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Chemical constituents, insecticidal and anthelmintic activities of *Gongronema latifolium* Leaf Petroleum Ether Extract

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Abstract
Gongronema latifolium is an edible vegetable which serves as spice and versatile African medicinal plant. The leaf is locally used against diabetes and high blood pressure. It also has antimicrobial and anti-inflammatory properties. The study is aimed at identifying the chemical constituents, anthelmintic and insecticidal property of the leaf petroleum ether extract. The leaf was air dried and extracted with petroleum ether to give the petroleum ether extract. The constituents of the petroleum ether extract were separated by gas chromatography and the individual constituents identified by mass spectrometry. Twenty organic compounds were identified and the extract exhibited insecticidal activity against cockroach and anthelmintic properties.

Keywords: chemical constituents, *Periplaneta americana*, *Lumbricus terrestris*, anthelmintic and insecticidal activities, *Gongronema latifolium*

Introduction
Gongronema latifolium is a perennial plant which serves as edible leafy vegetable, spice and medicinal herb. It is used in soup and yam porridge and it is rich in fats, proteins, minerals and vitamins [1]. The leaves are used against malaria, diabetes, hypertension, constipation and it is an anti-inflammatory agent. Some other authors [2, 3, 4, 5] have also reported its use in West Africa to treat cough, intestinal worms and dysentery. Morah and Okon [6] recommend it as a suitable local alternative to conventional hop in beer brewing. Ndukwe et al. [7] reported the activity of aqueous ethanol extract of *G. latifolium* against book destroying termite.
Cockroaches are found all over the globe. The cockroaches are carriers of human intestinal parasites and pathogenic microorganisms [8, 9]. These human parasites are mechanically transported on cockroach body parts as they roam about feeding on filth. Cockroaches also destroy books and hard copies of documents [9]. Most of the reported biological activities of this plant species is on its alcohol and aqueous extracts. The present study is therefore focused on the chemical constituents, anthelmintic and insecticidal properties of its petroleum ether extract against *Lumbricus terrestris* (earth worm) and *Periplaneta americana* (cockroach).

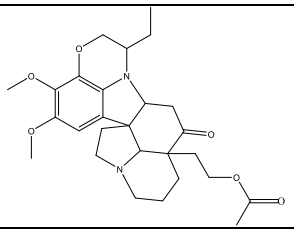
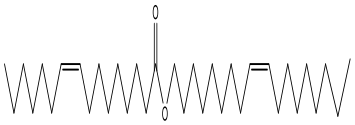
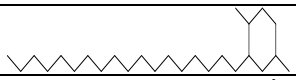
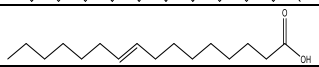
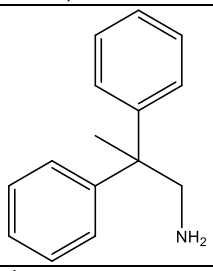
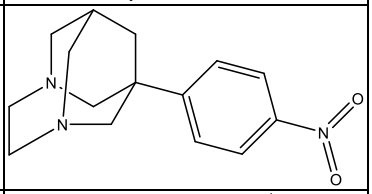
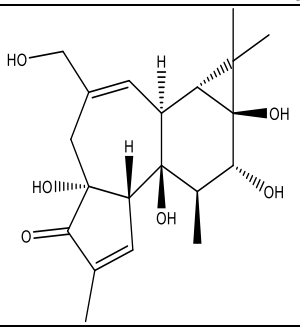
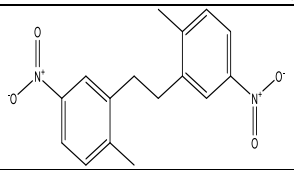
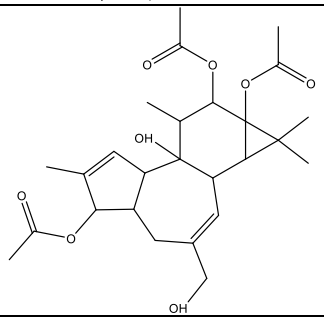
Materials and Methods
Fresh *Gongronema latifolium* was harvested from a farm in Calabar Municipality, Cross River State, Nigeria and was authenticated by staff of the Herbarium Unit, Botany Department, University of Calabar, Calabar, Cross River State, Nigeria. The fresh leaves were rinsed with deionized water. The leaves were air-dried in the laboratory for five days and crushed into powered form.
The powered dry leaf (200g) was weighed into one liter conical flask and 200cm^3 of petroleum ether (40-60^{0}C) added. The mixture was kept at room temperature for 48h. The extracted solution was collected by filtration and the residue washed twice with further 2 X 50cm^3 petroleum ether. The combined filtrate was concentrated at 40-50^{0}C over a hot water bath to give the petroleum ether extract (53.95g).

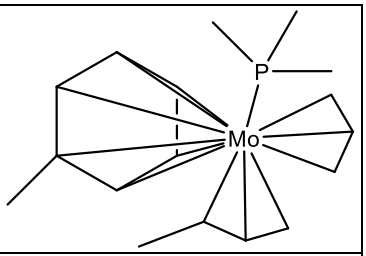
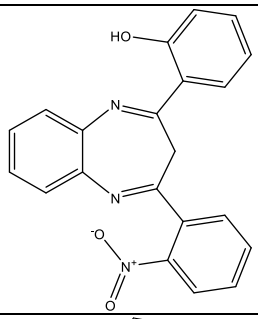
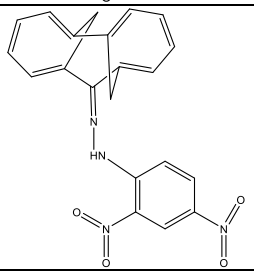
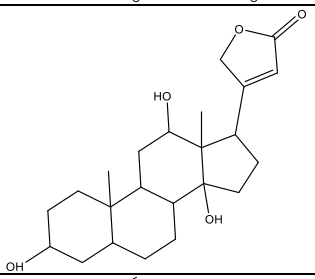
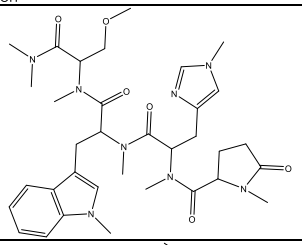
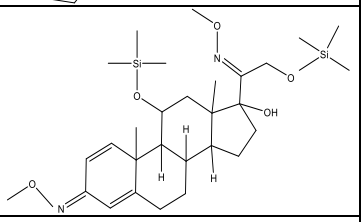
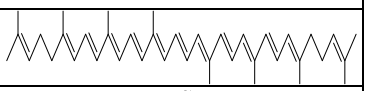
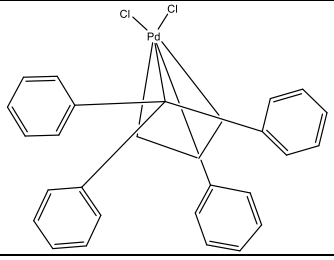
GC-MS analysis
Organic constituents of the petroleum ether extract were separated by gas chromatography while the individual constituents were identified by mass spectrometric analysis [10]. The identification was done by comparison of the obtained mass spectra with those of standard mass spectra of organic compounds from National Institute of Standard and Technologist, NIST.

Biological activities
25cm^3 of separate solutions containing 1%, 5% and 10% solutions of the extract were placed in separated Petri dishes. A fourth Petri dish containing 25% phosphate buffer saline, acted as the control. Five adult *Periplaneta americana* (cockroach) were placed in each of the four Petri dishes and kept for 3h at room temperature after which the number of dead cockroaches were recorded for each petri dish. From this, the percentage mortality of cockroach was calculated. The entire procedure was repeated using adult earth worm, *Lumbricus territris*. The number of dead earth worms were recorded after three hours and the percentage mortality of the earthworm in the different petri dishes were also calculated.

Results
Table 1 shows the result of GC-MS analysis of the petroleum extract of *Gongronema latifolium* leaf. It contained twenty organic compounds. Most of these compounds are oxygenated, two are hydrocarbons, two organometallic compounds while eight of them contain nitrogen heteroatom.

Table 1: GCMS Analysis of *Gongronema latifolium* leaf petroleum ether extract

S/N	Compound name	Time (min)	Mol. Formula	Molar macs	% composition	Chemical structure
	4,25-secoobscurinervan -4-one, O-acetyl-22-ethyl-15,16-dimethoxy-(22 ∝)-	2.55	$C_{27}H_{36}N_2O_6$	484	0.68%	
	9-hexadecenoic acid, 9-octadecenyl ester (Z, Z)	2.69	$C_{34}H_{64}O_2$	504	2.00	
	Cyclohexane, 1,4-dimethyl -2-octadecyl	2.99	$C_{26}H_{22}$	364	0.62	
	9-hexadecenoic acid	3.08	$C_{16}H_{30}O_2$	254	1.52	
	2, 2-diphenyl propylamine	3.14	$C_{15}H_{17}N$	211	1.54	
	1-(4-nitrophenylmethyl)-3,6-diazahomoadamantane	15.23	$C_{15}H_{19}N_3O_2$	273	1.10	
	Phorbol	16.21	$C_{20}H_{28}O_6$	384	1.56	
	7,10,13-eicosatrienoic methyl ester	16.68	C21 $H_{36}O_2$	320	1.18	
	Ethane, 1,2-bis(2-methyl-5-nitrophenyl)	17.42	$C_{16}H_{16}N_2O_4$	300	1.63	
10	1H-cyclopropa[3,4]benz [1,2-e] azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl. 5,9,9atriacetate [1a R-(1a∝, 1bβ, 4∝β, 5β, 7β, 8∝, 9β, 9a∝)]	17.4	$C_{25}H_{36}O_8$	476	3.02	

11	Molybdenum (σ- allyl)(n-3-butenyl)(toluene)(trimelhylphoshine)	17.55	$C_{17}H_{29}M_0P$	362	0.74	
12	2-[2-hydroxyphenyl]-4-(2-nitrophenyl)-3H-1, 5-benzodiazepine	17.95	$C_{21}H_{15}N_3O_3$	357	0.97	
13	Tricyclo[7.4.1.1 (3,8)] trideca-3,5,7, 9, 11,13-hexane, 2-(2', 4' dinitrophenylhydrazone)	19.90	$C_{21}H_{18}N_4O_4$	388	0.61	
14	Digoxigenin	20.49	$C_{23}H_{34}O_5$	390	5.33	
15	L-serinamide, 1-melhyl-5-oxo-L-propyl-N,1-dimethyl-L-hitidyl-N. 1-L-tryptophyl, N, N, N_2. O-tetramethyl	20.58	$C_{34}H_{48}N_8O6$	664	10.15	
16	Prednisolone, 11, 21-bis(trimethylsilyl) ether, bis(O-methyloxime	21.23	$C_{29}H_{50}N_2O_5Si_2$	562	2.34	
17	Iycopene	21.76	$C_{40}H_{56}$	536	4.01	
18	Palladium, dichbro (1,2,3,4-tetraphenyl-1,3-cyclobutadiene)	22.24	$C_{28}H_{20}Cl_2Pd$	532	27.00	

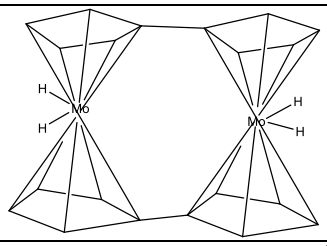
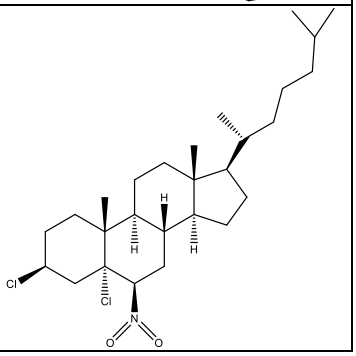
19	Bis(cyclopentadienylmolybdeum), di-hydro-μ (η5.n5-fluvalene)	23.91	$C_{20}H_{20}Mo_2$	456	24.34	
20	Cholestane -3, 5-dichloro-6-nitro- (3β, 5$\propto$, 6β)	24.74	$C_{27}H_{45}Cl_2NO_2$	485	4.08	

Table 2: Insecticidal activity of *Gongronema latifolium* leaf petroleum ether extract

Level of extract	No. of cockroach	No. dead	% mortality
1%	5	1	20%
5%	5	3	60%
10%	5	5	100%
Control (0%)	5	0	0.0%

Table 3: Anthelmintic activity of *Gongronema latifolium* leaf petroleum ether extract against *Lumbricus terretris*

Level of extract	No. of worms	No. dead	% mortality
1%	5	2	40%
5%	5	4	80%
10%	5	5	100%
(0.0%)	5	0	0.0%

Table 2 and 3 show the insecticidal and anthelmintic activities of *Gongronema latifolium* leaf petroleum ether extract against *Peripleneta americana* and *Lumbricus terrestis* respectively. In both cases 10% of the extract resulted in 100% mortality after three hours.

Discussion

The petroleum ether extract contains twenty organic compounds which include palladium dicholoro (1,2,3,4-tetraphenyl-1,3-cyclobutadiene) - (27.01%); bis (cyclopentadienylmolybdeum), dihydro-μ-(η5: η5-fulvalene) - (24.34%); cholestane -3,5-dichloro-6-nitro (3β, 5$\propto$, 6β), (4.02%); lycopene (4.01%); 9-hexadecenoic acid (1.52%); digoxigenin (5.33%); phorbol (1.56%) and 2,2-diphenylpropylamine (1.54%). It is these constituents that are individually or collectively responsible for the observed biological activity of *Gongronema latifolium*. Although the major constituents of the mixture may be considered to be responsible for its biological activities, it is a known fact that such biological activities are modulated by the minor constituents through several antagonistic, synergistic and additive effects [11].

The petroleum ether extract exhibited insecticidal activity against the cockroach (*Periplaneta americana*). The fact that the control, without the extract, had no insecticidal activity while the different concentrations of the extract showed strong activity points to the fact that the extract was responsible for the death of the insects. The insecticidal activity, as shown in Fig 2, is dose dependent as it increases with the level of the extract. At 10% concentration, all the cockroaches died within three hours. Cockroach is implicated in the spread of many human diseases [9, 11]. The work is therefore of public health interest.

Lumbricus terrestris is used as model study for intestinal round worms because it shows anatomical and physiological resemblance with intestinal round worm [12]. Similarly, table 3 shows that the petroleum ether extract has anthelmintic activity which is dose dependent and all the worms died at 10% concentration after three hours at room temperature while none died in the control indicating that the death was caused by the extract.

Conclusion

The petroleum ether extract of *Gongronema latifolium* has twenty organic constituents and these are being identified for the first time in the petroleum ether extract of *Gongronema latifolium*. The extract has strong activity against cockroach and also anthelmintic activity. The work also shows dose dependent nature of the crude drug.

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