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Chemical composition, antioxidant and larvicidal activity of *Alchornea laxiflora* (Benth) leaf extracts

Frank N. I. Morah, Dickson N. Uduagwu

ABSTRACT

Aims: To determine the chemical constituents, antioxidant activity and insecticidal potential of *Alchornea laxiflora* leaf extracts. Methods: The powdered dry leaf was Soxhlet-extracted with petroleum ether and the residue re-extracted with ethanol to give petroleum ether and ethanol extracts respectively. The chemical constituents were determined by GC-MS analysis. The antioxidant activity was measured using DPPH free-radical scavenging method. 25 *Anopheles* mosquito larvae were kept in each of the beakers containing different levels of the petroleum ether extract. The larvae were observed for a period of 96 h and the number of deaths recorded. From this the percentage mortality and probit mortality were calculated. The entire process was repeated for the ethanol extract. Results: Thirty constituents were identified from the petroleum ether extract and seven from the ethanol extract. These include batulin, octadecane, 6,10,14-trimethylpentadecan-2-one, methyl hexadecanoate, heptatricotan-1-01, ethyl iso-allocholate, rhodopin, glycocholic acid, dieicosyl oleate and ethyl linoleate. Both extracts have high antioxidant activity which is concentration dependent. Both extracts have higher antioxidant activity than ascorbic acid while the antioxidant activity of the petroleum ether extract is higher than that of the ethanol extract. The extracts also showed strong larvicidal activity against *Anopheles* mosquito larvae. Conclusion: *Alchornea laxiflora* leaf contains several natural products which have high antioxidant activity and strong larvicidal activity against *Anopheles* mosquito larvae.

Keywords: *Alchornea laxiflora*, Antioxidant activity, *Anopheles* mosquito, Chemical composition, Larvicidal activity

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INTRODUCTION

Alchornea laxiflora (Benth.) of the family Euphorbiaceae is a deciduous shrub or small tree and an African medicinal plant. Decoction of the leaf is used for control of inflammations, malaria, bacterial and fungal infections [1]. It is taken orally for the treatment of postpartum pains in Cameroun [2]. *Alchornea laxiflora* is known to contain a number of flavonoids which include quercetin, quercetin-3-O-β-D-glycopyranoside [3], laxiflorin glycoside [4] and alchornealaxine [5].

The use of natural products or medicinal plants and natural insecticides is becoming very popular because of development of resistance to the conventional synthetic drugs and insecticides. The natural products have the added advantage of being cheap, readily affordable and have reduced risk of side effects. They are also environmentally friendly [6]. *Alchornea laxiflora* leaf is employed in traditional medical practice for the management of different human health problems. The aim of the present investigation is to identify its chemical constituents which are believed to be responsible for its pharmacological activity. It is also aimed at evaluation of its antioxidant and larvicidal properties.

MATERIALS AND METHODS

Alchornea laxiflora leaves were harvested in the month of July from Ikwo in Eboyi State of Nigeria. It was authenticated by Frank Adeoye of the Herbarium unit, Botany Department, University of Calabar, Nigeria. The leaves were rinsed with distilled water, air dried and ground with a mill. The ground leaf was extracted with petroleum spirit (60–80°C) in a Soxhlet extractor and distilled down to give the petroleum ether extract. The leaf residue left after the petroleum ether extraction was re-extracted with ethanol to give the ethanol extract. The petroleum ether extract was fractionated over a silica gel column to give fractions A and B.

The gas chromatography-mass spectrometric analysis of these extracts was carried out with GC-MS instrument. The column consists of a 25 m x 0.23 mm fused silica capillary, coated with polydimethylsiloxane (DBP-1) of 1.5 μm film thickness filled in the GC-MS system. The column and oven temperatures were programmed to start from 60°C increasing up to 200°C at 3°C/min. The carrier gas used was helium and at a constant flow rate of 1 ml/min.

The antioxidant activity was determined by DPPH free-radical scavenging assay methods. The reduction capacity of the DDPH (2, 2-diphenyl-1-picrylhydrazyl) radical was determined by the decrease in absorbance induced by antioxidants according to the method of Brands-William et al. [7] with a few modifications. Solutions containing 40, 80, 100, 150 and 200 $mgdm^{-3}$ of both petroleum ether fractions and ethanol extract were separately prepared. 0.1 cm^3 of each of these and the standard (containing no extract) were each separately added to 2.9 cm^3 of 0.5 mM of DPPH in dimethyl sulfoxide, DMSO. Each mixture was shaken vigorously and left at room temperature for 30 min. The absorbance was taken at λ_{max} 517 nm against a blank. The ability to scavenge the DPPH radical was calculated from this using the following formula:

DPPH scavenging effect (%) = $A_o - A_1/A_o \times 100$

where:

A_o is the absorbance of the control at 30 min

A_1 is the absorbance of the sample at 30 min

Twenty milligrams (20 mg) of each of the crude extracts were dissolved in 100 cm^3 of dimethyl sulfoxide (DMSO) to obtain 200 $mgdm^{-3}$ of stock solution, which was diluted with distilled water to get different concentrations of 200 $mgdm^{-3}$, 150 $mgdm^{-3}$, 100 $mgdm^{-3}$, 80 $mgdm^{-3}$, 40 $mgdm^{-3}$ and 0.0 $mgdm^{-3}$. The control was prepared in a similar way without the extract.

Anopheles mosquito larvae were used for the larvicidal activity. Twenty-five, third and fourth instar larvae were kept in each of the different 500 cm^3 beakers, each containing 10 cm^3 of tap water. 1 cm^3 of each of the different concentrations of extract was added to the different beakers with exception of the control. Larval mortality was assessed at 24 h intervals for 96 h after which the percentage mortality and probit mortality were calculated.

RESULTS

Tables 1 and 2 give the chemical composition of fractions A and B of the petroleum ether extract of *Alchornea laxiflora* leaf respectively. Fraction A contains seven identified compounds while fraction B contains twenty-three compounds. With exception of 3-ethyl-5-(2-ethylbutyl)-octadecane which is a hydrocarbon, the rest are oxygenated compounds. Table 3 gives that the ethanol extract of this plant contains seven oxygenated natural products. The antioxidant activities of the ethanol and petroleum ether extracts of *Alchornea laxiflora* are given in Table 4 and Table 5 respectively. Table 6 gives the antioxidant activity of the ascorbic acid standard. Both extracts possess antioxidant activity which is dose dependent. Larvicidal activity of the leaf extracts against *Anopheles* mosquito larvae is shown in Figures 1 and 2. The extracts have strong larvicidal activity against *Anopheles* mosquito larvae (Table 7).

DISCUSSION

Thirty compounds were identified in the petroleum ether extract while seven compounds were identified in the ethanol extract of *Alchornea laxiflora* leaf. To the best of our knowledge, there is no earlier report of the occurrence of any of these compounds in *Alchornea laxiflora*. These include astaxanthin, 3-acetoxy-7,8-epoxylanostan-11-01,4,10,14-trimethylpentadecan-3-one, lycoxanthin, methyl hexadecanoate, rhodopin, linoleic acid, ethyl iso-allocholate, betulin and diisooctyl phthalate. Compounds identified in the ethanol extract include 2, 4-bis (1, 1-dimethylethyl) phenol, ethyl linoleate, eicosyl oleate and glycocholic acid. On the whole thirty-seven compounds were identified from the leaf extracts. This is the first report on the occurrence of these compounds in *Alchornea laxiflora*. Most of these compounds have pronounced biological activities which include antimicrobial, antiprotozoal, anticancer, antioxidant and anti-arthritic activities [8–12]. .

Table 1: Chemical composition of fraction A of the petroleum ether extract of *Alchornea laxiflora* leaf

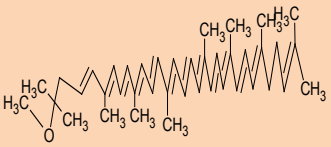
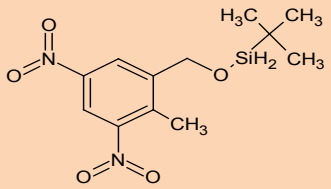
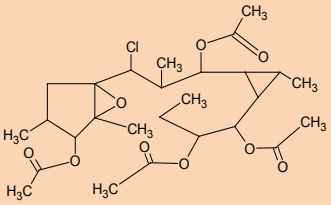
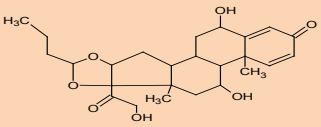
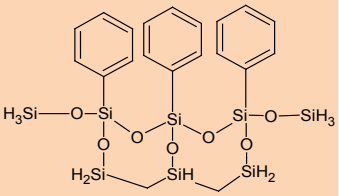
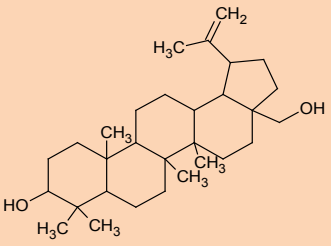
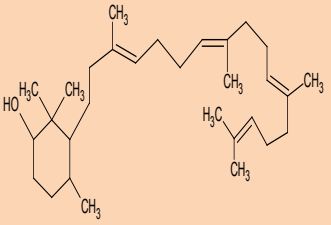
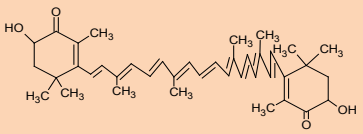
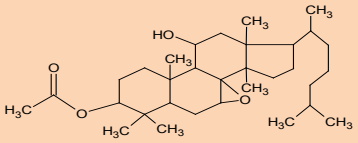
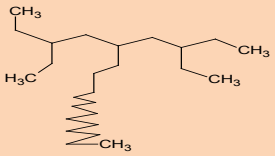
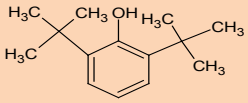
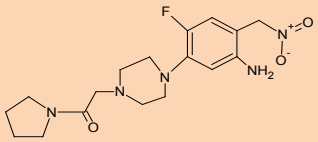
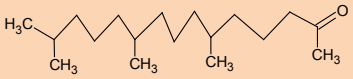
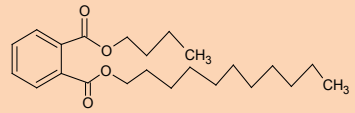
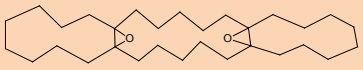
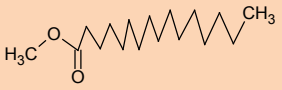
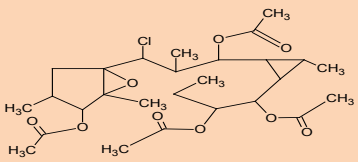
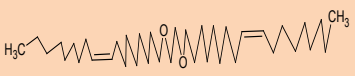
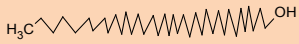
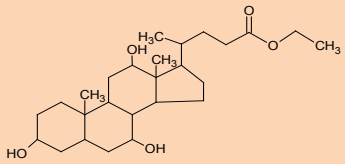
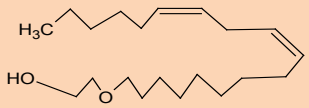
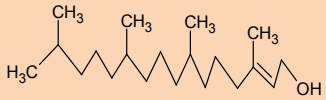
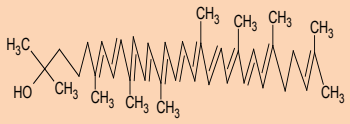
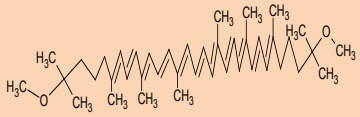
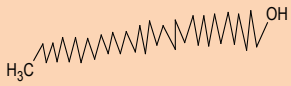
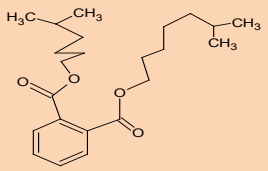
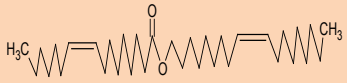
Retention time	% Composition	Compound	Molecular formula	Base peak	Relative molecular mass	Structure
5.864	1.72	.psi,.psi.-Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy-	$C_{41}H_{58}O$	91	566.90	
36.406	9.80	2-Methyl-3,5-dinitrobenzyl alcohol, tertbutyldimethylsilyl ether	$C_{12}H_{18}N_2O_5Si$	253	298.37	
39.116	1.93	9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate	$C_{27}H_{41}ClO_9$	135	545.06	
40.090	4.06	(22S)-6α,11β,21-Trihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione	$C_{25}H_{34}O_7$	135	446.53	
40.931	2.53	Bis[di(trimethylsiloxy) phenylsiloxy] trimethylsiloxyphenylsiloxane	$C_{20}H_{30}O_7Si_8$	135	607.13	
42.627	33.82	Betulin	$C_{30}H_{50}O_2$	147	444.72	
44.662	27.21	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	69	428.73	

Table 2: Chemical composition of fraction B of the petroleum ether extract of *Alchornea laxiflora* leaf

Retention time	% Composition	Compound	Molecular formula	Base peak	Relative molecular mass	Structure
21.057	0.64	Astaxanthin	$C_{40}H_{52}O_4$	133	596.84	
25.094	0.47	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{33}H_{54}O_4$	69	502.77	
25.346	0.83	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	57	366.71	
28.881	2.25	Phenol, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	191	206.32	
36.398	6.30	4-Fluoro-2-nitroaniline, 5-[4-(pyrrolidin-1-yl) carbonylmethylpiperazin-1-yl]-	$C_{17}H_{24}FN_5O_3$	253	365.40	
37.474	15.26	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	58	268.48	
37.694	1.61	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	149	376.53	
38.205	0.65	Lycoxanthin	$C_{40}H_{56}O$	91	552.87	
38.268	1.16	Tricyclo[20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	69	444.73	
38.362	2.93	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	74	270.45	
38.920	2.53	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	88	284.48	
39.116	1.40	9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate	$C_{27}H_{41}ClO_9$	135	545.06	
39.532	0.85	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)] bis-, (Z,Z)-	$C_{38}H_{74}O_2$	85	256.99	

39.635	3.34	1-Heptatriacotanol	$C_{37}H_{76}O$	85	537.00	
39.705	1.04	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	71	436.62	
39.784	1.34	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	74	298.50	
39.886	2.92	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	55	310.51	
39.933	4.84	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	81	296.53	
40.279	1.43	Rhodopin	$C_{40}H_{58}O$	207	554.89	
40.931	2.92	.psi, psi-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	207	600.96	
41.096	3.05	1-Heptatriacotanol	$C_{37}H_{76}O$	69	537.00	
42.054	12.93	Diisooctyl phthalate	$C_{23}H_{36}O_4$	149	376.53	
44.788	6.25	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	$C_{34}H_{64}O_2$	207	504.87	

Tables 4–6 show that both ethanol and petroleum ether extracts have high antioxidant activities which is concentration dependent as it increases with increases in concentration of the extracts. They have higher antioxidant activity than the ascorbic acid standard. The petroleum ether extract has higher antioxidant activity than the ethanol extract. The observed antioxidant activities are higher than what was reported for 50% aqueous alcohol extract of this plant [4]. This is attributable to the greater amount of antioxidant potential compounds present in the petroleum ether extract followed by the ethanol extract. The identified antioxidants include 3-acetoxy-7, 8-epoxylanostan-11-one, rhodopin, ethyl iso-allocholate, hexadecanoic acid, 9-octadecenyl hexanoate, eicosyl oleate and astaxanthin [8–12]. This explains the use of *Alchornea laxiflora* in traditional medicine to control oxidative stress [13].

Table 7 and Figures 1 and 2 show that both ethanol and petroleum ether extracts have larvicidal activity against *Anopheles* mosquito larva. Since the control showed no larvicidal activity, it is conceivable that the natural products in the *Alchornea laxiflora* leaf extracts are solely responsible for the observed larvicidal activity. This activity is concentration dependent as an increase in concentration of the extract resulted in higher percentage mortality and hence higher probit mortality of the *Anopheles* mosquito larvae. The petroleum ether extract generally showed higher larvicidal activity. Some of the identified compounds including methyl hexadecanoate and eicosyl oleate are known to have insecticidal activity [8, 10]. Hexadecanoic acid esters and di-n-octylphthalate identified in this plant species have been shown to have larvicidal activity against mosquito larvae [14, 15]. The natural substances from plants are more effective and more

Table 3: Composition of the ethanol extract of *Alchornea laxiflora* leaf

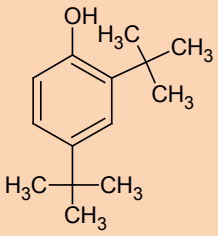
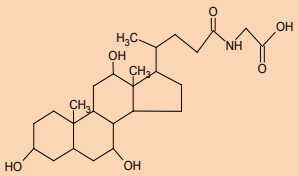
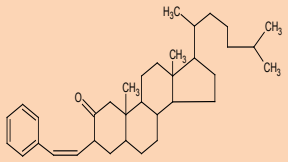
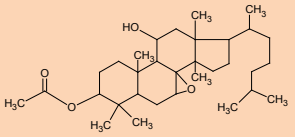
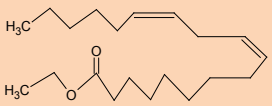
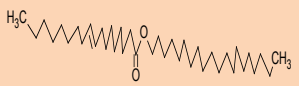
Retention time	% Composition	Compound	Molecular formula	Base peak	Relative molecular mass	Structure
28.865	37.44	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	191	206.32	
36.406	9.61	Glycocholic acid	$C_{26}H_{43}NO_6$	253	465.62	
37.396	3.97	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one		95		
38.362	2.33	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	74	366.62	
38.912	1.34	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	55	502.77	
39.580	1.71	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	55	308.50	
39.627	5.27	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	55	562.99	

Table 4: Antioxidant activity of the ethanol extract of *Alchornea laxiflora* leaves

Concentration	Mean absorbance±std	DRSA* (%)
200 mgdm^{-3}	0.967±0.002	42.95
150 mgdm^{-3}	1.055±0.001	37.76
100 mgdm^{-3}	1.286±0.001	24.13
80 mgdm^{-3}	1.480±0.003	12.68
40 mgdm^{-3}	1.554±0.001	8.32

*DRSA DPPH Radical Scavenging Ability

Table 5: Antioxidant activity of the petroleum ether extract of *Alchornea laxiflora* leaves

Concentration	Mean absorbance±std	DRSA (%)
200 mgdm^{-3}	0.839±0.002	50.50
150 mgdm^{-3}	0.901±0.001	46.84
100 mgdm^{-3}	0.983±0.003	42.01
80 mgdm^{-3}	0.998±0.002	41.12
40 mgdm^{-3}	1.044±0.001	39.24

Table 6: Antioxidant activity of the ascorbic acid used as standard

Concentration	Mean absorbance±std	DRSA (%)
200 mgdm^{-3}	1.098±0.002	35.22
150 mgdm^{-3}	1.198±0.002	29.32
100 mgdm^{-3}	1.389±0.002	18.05
80 mgdm^{-3}	1.555±0.001	8.26
40 mgdm^{-3}	1.611±0.001	4.96

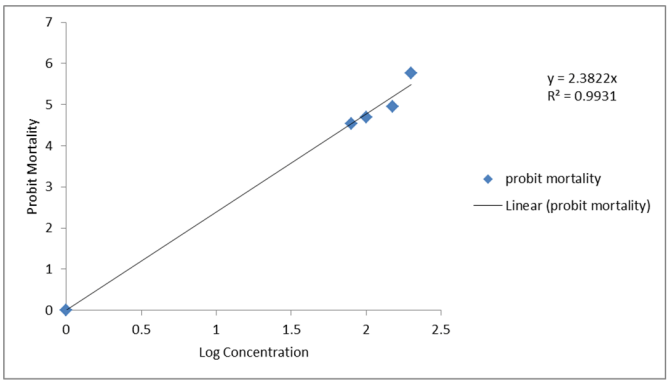


Figure 2: Mortality rate of mosquito larvae exposed to different levels of petroleum ether extract of *Alchornea laxiflora*.

Table 7: Larvicidal bioassay of *Alchornea laxiflora* leaf extracts

Concentration	Log_{10} conc.	% Mortality		Probit mortality	
		Ethanol extract	Petroleum ether extract	Ethanol extract	Petroleum ether extract
0.0 mgdm^{-3}	-	0.0	0.0	0.0	0.0
80 mgdm^{-3}	1.90	30.00	32.00	4.48	4.53
100 mgdm^{-3}	2.00	38.00	38.00	4.69	4.67
150 mgdm^{-3}	2.18	60.00	48.00	5.25	4.95
200 mgdm^{-3}	2.30	68.00	78.00	5.47	5.77

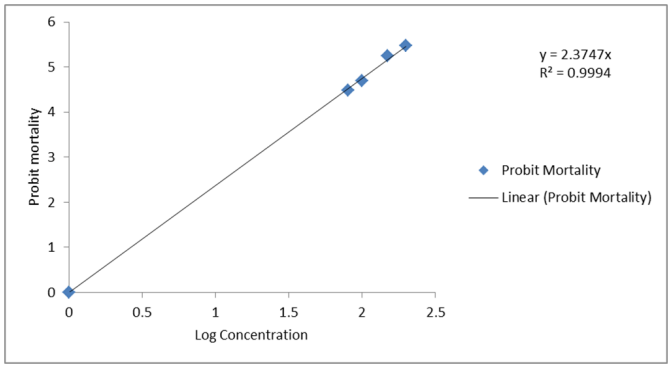


Figure 1: Mortality rate of mosquito larvae exposed to different levels of ethanol extract of *Alchornea laxiflora* leaves.

environmentally friendly than the synthetic commercial insecticides. Natural insecticides are, therefore, better alternatives to the conventional insecticides.

CONCLUSION

The leaf extracts of *Alchornea laxiflora* have high antioxidant and larvicidal activities. The present study has identified thirty-seven natural products from the plant. These compounds are being reported for the first time in *Alchornea laxiflora*. Many of the compounds are known to have bioactive activities and are responsible for the pharmacological activities of this plant.

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Author Contributions

Frank N. I. Morah – Substantial contribution to the conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important intellectual content, Final approval of the version to be publisher

Dickson N. Uduagwu – Acquisition of data Analysis and interpretation of data, Revising the article critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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