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# AN ANTIPLASMODIAL DEPSIDE FROM A NIGERIAN LICHEN *DIRINARIA PICTA*, EPIPHYTIC ON THE OIL PALM *ELAEIS GUINEENSE*

# UN DÉPSIDO ANTIPLASMODIAL DE UN LIQUEN NIGERIANO *DIRINARIA PICTA*, EPÍFITO EN LA PALMA DE ACEITE *ELAEIS GUINEENSE*

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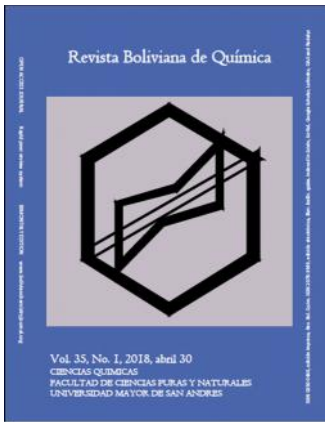


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## ABSTRACT

This study investigated the anti-plasmodial and cytotoxic potentials of the chloroform (LCE) and ethanol (LEE) extracts from the foliose lichen *Dirinaria picta* with the view of isolating anti-malarial drug lead compound(s). *In vitro* anti-plasmodial and cytotoxicity assays were done using the plasmodium lactate dehydrogenase assay and human HeLa cervica cell lines respectively. The structure of the isolated compound was elucidated using spectroscopic techniques. The LCE yielded a novel antiplasmodial depside **1** (antiplasmodial $IC_{50}$ ≈ 37 µg/mL; cytotoxicity ($IC_{50}$ >100 µg/mL; Selectivity index >2.7) and an impure fraction LC2 (antiplasmodial $IC_{50}$ ≈ 79 µg/mL; cytotoxicity ($IC_{50}$ >100 µg/mL; Selectivity index <1.3). The LEE (antiplasmodial $IC_{50}$ ≈ 17 µg/mL; cytotoxicity ($IC_{50}$ ≈ 62 µg/mL; Selectivity index ≈ 3.7) showed a significantly ($p < 0.05$) better anti-plasmodial activity though more cytotoxic compared to depside **1** and LC2.The depside **1**, LC2 and LEE were less cytotoxic compared to emetine (cytotoxicity ($IC_{50}$ = 0.02 µM ≈ 0.013 µg/mL) though not as active as the reference drugs chloroquine (antiplasmodial $IC_{50}$ = 0.031 µM ≈ 0.016 µg/mL). This is the first time report on the anti-malarial potential of Nigerian lichens and the isolation of a novel anti-plasmodial depside **1**.

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**RESUMEN**

Este estudio investigó los potenciales anti-plasmodiales y citotóxicos de los extractos de cloroformo (LCE) y etanol (LEE) de líquenes foliosos *Dirinaria picta* con el objetivo de aislar el (los) compuesto (s) principal (es) del fármaco antipalúdico. Los ensayos in vitro anti-plasmodial y de citotoxicidad se realizaron usando el ensayo de deshidrogenasa plasmodium lactato y las líneas celulares humanas HeLa cervica, respectivamente. La estructura del compuesto aislado se elucidó usando técnicas espectroscópicas. El LCE produjo un nuevo dépsido antiplasmodial 1 (antiplasmodial IC50 ≈ 37 µg / ml; citotoxicidad (IC50> 100 µg / ml; Índice de selectividad> 2,7) y una fracción impura LC2 (antiplasmodial IC50 ≈ 79 µg / ml; citotoxicidad (IC50> 100) µg / mL; Índice de selectividad <1.3). El LEE (IC50 antiplasmodial ≈ 17 µg / mL; citotoxicidad (IC50 ≈ 62 µg / mL; Índice de selectividad ≈ 3.7) mostró una actividad antiplamodial significativamente mejor (p <0.05) aunque fue más citotóxico en comparación con dépsido **1** y LC2. El dépsido **1**, LC2 y LEE fueron menos citotóxicos en comparación con emetina (citotoxicidad (IC50 = 0.02 µM ≈ 0.013 µg / mL) aunque no tan activos como los medicamentos de referencia chloroquine (antiplasmodial IC50 = 0.031 µM ≈ 0.016 µg / mL). Este es el primer informe sobre el potencial antipalúdico de los líquenes nigerianos y el aislamiento de un nuevo dépsido antiplamodial **1**.

## INTRODUCTION

The global plague of malaria which has been worsened by the prevalence of drug resistant *Plasmodium falciparum* strains of the causative parasite, limited access to quality health facilities and high cost of orthodox drugs, remains a threat to children, expectant mothers and the poor people living in endemic regions especially of Sub-Saharan Africa[1]. In view of these obstacles to receiving effective treatment for malaria, the continued search for new anti-malarial agents that are relatively non-toxic and easily affordable is an imperative. Bioactive metabolites from the flora and fauna in nature are good leads in drug development [2]. Lichens are a symbiotic form of life consisting of an alga (photobiont) and a fungus (mycobiont). They are found growing on rocks and as epiphytes on trees. Their harsh niche conditions pre-disposed them towards secreting protective metabolites against different ecological and biological influences. Lichens substances (the secondary metabolites) includes: depsides, benzofuranoids, terpenoids, xanthones, and anthraquinones [3-4]. They have been reported to have anti-viral, anti-bacterial, anti-fungal, anti-protozoan, anti-herbivore, antioxidant, anti-tumor, anti-ulcerogenic, anti-nociceptive, anti-pyretic, and anti-inflammatory activities [3-4]. With respect to the biological activities of Nigerian lichens, few literatures on the antiviral activities of Nigerian lichens are documented [5-8]. As a follow-up to our interest in the isolation and characterization of bioactive metabolites from Nigerian mycoflora [9-12], this present study reports the anti-plasmodial and cytotoxic activities of a novel depside **1** isolated from the chloroform extract of the lichen *Dirinaria picta* epiphytic on the oil palm tree *Elaeis guineensis.*

## RESULTS AND DISCUSSION

*Structural elucidation of compound **1***

The structure of compound **1** (Figure 1) isolated from the LCE after chromatography separation was elucidated using nuclear magnetic resonance (1D and 2D), mass spectrometric, UV-visible and infra-red spectroscopic techniques. Compound **1** showed $^{1}H$ and $^{13}C$-NMR chemical shift signals similar to the reported lichen compound atranorin [13] except for the replacement of the aldehyde functional group in atranorin with a carboxylic acid group. This was confirmed from the DEPT-135 and HSQC experiments with the characteristic carbonyl carbon and proton signals of the aldehyde functional group evidently absent. This is a confirmation that the carbon-13 chemical shift signal at δC = 193.8 ppm for $C_9$ is quaternary, indicative of a carboxylic acid. Infact, the $^{1}H$-NMR data ( see Table 3) of compound **1** has: two $sp^2$ hybridised methine (CH) protons ( one of which is the quartet at $H_{10}$; δH = 6.33 ppm, 1Hq J=0.6, and the other the singlet at $H_{6'}$; δH = 6.45 ppm, 1Hs ), one deshielded methyl proton singlet of a methoxyl ($H_{10'}$; δH = 3.92 ppm, 3Hs) which is rationalised for the methyl ester moiety, in addition to three other less deshielded methyl protons signals rationalised for the two singlets at $H_8$' (δH = 2.47 ppm, 3Hs) and $H_9$' (δH = 2.02 ppm, 3Hs), and the doublet at $H_{11}$ (δH = 2.62 ppm, 3Hd, J=0.6). Five characteristic hydroxyl (OH) proton singlets of phenols were also evident at: 2-OH (δH = 12.44 ppm), 3-OH (δH = 10.58 ppm), 6-OH (δH = 12.49 ppm), 8-OH (δH = 9.90 ppm) and 3'-OH (δH = 11.90 ppm). The presence of the methyl allylic enol residue was unambiguously assigned at the position para to the carboxylic acid moiety from the long range ($^{2}J_{C,H}$, $^{3}J_{C,H}$, $^{4}J_{C,H}$,) HMBC experiments rationalised in Table 1 for $H_{10}$ and $H_{11}$. A further confirmatory evidence was seen in that the doublet protons of this allylic methyl signals

($H_{11}$; δH = 2.62 ppm, 3Hd, J=0.6), were observed from COSY experiment (see Table 1) to be vicinally coupled to the olefinic proton quartet ($H_{10}$; δH = 6.33 ppm, 1Hq J=0.6). In all, a total of twenty-one (21) carbón signals were rationalised from the $^{13}$C-NMR data (see Table 3) for fifteen quartenary (in the región 102- 194 ppm, three of which have been assigned to the carbonyl group of the carboxylic acid moiety at $C_9$ and that of the ester moieties at $C_7$ and $C_7$'), two methine, and four methyl. One of the methyl is due to the methoxy group of the methyl ester moiety which is rationalised for the deshielded δC signal at 52.4 ppm for $C_{10}$'. The structure of compound **1** was thus confirmed to be a 4-(1-hydroxylprop-1-en-1-yl) atranorin-1-carboxylic acid derivative with the systemic name: 2,3,6-trihydroxy-5-((3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenoxy)carbonyl)-4-(1-hydroxyprop-1-en-1-yl)benzoic acid.

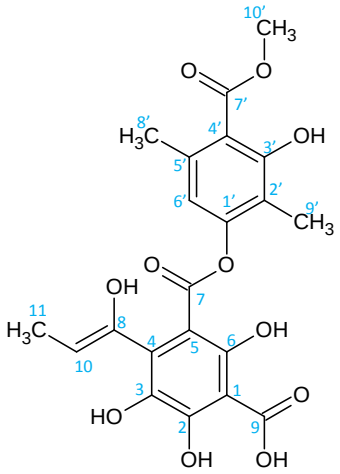


***Figure 1:*** *Chemical structure of compound **1**: 4-(1-hydroxylprop-1-en-1-yl) atranorin-1-carboxylic acid, Systemic name: 2,3,6-trihydroxy-5-((3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenoxy)carbonyl)-4-(1-hydroxyprop-1-en-1-yl)benzoic acid*

***Table 1 :.*** *$^1$H-NMR, H-H-COSY, and HMBC spectral data of compound **1** isolated from the LCE of Dirinaria picta*

| S/No | $\delta_H$ (ppm) | H-H-COSY | HMBC ($^2J_{C,H}$, $^3J_{C,H}$, $^4J_{C,H}$) |
|---|---|---|---|
| 2-OH | 12.44 | | $C_3$, $C_6$ |
| 3-OH | 10.58 | | $C_8$ |
| 6-OH | 12.49 | | $C_5$, $C_7$ |
| 8-OH | 9.9 | | $C_8$ |
| 10 | 6.33(1Hq) J=0.6 | $H_{11}$ | $C_3$, $C_4$, $C_5$, $C_{11}$ |
| 11 | 2.62(3Hd) J=0.6 | $H_{10}$ | $C_4$, $C_{10}$ |
| 3'-OH | 11.9 | | $C_{7'}$ |
| 6' | 6.45(1Hs) | | |
| 8' | 2.47(3Hs) | | $C_3$', $C_4$', $C_{6'}$,$C_7$' |
| 9' | 2.02(3Hs) | | $C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{6'}$ |
| 10'-OMe | 3.92(3Hs) | | C7' |

s: singlet, d: doublet, q: quartet. *Data obtained using a 300 MHz NMR spectrometer with the compound **1** dissolved in $CDCl_3$*

Its molecular formula of $C_{21}H_{10}O_{11}$, corresponded to the molecular mass of 448 g / mol as seen from its ESI (Positive mode)-Mass spectrum: [*m/z (rel. int)*]: 449(2) $[M+H]^+$, 373 (100) [M-75]. The base peak at m/z 373 is rationalised from cleavages at the olefinic bond of the methyl allylic group and loss of the carboxylic acid moiety.

***Table 3 :***. *$^{1}$H- and $^{13}$C-NMR spectral data of compound 1 isolated from the LCE of Dirinaria picta*

| S/No | $\delta_C$ ppm | $\delta_H$ (ppm) | H-Multiplicity |
|---|---|---|---|
| 1 | 102.8 | - | - |
| 2 | 139.9 | - | - |
| 2-OH | - | 12.44 | 1Hs |
| 3 | 102.9 | - | - |
| 3-OH | - | 10.58 | 1Hs |
| 4 | 152.5 | - | - |
| 5 | 108.5 | - | - |
| 6 | 169.1 | - | - |
| 6-OH | - | 12.49 | 1Hs |
| 7 | 167.5 | - | - |
| 8 | 169.4 | - | - |
| 8-OH | - | 9.9 | 1Hs |
| 9-COOH | 193.8 | - | - |
| 10 | 112.9 | 6.33(q) J=0.6 | 1Hq |
| 11 | 25.6 | 2.62(d) J=0.6 | 3Hd |
| 1’ | 151.9 | - | - |
| 2’ | 116.8 | | |
| 3’ | 162.9 | - | - |
| 3’-OH | - | 11.9 | 1Hs |
| 4’ | 110.2 | - | - |
| 5’ | 139.9 | - | - |
| 6’ | 116.0 | 6.45(s) | 1Hs |
| 7’-COOMe | 172.2 | - | - |
| 8’ | 24.1 | 2.47(s) | 3Hs |
| 9’ | 9.4 | 2.02(s) | 3Hs |
| 10’-OMe | 52.4 | 3.92(s) | 3Hs |

s: singlet, d: doublet, q: quartet. Data obtained using a 300 MHz NMR spectrometer with the compound **1** dissolved in $CDCl_3$

The UV spectra data in MeOH $\lambda_{max1}$ 281 nm and $\lambda_{max2}$ 340 nm, is a further confirmation for a highly conjugated compound typical of aromatic (phenolic) compounds. The IR spectrum showing vibrational frequency at: 2925 $cm^{-1}$ for aliphatic C-H stretching, 1650 $cm^{-1}$ for C=O, 1581, and 1451 $cm^{-1}$C=C deformation, 1106, 1077, 1031 $cm^{-1}$ for C-O stretching of ester, carboxylic acid,), and the out-of-plane CH deformation frequencies typical of olefin and substituted aromatic systems at 935, 862, 728 $cm^{-1}$, are also supporting the rationalised structure of compound **1** for a methyl allyl enol carboxylic acid derivative of the known lichen depside atranorin and is being reported here for the first time.

The novel antiplasmodial depside compound **1** (antiplasmodial $IC_{50}$ ≈ 37 µg/mL) was significantly ($p < 0.05$) less active compared to the standard drug chloroquine (antiplasmodial $IC_{50}$ = 0.031µM ≈ 0.016 µg/mL) and the other impure lichen fractions LEE (antiplasmodial $IC_{50}$ ≈ 17 µg/mL) and LC2 (antiplasmodial $IC_{50}$ ≈ 79 µg/mL). The trend in the dose response pLDH activity (Figure 2 and Table 2) revealed that a marked inhibition of the pLDH activity was evident as from 25 µg/mL for the compound **1** and LEE. This was however not so for the LC2 where a similar trend was not evident until 100 µg/mL. Generally, the trend in pLDH activity was observed to be of the order: Chloroquine > LEE> Compound **1** > LC2. The plasmodium parasite lactate dehydrogenase (pLDH) is the last enzyme in the parasite glycolytic pathway and is produced by both the sexual and asexual stages of parasites, as well as the mature gametocytes of all human *Plasmodium species* [14-16]. Due to its dependence on anaerobic glucose metabolism, the pLDH plays an important role in catalysing energy production in the parasite [16-17]. Its associated activity is reported to disappear within 24 hours of effective malaria treatment [17] thus the pLDH antigen is considered a specific marker for the presence of viable plasmodium in blood.

Generally, a marked onset of cytotoxicity was observed for the test lichen samples after 25 µg/mL. At 100 µg/mL, the trend in cytotoxicity (Figure 3 and Table 2) expressed as % cell viability was of the order: LEE (14.003 %) > Compound **1** (55.405%) > LC2 (71.037%). Their $IC_{50}$ compared to the reference drug emetine indicated relative lower cytotoxicity with a trend: emetine ($IC_{50}$ = 0.013µg/mL) > LEE ($IC_{50}$ = 62.38 µg/mL) > Compound **1**($IC_{50}$>100µg/mL) > LC2 ($IC_{50}$ > 100µg/mL). Report by the United States National Cancer Institute regards plant extract with cytotoxic $IC_{50}$ of 20µg/mL or lower as being highly cytotoxic [18-20]. Those with $IC_{50}$ greater than 100 µg/mL are regarded to be of low to non toxicity [18]. The observed low cytotoxicity of the isolated lichen compound **1**, and the impure lichen samples LEE and LC2 is suggestive that their anti-plasmodial activity may not necessarily be due to general cytotoxicity of the extract. This is an indication of their potential as non-toxic agents for drug development. Their trend in selectivity index was observed to be LEE (SI = 3.7) > Compound **1** (SI >2.7) > LC2 (SI >1.3). Selectivity index is a measure of how a drug substance is toxic to the parasite cells compared to the host (mammalian) cells. Higher SI values is therefore indicative of better selectivity [13].

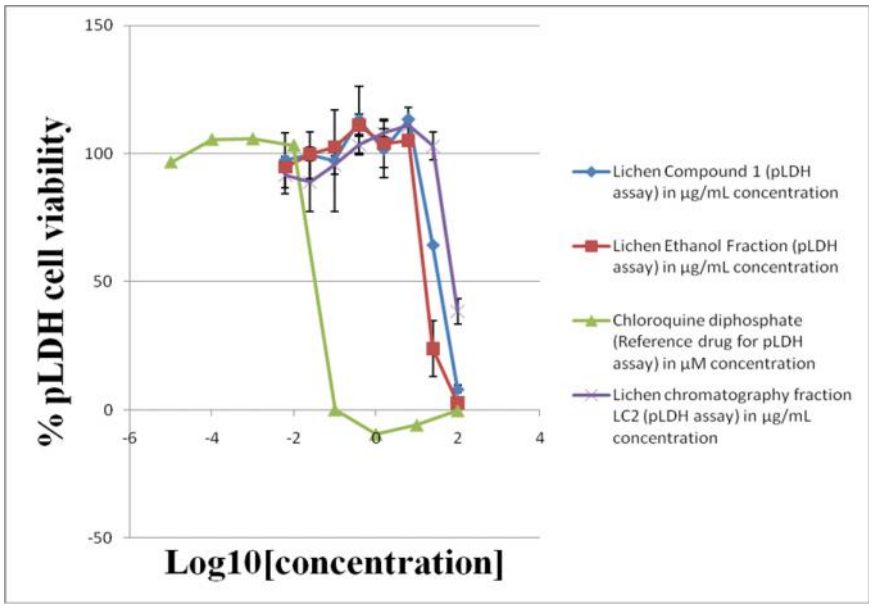


***Figure 2:*** *Dose response plasmodium parasite growth inhibition profile of Lichen compound 1, compound 1, chromatography fraction (LC2), and lichen ethanol extract (LEE) from Dirinaria picta epiphytic on E. guineense*

## EXPERIMENTAL

*Lichen species*

*Dirinaria picta* (thalli) epiphytic on oil palm tree *Elaeis guineense* were collected from the premises of the University park (Abuja campus, 4.9018ºN, 6.9205ºE) of the University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

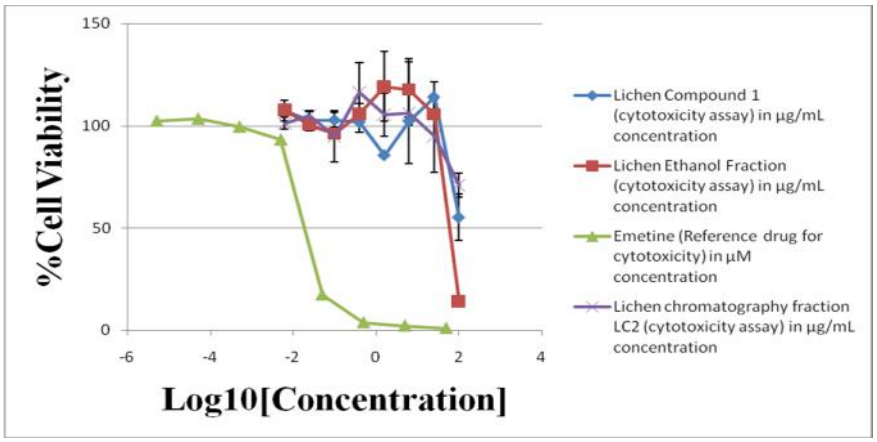


***Figure 3.*** *Dose response cytotoxicty profile of the Lichen compound 1,chromatography fraction (LC2), and lichen ethanol extract (LEE) from Dirinaria picta epiphytic on E. guineense*

***Table 2****. Plasmodium pLDH inhibition and mammalian cell (HeLa) cytotoxicity assay results for the Lichen compound 1 , chromatography fraction (LC2) and lichen ethanol extract (LEE) from Dirinaria picta epiphytic on E. guineense*

| Test samples' (**1**, LEE, LC2) µg/mL | Compound **1** isolated from the Lichen chloroform extract LCE | | Lichen ethanol extract (LEE) | | Lichen chromatography fraction (LC2) | | Reference drug Emetine | | Reference drug diphosphate | Chloroquine |
|---|---|---|---|---|---|---|---|---|---|---|
| | pLDH inhibition (% Parasite cell Viability) | Mammalian cell (HeLa) cytotoxicty (% cell Viability) | pLDH inhibition (% Parasite cell Viability) | Mammalian cell (HeLa) cytotoxicty (% cell Viability) | pLDH inhibition (% Parasite cell Viability) | Mammalian cell (HeLa) cytotoxicty (% cell Viability) | Concentration x $10^{-3}$µM | Mammalian cell (HeLa) cytotoxicty (% cell Viability) | Concentration x $10^{-2}$µM | % Parasite cell Viability |
| 0.006 | 97.442±10.689 | 107.288±5.159 | 94.822±1.646 | 107.901±0.246 | 91.574±7.302 | 101.23±2.974 | 0.005 | 102.571 | 0.001 | 96.775 |
| 0.024 | 99.413±9.175 | 102.818±4.450 | 99.967±2.656 | 100.628±3.122 | 88.860±11.299 | 105.057±1.740 | 0.050 | 103.607 | 0.010 | 105.657 |
| 0.098 | 97.301±19.816 | 102.819±3.630 | 102.622±1.235 | 96.222±0.893 | 95.611±3.665 | 94.922±12.423 | 0.500 | 99.648 | 0.100 | 105.901 |
| 0.391 | 113.158±13.026 | 101.945±5.054 | 111.177±4.302 | 105.935±4.893 | 103.413±3.997 | 116.407±14.455 | 5.000 | 93.383 | 1.000 | 103.328 |
| 1.563 | 101.629±11.167 | 85.709±1.114 | 103.995±9.255 | 119.306±17.120 | 108.098±1.593 | 105.460±10.475 | 50.000 | 17.465 | 10.000 | 0.033 |
| 6.250 | 113.365±0.199 | 102.371±1.929 | 105.103±2.005 | 117.891±15.111 | 110.990±6.878 | 106.411±25.052 | 500.000 | 3.878 | 100.000 | -9.469 |
| 25.000 | 64.365±0.239 | 114.066±7.295 | 23.946±10.848 | 105.612±1.495 | 102.934±5.470 | 94.936±17.728 | 5,000.000 | 2.282 | 1,000.000 | -5.920 |
| 100.000 | 8.173±1.527 | 55.405±11.286 | 2.831±1.062 | 14.003±0.504 | 38.442±5.032 | 71.037±5.970 | 50,000.000 | 1.032 | 10,000.000 | -0.249 |
| $IC_{50}$ | 36.730±0.910µg/mL (81.980* µM) | >100µg/mL (>223.210* µM) | 16.780 ± 0.700 µg/mL | 62.380±1.100µg/mL | 79.430±5.420µg/mL | >100µg/mL | | 0.020 µM (0.013µg/mL) | | 0.031 µM (0.016 µg/mL) |
| Selectivity Index | >2.7 | NA | 3.7 | NA | >1.3 | NA | | NA | | ND |

*calculated from the relative molecular mass of 448 for compound **1** from ESI- mass spectrometric analysis; NA= Not applicable, ND= Not determined

The specimen was authenticated by lichenologist Andre Aptroot and Louise Olley of the ABL Herbarium, The Netherlands and the Royal Botanic Garden, United Kingdom respectively. A voucher specimen (UPH-PCG/LC002) has been deposited at the herbarium of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt, Nigeria. The thalli were carefully scraped from the host plant and sorted out to remove insects and other foreign matters and then dried under a current of air in a de-humidified environment. The dried samples were pulverized using an electric blender.

*Materials*

All solvents used were of analytical grade (Sigma-Aldrich, Germany) or previously distilled. The spectra of 1D and 2D NMR were recorded on a Brucker Avance NMR spectrometer of 300 MHz in the Chemistry Department of the Rhodes University, Grahamstown, South Africa using $CDCl_3$ as solvent. The Infra-red (IR) spectra were recorded on 1600 ATI Matson Genesis series FTIR$^{TM}$ spectrometer. Mass spectra were recorded on ESI positive mode on a mass spectrometer equipped Waters Synapt G2. The chromatographic separation was performed using normal phase silica

gel (Mesh size 230-400) for column chromatography. The purity confirmation was done using Thin Layer Chromatography (TLC) using Iodine fume as developer, and by melting point determination.

*Extraction and isolation*

The dried pulverised lichen (60 g) was cold macerated for 72 hours with chloroform and ethanol in succession with fresh replacement of solvent at 24 hours interval to obtain the chloroform extract (LCE) and ethanol extract (LEE) used in this study after concentration using a rotary evaporator. The lichen chloroform extract LCE (3.2 g) was dissolved in chloroform and pre-adsorbed on silica gel in the ratio of 1:1 w/w to form a homogenous paste which was allowed to air dry in a fume cupboard. The mixture was loaded on a chromatography column (internal diameter 4.1 cm and packed with normal phase silica gel mesh 230-400 to a height of 30 cm). The column was eluted isocratically with chloroform (1200 mL) based on prior analytical Thin Layer Chromatography (TLC) evaluation. The eluted fractions were collected in 50 ml portion. From fractions 5-10 eluted with chloroform was isolated compound **1** after re-crystalisation from acetone. Its purity was confirmed from TLC and melting point determination. Fractions 13-17 were pooled together based on TLC as fraction LC2

Compound **1 (** a 4-(1-hydroxylprop-1-en-1-yl)atranorin-1-carboxylic acid derivative with the systemic name: systemic name: 2,3,6-trihydroxy-5-((3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenoxy)carbonyl)-4-(1-hydroxyprop-1-en-1-yl)benzoic acid), molecular formula: $C_{21}H_{10}O_{11}$, yield: 124 mg, white amorphous solid; Melting point: 103-108 $^{o}$C; Freely soluble in chloroform, dichloromethane; acetone, methanol and DMSO; Molecular Weight: 448 g / mol; UV in MeOH $\lambda_{max1}$ 281 nm and $\lambda_{max2}$ 340 nm; IR spectrum [*frequency, ν, cm$^{-1}$*]: 2925 C-H str aliphatic, 1650 (C=O), 1581, $1451_{def}$ (C=C) 1106, 1077, 1031 (C-O ester, carboxylic acid,), 935, 862, 728 (out-of-plane CH deformation of olefin and aromatic systems); $^{1}$H and $^{13}$C NMR (Tables 1 and 3); ESI (Positive mode)-Mass spectrum: [*m/z (rel. int)*]: 449(2) $[M+H]^{+}$, 373 (100)[M-75]

*Plasmodium falciparum growth inhibition assay*

This was done using the LEE, LC2 and the isolated compound **1**. Briefly, the *P. falciparum* (3D7 strain) parasites were maintained in a medium composed of RPMI 1640 supplemented with 2 mM L-glutamine, 25 mM Hepes (buffered between a pH of 7.2 and 7.4), 5%(w/v) Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamicin sulfate and 2-4% (v/v) human red blood cells, in an atmosphere containing a mixture of $O_2$, $CO_2$, $N_2$ (5:5:90 v/v/v). For the growth inhibition assays, parasite cultures were adjusted to 2% parasitaemia and 1% haematocrit (final) and incubated for 48 hours, after addition of the test samples [(final test concentrations range of 0.006104 -100 µg/mL prepared in duplicate following a 4-fold serial dilutions approach in 96-well plates (200 µL culture/well; two wells per test sample dilution)]. After the incubation period, the levels of parasite in the wells were determined by colorimetric determination of parasite lactate dehydrogenase activity[21]. Chloroquine (eight final test concentration within the range 0.00001 - 100 µM) prepared following a 10-fold serial dilutions approach in 96-well plates was used asstandard anti-malarial drug for comparison. The Abs 620 values in test sample's and standard drug (chloroquine) wells were converted to percentage parasite viability relative to wells containing untreated parasite cultures. The median pLDH inhibition concentration ($IC_{50}$) values were derived from graphs of mean % parasite viability against Log (test sample concentration) using the non-linear regression function of Microsoft Excel 2007 software.

*Mammalian cell growth inhibition assay*

This was done using the LEE, LC2 and the isolated compound **1**. Briefly mammalian HeLa cells were plated in 96-well plates at $2x10^4$ cell per well in 150 µL the culture medium. The culture medium was prepared from Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5mM L-glutamine, 10% (v/v) fetal bovine serum and antibiotics (penicillin/streptopmycin/amphotericin B). After an overnight incubation in a 5% $CO_2$ humidified incubator, the various concentration (0.006104 -100 µg/mL) of the test samples prepared following a 4-fold serial dilutions approach in 96-well plates were added to the cultures (duplicate wells; 200 µL final culture volume) and incubation continued for an additional 48 hours. The viability of cells in individual wells was assessed by adding 20 µL of resazurin toxicology reagent (Sigma-Aldrich) per well and measuring fluorescence intensity (exc. 560 nm/em. 590 nm) in a Spectramax M3 plate reader after an incubation of 2 hours. Fluorescence readings in experimental wells were converted to % cell viability relative to control wells containing untreated cells and used to obtain the dose-

response plots of mean % cell viability against log (test sample concentration) using the non-linear regression function of Microsoft Excel 2007 software with the median inhibition concentration $IC_{50}$ values derived from the plot by extrapolation. Emetine various concentration (0.000005 -50 µM) prepared following a 10-fold serial dilutions approach in 96-well plates was used as standard drug for comparison.

## CONCLUSIONS

This study is reporting for the first time, the anti-malarial potential of the Nigerian lichen *Dirinaria picta* epiphytic on *E. guineense* as well as the isolation and structural elucidation of the novel depside **1** –a methylallyl enol carboxylic acid derivative of atranorin as an anti-malarial drug lead compound. Further work is ongoing to isolate the constituents in LEE as well as LC2 and to evaluate them for anti-plasmodial activity.

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