Candida albicans</i>	00	21	00	00	00	24	00
<i>Candida stllatoidae</i>	00	00	00	00	00	27	00
<i>Candida tropicalis</i>	00	24	20	21	00	21	00

Results: Mean of three trials ± Standard error, R→ Resistance.

Sparflo = Sparfloxacin (Bacteria Control): 10µg/ml

Flucona = Fluconazole (Fungal control): 20µg/ml

The chloroform extract had the highest activity, followed by the ethyl acetate, then the methanol and lastly the petroleum ether. In (Table 2), the minimum inhibitory concentration (MIC) was 1.25 mg/mL on the average. MBC / MFC (Table 3) were carried out to check whether the bacteria and the fungi were killed or only their growth was inhibited with an average value of 2.25 mg/mL. MFC was found to be 5.00 mg / ml and was effective for chloroform, ethyl acetate and methanol.

**Table 2:** Minimum Inhibitory Concentration (MIC) of Petroleum ether, Chloroform, ethyl acetate and methanol extracts of the stem bark of *N. diderichii* on some fourteen micro-organisms

Micro-organisms	Concentration (mg/mL)																			
	Pet. Ether					Chloroform					Methanol					Ethyl acetate				
	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312
<b>Methicillin R.S.A</b>	-	-	-	-	-	-	-	MIC	+	++	-	-	MIC	+	++	-	-	-	-	-
<b>Staphylococcus aureus</b>	MIC	+	+	++	+++	-	-	MIC	++	+++	-	-	MIC	+	++	-	MIC	+	++	++
<b>Streptococcus pyrogenes</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Bacillus subtilis</b>	MIC	+	+	++	+++	-	-	MIC	+	++	-	MIC	-	+	++	-	-	MIC	+	++
<b>corynebacterium ulcereas</b>	-	-	-	-	-	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++
<b>Escherichia coli</b>	-	MIC	+	++	+++	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++
<b>Proteus mirabilis</b>	-	-	-	-	-	-	-	MIC	+	++	-	-	-	-	-	-	-	-	-	-
<b>Proteus vulgaris</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Pseudomonas aeruginosa</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Salmonella typhi</b>	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++
<b>Klebsella pneumoniae</b>	-	MIC	+	++	+++	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++
<b>Candida albicans</b>	-	-	-	-	-	-	-	MIC	-	-	-	-	-	-	-	-	-	-	-	-
<b>Candida stllatoideae</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Candida tropicalis</b>	-	-	-	-	-	-	-	MIC	+	++	-	-	MIC	+	++	-	-	MIC	+	++

Key: - = no turbidity, (No growth), + = turbidity (Light growth), ++ = (Moderate growth), +++ = (High growth) MIC= Minimum inhibitory concentration

Table 3: Minimum Bacteriocidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC) of Petroleum ether, Chloroform, ethyl acetate and methanol extracts of the stem bark of *N. diderichii* on some fourteen micro-organisms

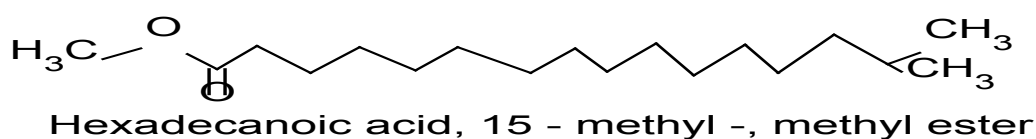
Micro-organisms	Concentration (mg/mL)																			
	Pet. Ether					Chloroform					Methanol					Ethyl acetate				
	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312	5.00	2.50	1.25	0.625	0.312
<b>Methicillin R.S.A</b>	-	-	-	-	-	-	-	MIC	+	++	MIC	+	++	+++	+++	-	-	-	-	-
<b>Staphylococcus aureus</b>	MIC	+	++	+++	++++	-	MIC	+	++	+++	MIC	+	++	+++	++++	MIC	+	++	+++	++++
<b>Streptococcus pyrogenes</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Bacillus subtilis</b>	MIC	+	++	+++	++++	-	MIC	+	++	+++	MIC	+	++	+++	++++	MIC	+	++	+++	++++
<b>corynebacterium ulcerans</b>	-	-	-	-	-	MIC	+	++	+++	++++	MIC	+	++	+++	++++	MIC	+	++	+++	++++
<b>Escherichia coli</b>	MIC	+	++	+++	++++	-	MIC	+	++	+++	MIC	+	++	+++	++++	MIC	+	++	+++	++++
<b>Proteus mirabilis</b>	-	-	-	-	-	MIC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Proteus vulgaris</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Pseudomonas aeruginosa</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Salmonella typhi</b>	MIC	+	++	+++	++++	-	MIC	+	++	+++	MIC	+	++	+++	++++	MIC	+	++	+++	++++
<b>Klebsella pneumoniae</b>	MIC	+	++	+++	++++	-	MIC	+	++	+++	-	MIC	+	++	+++	MIC	+	++	+++	++++
<b>Candida albicans</b>	-	-	-	-	-	MIC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Candida stllatoideae</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Candida tropicalis</b>	-	-	-	-	-	MIC	+	++	+++	++++	MIC	+	++	+++	++++	MIC	+	++	+++	++++

Key: - = no turbidity, (No growth), + = turbidity (Scanty colonies growth), ++ = (Moderate growth), +++ = (High growth), ++++ = (v. heavy colonies growth) MBC / MFC = Minimum Bacteriocidal Concentration / Minimum Fungicidal Concentration.

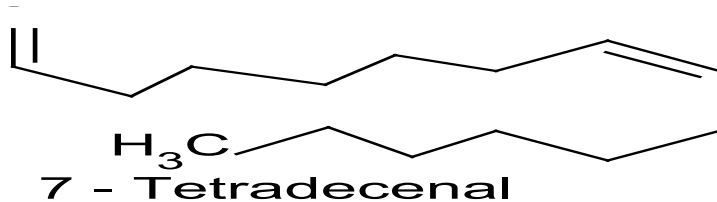
The alcoholic extract of *N. diderichii* showed anti-malarial activity against *Plasmodium berghei berghei* Nk65 to be  $\geq 3800$  mg kg<sup>-1</sup> body weight of Swiss Albino mice. The suppressive test, curative effect, prophylactic effect and the survival periods in parasitaemia against chloroquine tolerant strain of *Plasmodium berghei* NK65<sup>[18]</sup> were highly encouraging. Anti-malaria, anti-bacterial and anti-fungal activities of the stem-bark of *Nuclear diderichii* may have been due to the synergetic effect of their chemical constituents or any single chemical compound may have had toxic effects. In various ancient literatures related to healthcare *Clerodendrum* have been reported for its anti-malarial activities because of the presence of a bitter principle. Studies with different parasites supported these ancient claims. These plants may be useful as a source for novel anti-plasmodial drugs/compounds from natural origin.

**Component identification:** All findings with TLC, UV, IR and GC /GL mass confirmed with methyl and hydroxyl groupings as active components in the petroleum ether fraction of the *N. diderichii* as seen:

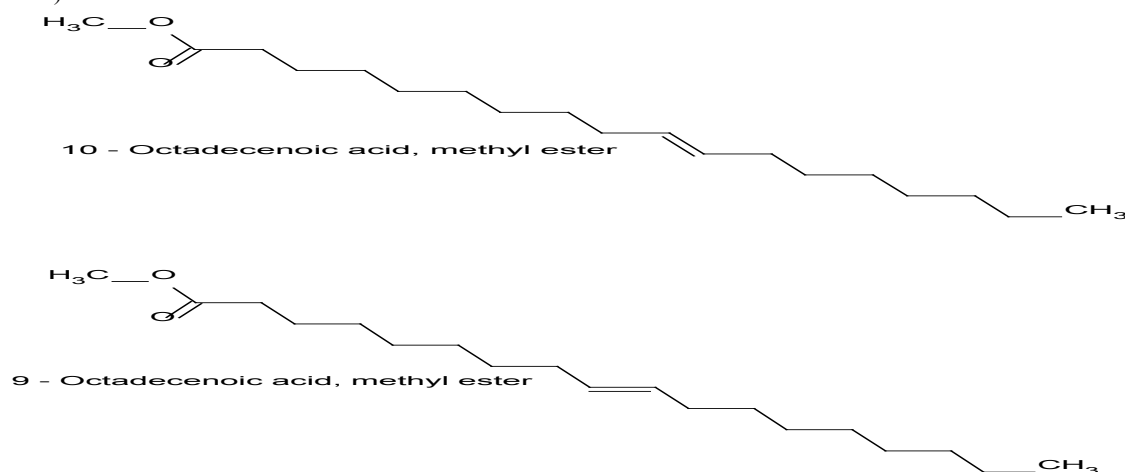
Structure 4



Structure 7



Structure 6 (a &amp; b)



Structures 6a and 6b are structural isomers with the difference only in the position of the ethylene moiety. One at position 9 and the other at position 10.

## Conclusion

From a natural chemist's viewpoint, all the structures that were extracted from the petroleum ether fraction of *N. diderichii* as the active components most have been partially/fully responsible for the activity in both microbes and the *plasmodium berghei* Nk 65 and were non alkaloidal or were all none alkaloids.

## Recommendation

More work should be done on the plant so as to have good quantity of purified compounds for further anti-malaria activity. This will help to back-track and compare the activity of both the crude and the pure samples. Also, other solvent extracts should be elucidated for their active principles' activities on the microbes and the plasmodium species.

Clinical and nutritional studies are recommended for the above indigenous plant so as to be able to advice locals in the various locations of the ill effects of the plant.

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