ISOLATION AND CHARACTERIZATION OF AN ANTI-MALARIAL CLIONASTEROL DERIVATIVE FROM THE CHLOROFORM-SOLUBLE FRACTION OF *Nauclea diderichii* (RUBIACEAE)

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Abstract: Chromatographic scrutiny of the chloroform soluble fraction of the stem bark extract of *Nauclea diderichii* using 'Vacuum liquid' column chromatography led to the isolation of a Clionasterol derivative. The structure of this new compound was determined using both 1 and 2D nuclear magnetic resonance (NMR). *Plasmodium berghei* NK 65 was obtained from the National Institute for Medical Research (NIMR) Lagos. The anti-malarial activity of the crude sample of the compound was investigated using four (4) weeks old-albino mice weighing 18 – 22 g, obtained from the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Oral acute toxicity of the extract with modified Lorke`s method was evaluated against early infection, curative effect, established infection, prophylactic effect, residual infection and their total mean survival period obtained. The Oral median lethal dose of the extract in mice was determined to be about 3800 mg kg^{-1} body weight. The extract at doses of 100, 200 and 400 mg kg^{-1} body weight produced significant ($P< 0.05$) dose dependent activity against the parasites in the suppressive, curative and prophylactic tests. The results suggest that the chloroform soluble extract of *N. diderichii* possesses anti-malarial activity against the susceptible organisms and corroborates the ethno medicinal uses of the plant in the treatment of various ailments, malaria inclusive.

Keywords: *Nauclea diderichii* extract, anti-malarial, *Plasmodium berghei*, oral acute toxicity

Introduction

Natural products are the most important sources of drugs and drug leads in history (Cragg and Newmann, 2001). The occurrence of multidrug resistance in man and animal pathogenic bacteria as well as detrimental side-effects of certain antibiotics has elicited immense interest in the search for novel antimicrobial drugs of plant origin (Ahmed and Beg, 2001). In theory and in practice, bacteria will continue to develop resistance every time it is exposed to any antimicrobial agent, thus imposing the need for a continuous search and development of new/novel drugs (Silver and Bostian, 1993). Included in the priority actions needed to hold back the spread of drug resistance and restrain its potential devastating effects is the continuing finding and development of novel and effective antimicrobial agents (European Commission (EC), 2011). Plants represent a potential and utmost source of newer antibiotic models (Meurer–Grimes *et al*., 1996; Abdullahi *et al*., 2014).

More so, it is an undeniable fact, that owing to their ethnomedicinal importance and numerous compounds isolated and identified from some of them, it becomes expedient to carry out research into these useful medicinal plants used in the therapeutic management of pains in order to obtain potent analgesics, with fewer side effects, cost effective, safe, efficient & effective and accessible, agents for the control of human and animal ailments. (Tor-Anyiin *et al.,* 2013; Sani *et al*., 2014).

Nauclea diderichii (De Wild & Dur) Meril (Rubiaceae). (Commercial name: *bilinga*) Common name; African peach is an evergreen tree that reaches a height of 30-40 m and a diameter of 0.9-1.5 m; bole cylindrical, slender, straight and branchless, rising to 20-30 m and a broad spherical crown with thick foliage. The shining leaves are 15 cm long and bigger when young, elliptic, acute at the ends, keeled towards the base, and stipulate, with a pair of distinct leafy stipules at the base. It is mostly deciduous except at the ends of shoots, and the nodes are often occupied by ants. Flowers small, green-white-yellow and tubular, in solitary terminal heads (unbranched), 3 cm across; stalks only about 1 cm. The fruit is yellow, fleshy, in a globose head deeply pitted between the deeply fused calyx lobes. There are about 250 fruit / kg

Subject: With the above in focus, the researchers seek to isolate and characterize anti-malarial chloroform-soluble fraction(s) of the stem bark extract of *Nauclea diderichii* for the remediation of some human and animal ailments.

Materials and Methods

Collection of plant materials

Fresh samples of the stem bark of the *African peach* were collected in Yola 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

200 g of the powdered stem bark was accurately weighed and percolated with 2.5 L of distilled ethanol for 72 h. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 35^0C to obtain ethanol soluble fraction, (F_E01), labeled, $F_{E0}R$. [30.5 g].

This crude fraction was macerated with petroleum ether, chloroform, ethyl acetate, and methanol respectively and separated to give the various fractions. The chloroform, ethyl acetate, and methanol fractions were phytochemically screened, and investigated for their toxic inhibition properties against early, residual (repository) and established malaria infections due to *Plasmodium berghei*.

Anti-malarial studies

Anti-malarial tests for the crude ethanol extract was carried out in National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria.

Animals

The animals used in the study were four (4) weeks old-albino mice weighing 18 – 22 g, obtained from the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State, Nigeria. The mice were carried in cages to NIPRD where they were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. They were kept in accordance with National institute for Health Lagos (NIH)

guide for the care and use of laboratory animals NIH Publication (No. 83 -23) revised (1985).

In vivo activity: Acute toxicity test (LD_{50})

The oral acute toxicity of *Nauclea diderichii* chloroform stem bark was carried out in mice using modified Lorke (1983). The study was carried out in two phases each. In phase one, nine mice were randomized into three groups of three mice each and were given five (5 mL) each of 10, 100 and 1000 mg kg^{-1} body weight (b. wt) of the extracts orally respectively. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four (4) h and subsequently daily for seven (7) days. In phase two, another fresh set of nine mice were randomized in to three groups of three mice each and were given 1600, 2900 and 5000 mg kg^{-1} b. wt of the extracts orally based on the results of the first phase.

They were observed for signs of toxicity and mortality for the first four critical hrs and thereafter, daily for 7 days. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose. I .E the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase, the oral median lethal dose was calculated using the formula:

LD_{50} =√ Minimum toxic dose X maximum toxic dose or

Oral median lethal (LD_{50}) dose = $\sqrt{2900X5000}$ = 3800 mg kg^{-1}

Reference drug

Positive control, 5 mg Chlorquine kg^{-1} b. wt while negative control was 5 mL kg^{-1} distilled water.

Rodent parasite

The rodent parasite, *Plasmodium berghei berghei* NK 65 was obtained from the National Institute for Medical Research (NIMR) Lagos, Nigeria. The parasites were kept alive by continuous intraperitoneal passage in mice (Adzu and Haruna, 2007), every 24 days. These infected mice were used for the study. Prior to the beginning of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection (English, 1996).

The Oral Acute toxicity, the suppressive, the curative/Rane, the prophylactic and the mean survival periods of the parasitaemia levels were calculated for each dose level by comparing the parasitaemia in non-infected control with those of treated mice. I.E

$$\text{Average \% suppression} = \frac{A-B}{A}$$

Where A = Average percentage parasitaemia in negative control group, and B = Average percentage parasitaemia in test group (extract).

Statistical analysis

The one way ANOVA test was used to analyze and compare the results at a 95% confident level, values of $P \geq 0.05$ were considered significant, results were expressed as Mean ± SE of mean.

Vacuum liquid chromatography (VLC)

As a result of the above guided bioassay, the crude chloroform extract (2.1g) of the potent plant *N. diderichii* stem bark was extracted and pre-adsorbed on celite, in a vacuum liquid chromatography (VLC) apparatus. The sintered glass funnel (porosity 3) used for the (VLC) was loaded with silica gel under vacuum ensuring that it was compacted and uniformly spread (Leopold, 1982; Hostettmann and Marston, 1998). Suction was applied to compress the slurry to the silica gel and a piece of Whatman(R) filter paper was used to cover the surface to prevent disturbance during the course of the elution. Gradient elution was used. The slurry was first defatted with N-hexane (100%). This was done several times. After defatting, the column was eluted with n-hexane: chloroform in ratio of 19.8: 0.2) and gradually increasing the polarity in the ratio, 19.6: 0.4, 19.4: 0.6, 19.2: 0.8 etc to 100% chloroform.

The column was finally washed with 10% methanol: 90% chloroform. A total of one hundred and seventy – three (173) fractions were collected in 20 cm^3 aliquot.

These extracts collected were concentrated *in vacuo* and allowed to dry. They were subjected to thin layer chromatographic analysis to find the solvent system that will give good separation of the components therein. This was achieved using pre-coated TLC plates. A micro quantity of the sample solution was spotted on TLC pre-coated (MERCK) 20 x 20 cm^2 plates, and developed with various ratios of organic solvents (petroleum spirit, ethyl acetate, methanol, chloroform) taking into consideration their polarities. The solvent system that separated the components to high degree of resolution was considered. The plates were visualized under visible and UV-light (366 nm and 254 nm). The plates were sprayed with 10% ethanol / 1% Vanillin in sulphuric acid and heated at 100ºC for 5-10 minutes to char. Appropriate fractions collected from the column were monitored by using TLC. The fractions with the same R_f values were pooled together and re-concentrated. From the total concentrated samples, the percentage recovery from the chloroform column was 77.8 (Hostettmann and Marston, 1998).

Following their gel filtration, further purification was carried out on the sample and from their TLC and UV analyses there was identification/isolation of pure sample coded NDK01 isolated from the chloroform fraction white in colour, solvent ratio chloroform / methanol 9:1 with R_f 11 cm, and solid in nature.

Spectral analysis

UV spectra were obtained in ethanol on a Helios-zeta, UV-VIS Spectrophotometer. IR spectra was recorded (KBr) on Shimadzu FTIR8 400S Fourier Transform Infrared Spectrophotometer. NMR spectra (both 1D and 2D) were obtained on a Bruker AVANCE (600 MHz for 1H and 300 MHz) for 13C spectrometer, using the residual solvent peaks as internal standard. Chemical shift values were reported in parts per million (ppm) relative to internal solvent standard and coupling constants (*J* values) were given in Hertz. HSQC, HMBC, COSY, DEPT, NOSEY and LC-MS spectroscopy spectra were optimized for a long range *J*H-C of 7Hz (d6 = 0.07s). The solvent used was deutrated chloroform (CDCl3)

Results and Discussion

The mice were treated orally with single dose each of 10 – 5000 mg kg^{-1} b. wt. of *N. diderichii* stem bark after being starved for 24 h (Table 1 [i – ii]). The route was chosen because of its sensitivity and rapid results. The extract at 10 – 1000 mg kg^{-1} (phase 1) produced no physical signs of toxicity in the mice 24 h after administration. From 1600 to 5000 mg kg^{-1} (phase 2) there were no physical signs within the first minutes of administration. But before the end of seven days, some physical signs: salivation, paw licking, stretching/writing, calmness, sleep and even death, manifested. There was however no mortality at all dose levels used below 5000 mg kg^{-1} b wt, but at 5000 mg kg^{-1} b. wt. all died within the period of observation. The median lethal dose LD_{50} was estimated to be ≥ 3800 mg kg^{-1} b. wt. However, the observed reduced activity of the treated mice showed that the extract possessed central depressant effect. The absence of death following oral administration of the extract, at below 5000 mg extract kg^{-1} b. wt. observed in mice suggested that the extracts were practically non-toxic acutely (Salawu *et al*., 2009). This high safety profile may have been responsible for the wide spread use of this indigenous medicinal plants in

different ethno-therapeutic interventions. Although, primate models provide a better prediction of anti-malaria efficacy in human than in rodent models, the later have also been validated through the identification of several conventional anti-malaria, drugs such as chloroquine, halofantrine, mefloquine, maldox and more recently artemisinin derivatives (Ryley and Peters, 1970).

Suppressive test

N. diderichii stem bark extract exerted dose dependent chemo-suppressive effect against *Plasmodium berghei berghei* NK 65 malaria parasite. The extract caused a significant ($P<0.05$) chemo-suppression of 59.20, 67.62 & 77.00 when compared to the controls. The standard drug chloroquine caused chemo-suppression of 96.01, (Table 2 [i]) which were higher than the groups treated with the plant extracts. The observed higher efficacy of chloroquine may in part be due to non selectivity of the extracts or slow absorption and poor bioavailability of the crude extracts and even the crude nature of the extracts. This is common with medicinal plants extracts (Adzu and Haruna, 2007).

The significant chemo-suppression produced by the extracts on day 4 is consistent with the traditional use of the plant as an herbal medicament against malaria in Northern Nigeria.

Curative effect

Stem bark of *N. diderichii* extract produced significant ($P< 0.05$) 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 average percentage parasitaemia reduction of the extract treated groups on day 7 was 65.57, 87.70, 99.18% for the 100, 200 and 400 mg/kg/day. Chloroquine 5 mg/kg b. wt exerted 99.59% reduction of the parasite (Table 2 [ii]). While there was a daily increase in the parasitaemia in the negative control group, the average percentage prasitaemia decreases in the extract and the positive control. This is in consonance with the earlier reports (Tantchou *et al*., 1986; Adjanohoun *et al*., 1996; Odeku *et al*., 2008; Titanji *et al*., 2008; Idowu *et al*., 2010), using the plant *Alstonia boonei* and (Agbedahunsi et *al*., 1998), using *Khaya senegalensis*. This is consistent with natural products of plant origin due to the crude nature of the extract.

Table 1: Acute toxicity test of the chloroform extract of the stem bark of *N. diderichii*

(i) Phase I

10 mg / kg (1 mg /mL)	Vol. (mL)	Signs of toxicity	Survival
18 g	0.18	x	√
20 g	0.20	x	√
21 g	0.21	x	√
100 mg/kg (10 mg /mL)			
20 g	0.20	x	√
22 g	0.22	x	√
18 g	0.18	x	√
1000 mg/Kg(100 mg/mL)			
21 g	0.21	Paw licking	√
19 g	0.19	Erect fur	√
18 g	0.18	salivation	√

Prophylactic effect

The chloroform extract of stem bark of *N. diderichii* produced significant ($P< 0.05$) dose dependent reduction in parasitaemia levels in the extracts treated groups of *Plasmodium berghei berghei* NK 65 malaria of 42.24, 47.28 & 59.08% while 5 mg chloroquine kg^{-1} b. wt. caused 89.40% reduction in parasite count (Table 2 [iii]). The results indicated that the stem bark and leaf extract of *N. diderichii* possessed blood schizontcidal activity as evident from the chemo-suppression obtained during the four day early infection test and the 30 days curative/established infection which is comparable to the standard drug chloroquine, 5 mg/kg/day.

(ii) Phase II: (Concentrations based on phase I)

1600 mg /kg	Vol., (m L).	Signs of toxicity	Survival	
20 g	0.20	salivation	√	
18 g	0.18	Paw licking	√	
21 g	0.21	Stretching/writhing	√	3/3
2900 mg /kg				
22 g	0.22	Erect fur	√	
19 g	0.19	calmness	√	
20 g	0.20	Reduced locomotion	√	3/3
5000 mg / kg				
19 g	0.19	Erect fur	death	
18 g	0.18	weakness	death	
21 g	0.21	Writhing, comatose, convulsion, death	death	0/3

x =absent √ = present; Thus, Oral median Lethal (LD_{50}) dose = √ Minimum toxic dose X maximum toxic dose or Oral median lethal (LD_{50}) dose = √ 2900 x5000 = 3800mg kg^{-1}

Table 2: (i) Suppressive Effect of *N. diderichii* chloroform stem bark extract and chloroquine against *P. berghei berghei* infection in Swiss Albino Mice

Treatment	Parasite count	%. Chemo-suppression
Distilled water 5 $mLkg^{-1}$(control)	5.72 ± 1.31	-
Extract 100 mg Kg^{-1}	3.42 ± 1.12*	59.02
Extract 200 mg Kg^{-1}	2.65 ± 0.98*	67.62
Extract 400 mg Kg^{-1}	1.51 ± 0.86**	77.05
Chloroquine (CQ) 5 mg kg^{-1}	0.44 ± 0.29**	96.01

*Significant different from control at P≤ 0.05 and **at P≤ 0.01

(ii) Curative Effect

Treatment	Parasite count	%. Chemo-suppression
Distilled water 5 $mLkg^{-1}$(control)	48.80 ± 2.22	-
Extract 100 mg Kg^{-1}	16.80 ± 2.12*	65.57
Extract 200 mg Kg^{-1}	6.00 ± 0.82*	87.70
Extract 400 mg Kg^{-1}	0.40 ± 0.16**	99.18
CQ 5 mg kg^{-1}	0.21 ± 0.14**	99.59

*Significant different from control at P≤ 0.05 and **at P≤ 0.01, Strain of parasite =*Plasmodium berghei berghei* NK 65 Specie of animal =Swiss albino mice, Model =Protective Parameter evaluated =Body wt., survival time, parasite count and body temp.

(iii) Prophylactic Effect

Treatment	Parasitemia %	%. Chemo-suppression
Distilled water 5 $mLkg^{-1}$(control)	7.89 ± 1.41	-
Extract 100 mg Kg^{-1}	4.52 ± 1.32*	42.24
Extract 200 mg Kg^{-1}	2.84 ± 0.88*	47.28
Extract 400 mg Kg^{-1}	1.81 ± 0.96**	59.08
CQ 5 mg kg^{-1}	0.64 ± 0.31**	89.40

*Significant different from control at P≤ 0.05 and **at P≤ 0.01

(iv) Mean survival period of Swiss Albino

Dose of extract (mg / kg / day)	Survival time (days)
Distilled water 5 $mLkg^{-1}$(control)	09
Extract 100 mg Kg^{-1}	23
Extract 200 mg Kg^{-1}	26
Extract 400 mg Kg^{-1}	28
CQ 5 mg kg^{-1}	30

Survival period

From (Tables 11 and 12), the extracts appear to be highly effective against the species of *Plasmodium berghei berghei* (NK 65). The mean survival period of the Swiss albino mice treated with the extracts in established infection during a

period of one month showed that as the dose increases, the survival time increases. Mice treated with chloroquine 5 mg/kg b. wt. per day survived for 30 days. Those treated with the extract at 100 mg, 200 mg and 400 mg/kg b. wt. per day survived for 23, 26 & 28 days. The animals in the negative control group, which were treated with distilled water /normal saline, were found to have a mean survival period of 9 days. This confirms with literature as these secondary metabolites are used to treat malaria, asthma, dysentery/diarrhea and other hay fevers (Gill 1992, Burkill 1994, Rahila *et al*., 1994).

Plasmodium berghei berghei parasite is used in predicting the treatment outcomes of any suspected anti-malaria agent due to its high sensitivity to chloroquine making it the most appropriate parasite for this research (Peter and Anatoli, 1998a). However, present findings seem to deviate from the previous study by (Oze *et al*., 2007), who demonstrated that some plants may be nephrotoxic when administered in at higher doses.

Currently, no single drug is effective for the treatment of multidrug resistant malaria and combination therapy includes artemisinin derivatives such as artesunate (David *et al.*, 2004) or mixtures with older drugs such as atovaquone (Deprez-Poulain and Melnyk, 2005), proguaml (Jones and Good, 2006) combination malarone/ maldox (Winter *et al*., 2006; Taylor and white, 2004). Unfortunately, first report on drug resistance to arteminin-derivatives (Jambou *et al*. 2005) and to drug combination therapies (Wichmann *et al*., 2004) have already appeared. So, in the absence of a functional, safe and widely available malaria vaccine, efforts to develop new anti-malaria drugs continue.

In NDK01, the UV absorption band using a 356 nm UV spectral lamp, giving a white spot is indicative of weak auxochromes that are P^H dependent like OH. The C=C stretching frequency of the cyclic molecule could be seen at 1604cm^{-1} The IR spectra of the isolated compounds indicated prominent absorption frequencies characteristic of certain functional groups:-

The band of medium intensity at 3239.9 cm^{-1} (in the IR) suggests the presence of hydroxyl group(s). The broad band of very strong intensities at 2950 and 2852.76 cm^{-1} are characteristic of C-H stretching of aliphatic groups e.g. CH_3, CH_2, and CH etc. It is obvious that there is the absence of carbonyl stretching vibration in the spectrum of NDK01. Overtones and combination tones of lower and weak frequency bands were displayed at 1530.8 cm^{-1}. Their appearance and position in a spectrum can be gainfully used for identification of some structural features. The C-H bending vibrations of the CH_3 and CH_2 groups were observed at 1429.6 and 1359.0 cm^{-1}.

Nuclear Magnetic Resonance (NMR)

The chemical shift of any proton is determined by its chemical environment. The cluster of methyl and methylene protons seen in the spectra suggests the steroidal nature of the compound, While the cluster of methyl and methylene protons, of course suggest the steroidal nature of the compound, this cluster makes it complicated to pick out the various CH_3 and CH_2 groups. DEPT, 90, 45 and 135, provides a clue to the various methyls, methylenes, methines and quarternary carbons. The hydroxyl proton is again confirmed at 4.0969 ppm in the proton nmr spectral.

Compound NDK01: The proton decoupled spectra supports the proposed structure of Clionaterol derivative by the display of the right number of signals at proper values for the carbon resonance as compared with model literature values Obviously, the spectra revealed two compounds each with seven methyl groups, thirty carbon atoms: clionasteroidal/sitosteroidal-type tetracylic steroid nucleus with three hydroxyl groups at C-3, C-5 and C-6 for both and a carbon- carbon double bond in the cycle, between C-12 and C-13. Worthy of note is the fact that when a molecule contains several asymmetric carbon atoms, then the diastereomer is bound to show diatereotopic shifts. For instance, clionasterol and sitosterol differ only in their absolute configuration at the one carbon, C-24. This carbon is S (sinister = left i.e. counter-clockwise rotation) for clionasterol while R (rectus = right i.e. clockwise) for sitosterol.

Table 3: NMR spectral data of isolated compound [NDK01] in DMSO-D6 (5 mmBBoz/av600), (δ in ppm) compared with a modified tetracyclic steroid, Clionasterol

^{13}C position	^{13}C Peaks of Clionasterol (Lit)	^{13}C of isolated Compound	^{13}C Peaks of Modified Clionasterol (MestReNova)	Code
1	71.7	70.6	70.7	CH
2	36.6	36.2	36.4	CH_2
3	36.5	36.7	34.8	C
4	50.2	50.1	50.0	CH
5	31.9	31.1	32.2	CH
6	56.6	56.6	57.1	CH
7	42.3	42.3	43.1	C
8	56.1	55.6	56.5	CH
9	20.4	20.2	20.6	CH_2
9	26.3	28.3	28.3	CH_2
11	24.3	24.3	24.9	CH_2
12	121.6	101.2	124.6	CH_2
13	140.8	140.9	140.4	C
14	121.8	121.7	124.4	CH
15	31.6	31.8	32.3	CH_2
16	32.0	77.4	77.2	CH
17	39.8	38.7	39.7	CH_2
18	12.8	12.1	12.1	CH_3
19	11.9	19.1	19.8	CH_3
20	36.3	35.9	36.1	CH
21	34.0	33.8	35.4	CH_2
22	26.4	25.8	24.9	CH_2
23	46.1	45.6	45.2	CH
24	29..5	72.2	72.3	CH
25	19.6	19.5	19.8	CH_3
26	18.8	19.0	18.7	CH_3
27	23.1	23.0	21.9	CH_2
28	12.3	12.2	12.3	CH_3
29	19.1	19.4	19.7	CH_3
30	59.8	61.6	63.2	CH_2

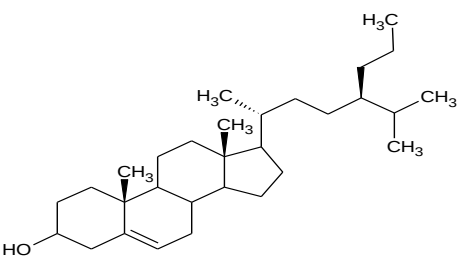
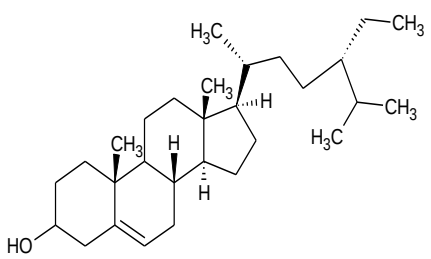


Fig. 1: (a) Clionasterol structure



(b) Sitosterol structure, a diastereomer of clionasterol

Thus, Compound NDK01 supports the proposed derivative structures of Clionasterol/sitosterol by the display of the right number of signals at proper values for the carbon resonance as compared with model literature values, for clionasterol and that simulated by MestReNova (R) (Table 3). The mass spectrum of peak at retention time 0.10 minutes of the Liquid Chromatogram showed the parent ion[M^+] peak at M/Z 437.8 which corresponds to the molecular formula $C_{30}H_{52}O_3$ and the other peaks showing characteristics reminiscent of a derivative of Clionasterol / sitosterol.
Spectra data of ^1H, ^{13}C, HSQC, HMBC, COSY, DEPT, NOSEY and LC-MS spectroscopy confirm the structure to be a clionasterol derivative.

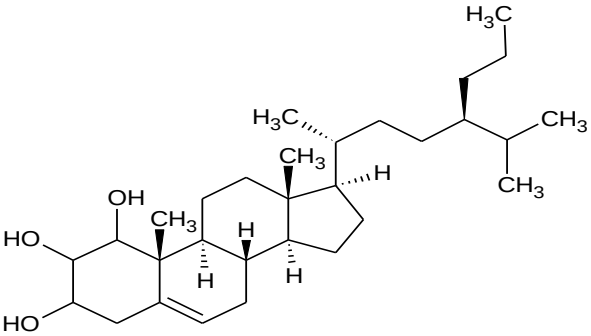


Fig. 2: Clionasterol Derivative

This is further justified as 90% ^{13}C peaks were within ±2 ppm (Tinto, Blair, Reynolds, and Macleans, 1992 $ Jain and Bari, 2010). It can be seen and concluded from the above table that the ^{13}C spectrum is a composite of clionasterol/sitosterol spectra since nearly every signal has been accounted for in the isolated compound.

Conclusion

Anti-malaria activities of the stem-bark of *Nuclear diderichii* may be due to the synergetic effect of their chemical constituents or any single chemical compound may have toxic effects. Most steroids have equally been investigated to have diverse medicinal properties. They are seen to be some of the most economically important pharmaceuticals or their precursors derived from plants e.g. Ergosterol -5, 8-endoperoxide has been isolated as an anti-mycobacterial component of some micro-organisms (Li, 2000; Okwu & Nnamd, 2008). This lends credence for the use of the stem bark for the treatment of malaria and other diseases in Adamawa, Borno and Taraba states in Nigeria (Olajide, 2000). Thus the isolated compounds, modified clionasterol from the chloroform methanol fraction (9:1) is one of the active components found in the plant *N. diderichii* that is obviously responsible for the anti-malarial activity in the Swiss mice.

There is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newmann *et al*., 2003). Indeed, the vast majority of the existing anti-malaria chemotherapeutic agents are based on natural products and this fact anticipates that new anti-malaria may constantly emerge from our tropical plants sources if well harnessed, since biological chemo diversity continue to be an important source of molecular templates in the search for drugs (Portet *et al.*, 2007). The results of the present investigation, suggests that the extracts of the indigenous plants *N. diderichii*, possess potent medicinal activity which justifies their continuous folkloric usage as anti-malaria and other human remedies especially when full toxicological analysis would have be carried out.

Recommendation

The isolated component should be scaled- up and further work carried out with it on the malaria parasite to confirm whether the active metabolites would be effective singly or in synergy. Also, clinical and nutritional studies are recommended for the above indigenous plants so as to be able to advice locals in the various locations of the ill effects of the plants.

Conflict of Interest

Authors declare that there are no conflicts of interest.

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