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In vivo anthelmintic potentials of *Gongronema latifolium* and *Picralima nitida* against gastrointestinal parasite (*Heligmosomoides bakeri*)

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Summary

Gastrointestinal parasites which are responsible for soil-transmitted helminthiases cause illness globally. The control of helminthiasis depends on mass distribution of anthelmintics which has been reported for its resistance, toxicity and low efficacy. In this study, anthelmintic potentials of *Gongronema latifolium* and *Picralima nitida* which have wide application in traditional medicine were determined *in vivo* using *Heligmosomoides bakeri* a naturally occurring gastro-intestinal parasite of rodents that is closely related to highly prevalent human nematode parasites.

Extracts of *P. nitida* at 500 mg/kg had higher ($p < 0.05$) chemosuppression (92.45 %) than extracts of *G. latifolium* (65.82 %) and was highly comparable to albendazole (92.61 %). As the dose of the extracts increased from 300 to 500 mg/kg body weight, chemosuppression of 84.91 % and 92.45 % (*P. nitida*) and 43.54 % and 65.82 % (*G. latifolium*) respectively were produced. The extract of *P. nitida* gave deparasitization rates ($p<0.05$) of 72.60 % and 77.16 % at 300g/kg and 500mg/kg of body weight respectively. The glucose level and protein content reduced ($p<0.05$) in mice treated with extract of *P. nitida* when compared with extract of *G. latifolium* and untreated mice. Phytochemical screening revealed that *P. nitida* and *G. latifolium* contained flavonoids, alkaloids, saponins, tannins and polyphenols. Acute toxicity studies showed that *Gongronema latifolium* and *Picralima nitida* have no apparent toxic effect in mice even at the dose of 5000 mg/kg.

Extracts of *P. nitida* and *G. latifolium* have anthelmintic properties that are dose-dependent, and this could offer potential lead for the development of safe, effective and affordable anthelmintics.

Keywords: *Picralima nitida*; *Gongronema latifolium*; *H. bakeri*; anthelmintic activity

Introduction

Intestinal parasitic infections remain a significant challenge to global health. These infections are mostly caused by helminthes which are classified into three taxonomic groups: nematodes, cestodes and trematodes (Salazar-Castañon, *et al*., 2014). Intestinal parasitic nematodes cause most common infection that continue to pose problems in human and veterinary medicine (Little *et al.,* 2009; Bogitsh *et al.,* 2012; Castro *et al.,* 2019). These infections lead to malnutrition, abdominal pain, physical disabilities, delayed growth and development in children, while parasitized pregnant women are anemic and their newborns have low birth weight. However, in animals it causes serious economic losses in ruminants ranging from decrease in growth rate and reductions in the

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production of milk, meat and wool (Qamar, *et al*., 2011; Charlier *et al.,* 2014; Coghlan, *et al*., 2019). It is estimated that approximately 1.5 billion people worldwide are infected with soil transmitted helminth, predominantly in tropical areas (WHO, 2020). In Nigeria, intestinal helminth infections are endemic with young children between the ages of 5 and 14years living in rural and urban slums majorly at risk (Oluwole *et al.,* 2015; Oyebamiji *et al.,* 2018; Isaac *et al.,* 2019). In the absence of vaccines for most parasite species, the control of these infections has been dependent on mass distribution of single-dose of broad-spectrum anthelmintics which has led to sporadic incidence of anthelmintic resistance and adverse effects (Humphries *et al.,* 2011).

However, one of the laboratory models for exploring host-parasite relationship for gastrointestinal nematode is *Heligmosomoides bakeri* (Behnke & Harris, 2010). It belongs to the order Strongylida that includes the highly-prevalent human hookworm parasite and causes chronic gastrointestinal nematode infections in rodents. It is of importance in the screening of anthelmintics and modeling of immune response to gastrointestinal infections of humans and animals (Gouy de Bellocq *et al*., 2001; Reynolds, *et al*., 2012). This parasite has direct life cycle with its third stage larva (L_3) being the infective stage. The infective third stage larvae are ingested by faecal-oral transmission or administered by oral gavage in the laboratory setting (Johnston *et al.,* 2015). However, mice are used for this research because their biology is similar to humans which makes it good model for helminth infections and due to host-specificity of the parasite (Morse, 2007; Reynolds *et al.,* 2012).

In recent times, natural products of plant sources are directly or indirectly used in modern medicine because it is cheap, efficacious and safe (Newman & Cragg, 2020). Medicinal plants are used to cure many infectious diseases including intestinal infections. Studies have shown the efficacy of medicinal plants in intestinal infections (Pone *et al.,* 2011; Debebe *et al.,* 2015; Banerjee *et al.,* 2019).

Gongronema latifolium (GL) which belongs to family Apocynaceae, Subfamily Asclepiadoideceae is a climbing perennial plant that is edible with sharp, bitter and slightly sweet taste, especially when eaten fresh. It is commonly known as Amaranth globe or Bush busk, locally called 'utazi' in South eastern Nigeria and 'Arokeke' or 'Madunmaro' in South western Nigeria (Eleyinmi, 2007; Nazia *et al.,* 2013). *G. latifolium* has been reported for its antibacterial activity (Eleyinmi, 2007; Enyi-Idoh *et al.,* 2017), anticancer (Iweala, 2015), hepatoprotective effects (Imo, 2017; Aloke, Ogbodo and Ejike, 2018), hypoglycemic and hypolipidemic activities (Sylvester, *et al*., 2015; Eyo & Chukwu, 2016) due to its different active constituents.

Picralima nitida belonging to the family Apocynaceae is commonly called Picralima, Akuamma or Pile plant and its distribution is restricted to African rain forest regions. It is known as limeme (Congo), Eban or Obero (Gabon), Erin or Abeere (Yoruba), Osuigwe (Igbo) and Bamborutuk or Eban (Cameroon) (Mbegbu, *et al.,* 2014). The various parts of the plants have been used for the treatment of hypertension, jaundice, dysmenorrheal, gastrointestinal disorders and malaria in West African traditional medicine. The extract from different parts of the plant exhibited a broad range of pharmacological activities which gives credence to its ethnomedicinal uses (Erharuyi, *et al*., 2014). However, *G. latifolium* and *P. nitida* are listed among the plants used by herb sellers in Nigeria for the treatment of many diseases including intestinal helminth infections (Borokini *et al.,* 2013a; Borokini *et al.,* 2013b) but there is paucity of information on the activities of these plants. Therefore, the study was therefore designed to ascertain the safety and efficacy of *G. latifolium* and *P. nitida* when used in the treatment of helminth infections.

Material and Methods

Plant materials

The fresh roots of *G. latifolium* and seeds of *P. nitida* were collected from Ogunmakin, Ogun State, Nigeria. The plant materials were identified and authenticated by plant taxonomists Mr. Nodza, G.I and Adeyemo, A. at the herbarium unit of University of Lagos, Nigeria and Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria respectively where the plant specimens were deposited with voucher numbers.

Preparation of extract

The plants materials were rapidly washed under running tap water and air-dried in the laboratory at room temperature. The dried plant materials were then crushed into coarse powder with plant grinder. Two hundred and fifty grams (200g) of each the resulting powder was soaked in 2000 ml 95 % ethanol stirred intermittently for 48 hours at room temperature and later filtered. Clean muslin cloth and Whatman filter paper 1 were used to filter to obtain a clear

Table 1. Acute toxicity studies of mice treated with extracts of *G. latifolium* and *P. nitida*.

	No.Dead/No.Alive		Clinicalsigns	
Dose(mg/kg)	***G.latifolium***	***P.nitida***	***G.latifolium***	***P.nitida***
10	0/3	0/3	Noobservablesign	Noobservablesign
100	0/3	0/3	,,	,,
1000	0/3	0/3	,,	,,
1600	0/3	0/3	,,	,,
2900	0/3	0/3	,,	,,
5000	0/3	0/3	,,	,,

solution. The filtrate was evaporated to dryness over water bath at 45°C and the residue was stored for further use. The choice of the solvent was based on the mode of preparation mentioned by the herb sellers during questionnaire administration.

Phytochemical Screening

The quantitative phytochemical tests for plant secondary metabolites such as flavonoids, tannins, alkaloids, saponin and total polyphenol were evaluated. Flavonoid was determined by repeated extraction with aqueous methanol at room temperature, as described previously Boham and Kocipai (1974). Tannin determination was carried out by using Folin – Dennis titration method as described by Pearson and Cox (1974). Alkaloids and saponin were estimated according to the methods of Harborne (1973) and Bruner (1984) respectively. Total polyphenol content was determined according to Attard (2013).

Proximate composition

The crude protein, ash, crude fat, carbohydrate and moisture content of the *G. latifolium* roots and *P. nitida* seeds were determined according to AOAC (2005).

Source of animals

A total of 74 *Mus musculus* BALB/c strain each weighing between 26 and 28g of similar age commercially obtained from Central Animal house, University of Ibadan, Ibadan, Nigeria was used for this study. The mice were free of pathogens and have not been used for any initial experiment.

Animal housing and husbandry

The mice were kept in plastic cages which contained dried wood shavings as beddings at room temperature (28 ± 2°C), fed with growers' mash (Top Feeds Limited, Ibadan) and clean water in the animal house in the Department of Zoology, Federal University of Agriculture, Abeokuta, Nigeria. The mice were acclimatized for two weeks before the commencement of the experiment and kept at 12 h light and dark cycle.

Acute toxicity studies

Thirty-nine BALB/c male mice weighing 26 – 28g were used to determine lethal dose (LD_{50}) of the extracts. The toxicity study was conducted in two phases. In phase one, eighteen mice divided into six groups of three mice were used for the two plant extracts. The mice received orally 10, 100 and 1000mg/kg body weight for each of the extracts (Lorke, 1983). Another three mice received distilled water. The animals were observed for signs of toxicity and mortality. Based on the results of phase one, another set of eighteen mice were randomized into six groups of three mice for the two extracts. The mice were given 1600, 2900 and 5000mg/kg body weight for each of the extracts. Immediately after dosing, the animals were observed for any behavioural changes, any sign of toxicity and mortality for critical 4hrs and thereafter, daily for 7 days.

Parasite

The gastrointestinal nematode parasite, *Heligmosomoides bakeri* was obtained from the Department of Veterinary Parasitology and Entomology, Micheal Okpara University, Abia State where it was maintained in albino mice.

Table 2. Phytochemicals, proximate composition and mineral contents of *G. latifolium* (roots) and *P. nitida* (seeds).

	G. latifolium	***P. nitida***	**p-value**
Phytochemicals (mg/100g)			
Saponin	579.67 ± 0.88	115.00 ± 0.58	0.00
Alkaloid	396.67 ± 0.88	290.33 ± 0.89	0.00
Tannin	2.00 ± 0.12	2.90 ± 0.12	0.01
Flavonoid	3.27 ± 0.08	5.33 ± 0.07	0.00
Total Polyphenol	138.67 ± 0.89	217.00 ± 0.57	0.00
Proximate composition (%)			
Crude protein	10.50 ± 0.00	14.43 ± 0.22	0.00
Ash	11.70 ± 0.35	3.37 ± 0.33	0.00
Crude fat	2.22 ± 0.52	3.36 ± 0.33	0.14
Carbohydrate	41.90 ± 0.02	29.70 ± 0.01	0.00
Moisture	7.05 ± 0.03	8.61 ± 0.00	0.00
Mineral content (mg/kg)			
Calcium	48843.33 ± 83.33	473.33 ± 23.33	0.00
Magnesium	11846.67 ± 43.33	1543.33 ± 28.40	0.01
Zinc	127.13 ± 0.00	82.56 ± 0.00	0.00
Copper	35.19 ± 0.00	25.91 ± 0.01	0.00
Iron	2173.91 ± 0.01	445.44 ± 0.22	0.00
Manganese	423.0 ± 0.58	72.25 ± 0.32	0.00
Potassium	19891.0 ± 0.58	22217.59 ± 3.13	0.00
Phosphorus	217.02 ± 0.58	598.19 ± 0.95	0.00

Coproculture of third-stage larvae (L_3)

The culture was set according to the method of Fakae *et al.,* (1994). Fresh faecal pellets were collected from the infected mice and broken up in distilled water in a coffee strainer. The supernatant was decanted while the pasty sediment was smeared on moistened filter paper placed in plain petri dishes. The cultures were left for 7 days in a refrigerator (4°C) to ensure optimal hatching of the *H. bakeri* eggs.

Harvesting and enumeration of infective larvae (L_3)

At the end of the culture period, a few drops of distilled water were sprinkled on and underneath the filter paper and left for 1 hour. The water obtained from the recovered infective larvae was pipetted into clean test tube, then allowed to settle under laboratory conditions and excess water was decanted using Pasteur pipette. The suspension was mixed thoroughly by gently shaking of the tube to ensure an even distribution of larvae. Thereafter, an aliquot of 0.2ml was drawn into a clean microscope slide for five times and larvae were counted under 4x objective lens.

Infection with H. bakeri

Each worm free mouse was infected orally with 150 L_3 *H. bakeri* suspended in 0.2ml of distilled water (Ngogeh & Fakae, 2011). The larvae suspension was inoculated by properly restraining the mice and using blunted tip slightly curved 18gauge needle mounted on tuberculin (1 ml) syringe.

Experimental design for in vivo anthelmintic studies

Thirty-five male BALB/c mice each weighing between 26g and 28g were randomly divided into seven groups consisting five mice each. Five animals were used per group in order to prevent loss of animals or data due to the infection. Groups 1 – 4 were infected but treated with graded doses (300 and 500mg/kg) of *G. latifolium* and *P. nitida* extracts orally for five consecutive days, group 5 was infected but treated with 10mg/kg albendazole once (positive control), group 6 was infected untreated (negative control) while group 7 was uninfected control group. At day 12 post treatment, mice in each group were euthanized by cervical dislocation. The *in vivo* anthelmintic studies were conducted three times for quality control.

Faecal egg counts

Faecal egg counts (FEC) were monitored on day 16 post infection using a modified McMaster technique (Fakae *et al.,* 1994). Briefly, 0.1g of fresh faecal pellet from each mouse was homogenised in 1.4ml of saturated sodium chloride solution and faecal suspension was filtered through a tea strainer. The filtrate was collected into a test tube and then adequately stirred. Pasteur pipette was used to withdraw the filtrate and rapidly used to fill the two chambers of the McMaster slide. This was then mounted under the light microscope and allowed for 5 min to settle. All eggs within the square on each of the chambers were counted at 10× objective. The number of eggs per gram (EPG) was calculated using formula described by Zajac and Conboy (2012) in equation (1).

$$Eggs\ per\ gram = \left\{ Number\ of\ eggs\ counted \times \left(\frac{T}{V} \right) \right\} \Big/ F$$

Where T = total volume of faeces/flotation solution mixture, V = volume of aliquot examined in slide, and F = grams of faeces used. The anthelmintic effects of each of the plant extracts were determined by comparing the EPG of the treated groups with that of those treated with distilled water (untreated control) using the formula described by Suleman *et al*. (2005) in equation 2

$$\% Chemosuppression \Big/ Deparasitisation = \frac{N - n}{N} \times 100$$

Postmortem worm count

On day 27 post infection, all the mice were deprived of food but not water for 24 hours so as to empty the gastrointestinal tract to make worm counting easier. Mice were euthanized by cervical dislocation. Modified method of Ngongeh (2013) was used for worm count. Summarily, the entire length of the small intestine of each mouse was removed and opened by cutting it longitudinally with a pair of surgical scissors. The adult worms were recovered individually by suspending each intestine with fine threads, then dipping into normal saline and incubating at room temperature (28 ± 2°C) for 20 hours to enable the migration of the worms into the solution.

Table 3. Daily chemosuppression (%) of *H. bakeri* infection in mice administered single extract of *G. latifolium*, *P. nitida* and albendazole. Values presented are mean from three mice in a group.

	Days										
	During treatment					Post treatment					
Treatments	1	2	3	4	5	6	7	8	9	10	11
300mg/kg GL	17.38	19.22	23.76	24.70	33.66	36.05	39.13	43.54	42.87	41.14	40.88
500mg/kg GL	40.00	44.39	53.57	56.23	56.73	60.52	63.12	64.11	65.03	65.82	63.84
300mg/kg PN	47.24	67.00	70.65	72.01	76.96	77.65	81.00	81.81	83.41	84.17	84.91
500mg/kg PN	54.17	70.71	77.48	77.64	88.73	89.23	90.50	90.75	91.46	91.77	92.45
Albendazole	27.07	56.47	72.98	73.29	73.69	86.79	90.01	90.91	91.62	92.41	92.61

At the end of incubation, the suspensions were centrifuged at 250 rpm (11 g) for 2 minutes. The supernatant was discarded and the sediment containing the worms was viewed using the magnifying lens where the reddish worms were identified, counted and recorded.

Biochemical analysis

The blood samples obtained from retro-orbital sinus of the mice were collected without anticoagulant to obtain non-haemolysed serum used for the biochemical analysis. Cholesterol was determined by enzymatic method (Allain *et al.,*1974), glucose was estimated by glucose oxidase method (Trinder, 1969) , total protein and albumin were estimated by Biuret and bromocresol green method (Doumas *et al.,*1971), triglyceride concentration was determined enzymatically by glycerol-3-phosphate oxidase-phenol+ aminophenazone method (Jacobs & VanDemark, 1960; Schettler & Nussel, 1975). High density lipoprotein cholesterol (HDL-C) was determined by precipitation methods of Friedewald, Levy and Frieddrickson 1972; Gordon *et al.,* 1977 using test kits by Agappe Diagnostics Limited. LDL-C was calculated using Friedewald formula:

$$LDL_c = TC - \left(HDL_c + \frac{TG}{5} \right)$$

Histological analyses

Mice were euthanized by cervical dislocation, intestines of the mice that received highest dose (500mg/kg) of *G. latifolium* and *P. nitida*, albendazole and untreated were removed, washed with saline and fixed in 10 % buffered neutral formalin for 24 hours. Subsequently, the tissue was subjected to histological techniques, including dehydration in ascending series of alcohols, clearing in Xylol and embedding in paraffin. Then, 5µm-thick sections were obtained in a manual rotation microtome and stained with Haematoxylin and Eosin (HE).

Data analyses

Data obtained were subjected to Shapiro-Wilk test for normality and was analysed using one-way analysis of variance (ANOVA) to compare the means across the groups using Statistical Package for Social Science (SPSS) version 20. The results were expressed as Mean ± SE and values of $p<0.05$ were considered significant. Independent sample t-test was used to analyse proximate composition and phytochemical constituents by comparing the means of *G. latifolium* and *P. nitida*.

Ethical Approval and/or Informed Consent

The ethical conditions governing the use laboratory animals were maintained in accordance with the recommendations in the Guide for Animal Research: Reporting of In Vivo Experiments (ARRIVE) and the experimental protocol was approved by College of Veterinary Medicine Research Ethics Committee (CREC), Federal University of Agriculture, Abeokuta, Nigeria with approval reference number FUNAAB/COLVET/CREC/005/19.

Results

Acute toxicity studies of G. latifolium and P. nitida in mice

In acute toxicity study, the roots extract of *G. latifolium* and seeds extract of *P. nitida* up to 5000mg/kg neither produced mortality nor

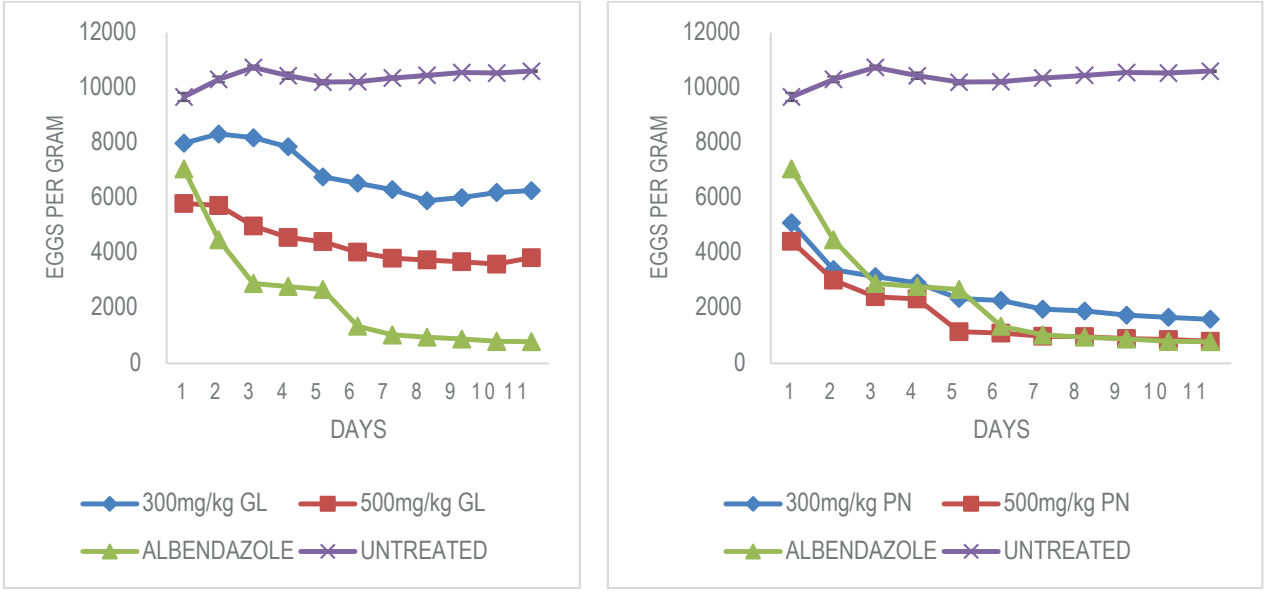

Fig. 1. Egg per gram of faeces of *H. bakeri* in infected mice treated with (A) extract of *G. latifolium* at 300 and 500 mg/kg body weight (B) *P. nitida* at 300 and 500 mg/kg body weight.

any sign of toxicity. Hence, the oral LD_{50} of *G. latifolium* and *P. nitida* was estimated to be greater than 5000mg/kg body weight (Table 1).

Phytochemicals, proximate composition and mineral contents of the roots of G. latifolium and seeds of P. nitida

Total polyphenol, flavonoid and tannin content were significantly higher ($p<0.05$) in *P. nitida* than *G. latifolium*. However, saponin was most abundant followed by alkaloid in *G. latifolium* root while alkaloid was most abundant in *P. nitida* seeds (Table 2). Proximate analysis revealed that the root of *G. latifolium* contained significantly higher ($p<0.05$) carbohydrate (41.90 ± 0.02 %) and ash content (11.70±0.35 %) when compared with seeds of *P. nitida*. Higher crude protein and moisture were recorded for *P. nitida* seeds (Table 2). Calcium, Magnesium, Zinc, Copper, Iron and Manganese were significantly ($p< 0.05$) higher in *G. latifolium* than *P. nitida*. However, Potassium and Phosphorus were significantly ($p< 0.05$) higher in the seeds of *P. nitida.* Calcium is the dominant mineral in the roots of *G. latifolium* while Potassium is the dominant in *P. nitida* seeds (Table 2).

Egg per gram of faeces of H. bakeri in infected mice treated with single extracts of G. latifolium and P. nitida

The faecal egg count of *H. bakeri* was significantly higher ($p<0.05$) in untreated mice throughout the experiment than mice treated with *G. latifolium* (Fig. 1a). Meanwhile, the faecal egg count of *H. bakeri* reduced in mice treated with albendazole when compared with the extract of *G. latifolium*. Although a decline was recorded in the faecal egg output of *H. bakeri* in mice that received varying doses of *G. latifolium*, however the decline was not as steep as that of mice that were treated with albendazole. Furthermore, the extract of *G. latifolium* at 300mg/kg reduced the faecal egg count of *H. bakeri* till day 8. However, on day 9 the faecal egg count increased till day 11 (Fig. 1a). There was reduction in the faecal egg count of *H. bakeri* in mice treated with extract and albendazole throughout the experiment (Fig. 1b). The faecal egg output of *H. bakeri* was significantly ($p<0.05$) lower from day 1 till day 7 in mice treated with extract of *P. nitida* at 500mg/kg when compared with other treatment groups. However, from day 8, there was no significant ($p>0.05$) difference in the faecal egg count of mice treated with extract of *P. nitida* at 500mg/kg and albendazole (Fig. 1b).

Daily chemo suppression of H. bakeri infections *in mice administered single extracts of P. nitida*, *G. latifolium and albendazole*

There was significant ($p<0.05$) difference in the percentage chemo suppression among all the treatment groups. Mice treated with *P. nitida* extract recorded higher level of chemo suppression ($p<0.05$) when compared with *G. latifolium* at the same doses (Table 3). Extract of *P. nitida* at 500mg/kg produced higher chemo suppression from day 1 till day 7 post treatment when compared to albendazole. On the other hand, albendazole gave higher chemo suppression ($p>0.05$) from day 8 till day 11 post treatment compared to *P. nitida* (Table 3). There was dose dependency in chemo suppression of mice treated with *P. nitida* throughout the experiment. At 300 and 500mg/kg body weight, extracts of *P. nitida* produced highest chemo suppression of 84.91 % and 92.45 % respectively while *G. latifolium* gave 43.54 % and 65.82 % respectively ($p<0.05$). The extract of *G. latifolium* at 300 and 500mg/kg recorded highest chemo suppression at day 8 and 10 post treatment respectively. However, extract of *P. nitida* recorded highest chemo suppression at day 11 at 300 and 500mg/kg (Table 3).

Worm burden of infected mice treated with single extracts of G. latifolium, *P. nitida and albendazole*

A significant reduction ($p<0.05$) was recorded in the worm burden of mice treated with different doses of the plant extracts when compared with the untreated mice. The reduction in worm burden was dose dependent (Table 4). However, mice treated with *P. nitida* at 500 mg/kg body weight had the lowest worm burden followed by mice treated with albendazole. The deparasitization rate produced by different doses of *G. latifolium* was significantly lower than that of *P. nitida* and albendazole. There was no significant ($p>0.05$) difference in the worm burden of mice treated with extract of *P. nitida* and albendazole (Table 4).

Biochemical parameters of H. bakeri infected mice treated with extracts of G. latifolium and P. nitida, albendazole and untreated

The level of glucose increased significantly ($p<0.05$) in infected mice treated with extract of *G. latifolium* than infected mice treated with *P. nitida* and the untreated. However, extract of PN reduced the glucose level of infected mice when compared with untreated and uninfected treated mice (Table 5). On the other hand, cholesterol level was significantly ($p<0.05$) higher in all infected mice

Table 4. Worm burden of infected mice treated with extract of *G. latifolium, P. nitida* and albendazole

Values were expressed as mean ± standard error of means. Columns with values bearing the same superscript are not significantly different ($p<0.05$). Mean values presented were from three mice in a group.

Treatment	Worm burden	Deparasitization (%)
300mg/kg GL	57.50 ± 0.50^{c}	21.23
500mg/kg GL	40.75 ± 1.11^{b}	44.17
300mg/kg PN	20.00 ± 2.08^{a}	72.60
500mg/kg PN	16.67 ± 0.33^{a}	77.16
Albendazole	19.33 ± 0.67^{a}	73.52
Untreated	73.00 ± 1.15^{d}	

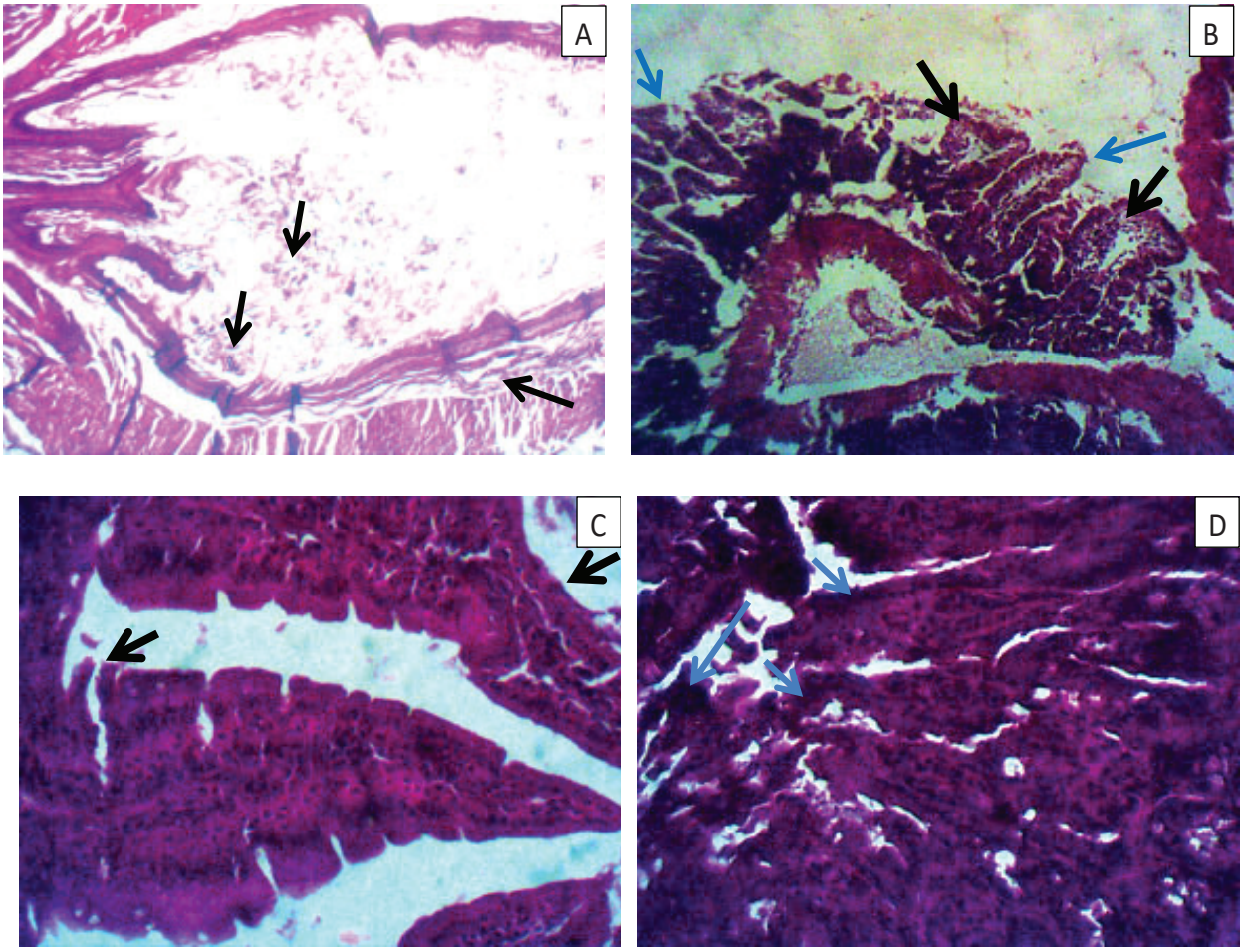

Fig. 2. Micrographs of Intestinal segments of (A) untreated mice with severe villi atrophy and intra-luminal parasite (black arrow).
(B) mice treated with 500mg/kg *G. latifolium* showing moderate villi atrophy (black arrow) and cryptal hyperplasia (blue arrow).
(C) mice treated with 500mg/kg *P. nitida* showing moderate villi (black arrows) atrophy (D) positive control showing cryptal hyperplasia (blue arrow) HE x400.

compared with uninfected mice. The protein content was lower in mice treated with *P. nitida* compared to mice treated with *G. latifolium.* Meanwhile, there was no significant difference (p >0 05) in protein content of both untreated and infected untreated. Higher albumin level was recorded in uninfected untreated mice when compared to infected but treated with plant extract (Table 5). Generally, triglyceride and HDL levels were significantly ($p<0.05$) higher in infected mice when compared with uninfected mice. However, the levels of triglyceride and HDL increased in both *G. latifolium* and *P. nitida* as the doses increased (Table 5). Furthermore, the LDL was in significantly reduced in infected but treated mice when compared to untreated and uninfected untreated mice (Table 5).

Histopathological findings of the small intestine of infected mice, treated with 500mg/kg of P. nitida and G. latifolium

Several villi atrophy and intra-luminal parasites were observed in the intestinal segments of mice that did not receive treatment (Fig. 2). However, there was moderate villi atrophy and cryptal hyperplasia in intestine of mice that received 500mg/kg *G. latifolium* when compared to the untreated. Furthermore, no cryptal hyperplasia was observed in the intestine of mice that received 500mg/kg *P. nitida* (Fig. 2).

Discussion

In this study, the oral lethal dose (LD_{50}) of *G. latifolium* and *P. nitida* was greater than 5000 mg/kg body weight this suggests the plant is not toxic at this dose. However, this corroborates the findings of Sylvester, *et al*. (2015) on *G. latifolium* and N'dri (2015) on *P. nitida.*

Many secondary metabolites have exhibited a wide range of biological activity and protection against various ailments. The presence and varying quantity of flavonoid, saponin, tannin, alkaloid and polyphenol in *G. latifolium* root and *P. nitida* seed indicates that they contain some useful bioactive substances which could have contributed to their acclaimed medicinal efficacy. This is in consonance the finding of Antai *et al.,* (2009), Nwabor *et al.,* (2014), Bruce, *et al*. (2016) and Georgy *et al.,* (2019).

Table 5. Biochemical parameters of *H. bakeri* infected mice treated with extracts of *G. latifolium* and *P. nitida*
Rows with values bearing the same superscript are not significantly different (p>0.05).
Values were expressed as mean ± standard error of means. Mean values presented were from three mice in a group.

Parameter	300mg/kg GL	500mg/kg GL	300mg/kg PN	500mg/kg PN	Albendazole	Untreated	Uninfected untreated
Glucose (mg/dl)	117.61 ± 4.71^{d}	123.21 ± 4.46^{d}	75.45 ± 2.94^{a}	88.63 ± 3.86^{b}	81.50 ± 1.21^{ab}	100.28 ± 2.61^{c}	122.00 ± 1.53^{d}
Cholesterol (mg/dl)	80.17 ± 1.80^{bc}	81.53 ± 4.50^{bc}	68.90 ± 4.04^{b}	89.29 ± 5.31^{c}	78.30 ± 5.29^{bc}	82.37 ± 8.76^{bc}	40.67 ± 0.67^{a}
T. Protein (g/dl)	3.77 ± 0.27^{ab}	6.53 ± 1.42^{bc}	2.11 ± 0.38^{a}	3.62 ± 0.38^{ab}	4.61 ± 1.07^{ab}	6.49 ± 1.38^{bc}	8.27 ± 0.27^{c}
Albumin (g/dl)	2.21 ± 0.09^{ab}	2.55 ± 0.45^{ab}	1.37 ± 0.51^{a}	1.96 ± 0.15^{ab}	2.78 ± 0.44^{ab}	2.49 ± 0.31^{ab}	3.27 ± 0.07^{b}
Triglyceride (mg/dl)	83.68 ± 5.67^{b}	122.32 ± 6.58^{e}	116.30 ± 5.67^{de}	119.62 ± 3.92^{de}	103.21 ± 7.12^{cd}	98.35 ± 4.72^{bc}	24.67 ± 0.67^{a}
HDL (mg/dl)	52.39 ± 0.06^{c}	41.51 ± 1.88^{b}	33.94 ± 1.33^{b}	50.43 ± 4.82^{c}	32.68 ± 4.37^{b}	38.22 ± 3.40^{b}	10.33 ± 0.33^{a}
LDL (mg/dl)	11.05 ± 0.71^{a}	15.55 ± 1.30^{a}	11.70 ± 1.58^{a}	14.94 ± 1.27^{a}	24.87 ± 0.48^{b}	24.48 ± 6.30^{b}	25.40 ± 0.20^{b}

However, abundance of saponin and low tannin content in *G. latifolium* roots in the study support the findings of Egbung *et al.,* (2011), and Enemor, *et al*. (2014). Furthermore, presence of saponin in *G. latifolium* root also indicate that intake of the plant can remove fatty compounds from the body, lowering the blood cholesterol and also may be responsible for the bitter taste of the plant (Vaghasiya, *et al*., 2011; Osuagwu *et al.,* 2013). On the other hand, predominance of alkaloid in *P. nitida* corroborates that of Erharuyi, *et al*. (2014) and Igwebuike (2019) that alkaloids are the major class of phytochemicals isolated from *P. nitida* and followed by polyphenols. The low quantitiy of tannin in *P. nitida* recorded in this study agrees with Bruce, *et al*. (2016).

The ash content is a reflection of the mineral elements preserved in any food materials (Iniaghe *et al.,* 2009). The high ash content of the roots of *G. latifolium* indicates high inorganic content from where the mineral content could be obtained. Mineral elements speed up metabolic processes, improve growth and development (Opara *et al.,* 2018). High carbohydrate in the roots of *G. latifolium* is an indication that it is a good source of energy because carbohydrates are polar compounds which are readily converted into glucose. The carbohydrate and protein content of *P. nitida* and *G. latifolium* was higher than those recorded for bulb of *Alum sativum* (24.82 % and 10.15 % respectively) and rhizome of *Zingiber officinale* (7.59 % and 7.52 % respectively) (Lawal *et al.,* 2018). However, the seeds of *P. nitida* are rich in protein when compared to *Garcina kola* (3.19 %), and *Persea americana* seeds (2.17 %) (Omeh *et al.,* 2014; Umeaku *et al.,* 2018).The crude protein of *P. nitida* recorded in this study is higher than value (10.20 %) reported by Adebowale *et al.,* (2012). The variation in composition may be as a result of variation in soil nutrient, environmental factors, age of plant at harvest, geographic location, diurnal and seasonal variations, method of cultivation, time of harvesting and extraction procedure (Mgbeje, *et al*., 2019). The moisture contents of *P. nitida* seeds in the present study are higher than that of earlier reports of 3.73 % (Adebowale *et al.,* 2012), 1.2 % (Bruce, *et al*., 2016); 3.0 % (Osuala *et al.,* 2018).

The sporadic incidence of drug resistance or low efficacy, toxicity and the risk of drug residues in animal products has led to a renewed interest in the use of plant-based drugs (Zenebe, *et al*., 2017). In this study, daily decrease in faecal egg output of *H. bakeri* in infected animals treated with plant extracts when compared with the untreated is an indication that *G. latifolium* and *P. nitida* have anthelmintic activity. The anthelmintic activity could be attributed to the bioactive substances in these plants. The anthelmintic activity of *G. latifolium* could be due to high concentration of saponin and alkaloid in this plant as recorded in the study. Studies have linked anthelmintic activity of some plants to presence of alkaloids and saponin (Simelane *et al.,* 2010; D'Addabbo *et al*., 2011; Simon, *et al*., 2012; Maestrini *et al.,* 2019). Similarly, saponins and alkaloids could have contributed to the anthelmintic potential of the extracts since the saponin causes changes in cell wall permeability which could induce cell death by inhibiting proteins while digestion of the alkaloid could hydrolyze the compound (Daniel *et al.,* 2013).

On the other hand, higher anthelmintic efficacy of *P. nitida* could be due to high concentration of alkaloids and polyphenol compounds as revealed in the phytochemical screening in this study. Alkaloids are known to act on central nervous system and caused paralysis of the worms by suppressing the transportation of glucose from stomach to small intestine (Jain *et al.,* 2013). Akkari *et al.,* (2016a; 2016b) has related the anthelmintic activity of some plants to their polyphenolic content. Polyphenolic compounds are known to interfere with enzymes secreted or excreted by the worms in the local environment, or with enzymes involved in metabolic pathways that are essential for nematode functions. They also inhibit parasite growth by causing apoptotic cell death, leading to the reduction of parasite and egg burden (Athanasiadou *et al*., 2001; Adedapo *et al.,* 2005). The variation in activity of the plant extracts of *G. latifolium* and *P. nitida* at different dosages might be due to differ-

ence in the proportion of the active components responsible for the anthelmintic activity in the plant extract.

The reduction in the worm burden of animals treated with extract of *G. latifolium* when compared with the untreated could be due to alkaloid, as treatment of rats with alkaloids rich solvent-partitioned extracts of *Afzelia africana* gave reduction of worm count (Simon, *et al*., 2012). However, the percentage deparasitisation of 50 % or more observed in the extract of *P. nitida* showed that the extract at different doses were effective against *H. bakeri* in mice. An anthelmintic is considered effective only when it can reduce 50 % or more of the worm burden in an infected animal (Suleiman *et al.,* 2005).

Biochemical parameters are good indicators of the health assessment for both animals and humans. The cholesterol level was elevated in infected mice compared with uninfected mice. This is in consonance with findings of Zaman *et al.,* (2018) who reported elevated cholesterol in *Ascaris* infected patients. The higher cholesterol, triglyceride and HDL level in the infected treated mice may be attributed to the extensive synthesis of bile acid from cholesterol in the liver. The increased output of epinephrine and corticosteroid could be responsible for elevation of serum cholesterol (Atasoy *et al.,* 2015). The glucose reduction in mice treated with single extract of *P. nitida* may be attributed to depression in voluntary feed intake thereby leading to decrease in blood glucose. However, the higher glucose level observed in mice treated with *G. latifolium* when compared to untreated mice could be due to abundance of zinc and iron in the plant extract and these minerals stimulate appetite. Studies have shown that supplementation of zinc and iron increase the eating frequency (Kusumastuti, *et al*., 2018). Similarly, total protein content and albumin reduced in the infected animals compared with uninfected mice. This is in agreement with other studies that reported low level of serum proteins during parasitic infection (Solanki *et al.,* 2017). This may be attributed to increased plasma leakage through the injured gut caused by the parasites and albumin loss could be due their smaller size and osmotic sensitivity to fluid movement (Radostits *et al.,* 2007; Ebrahim, 2018).

However, the total protein content reduced in animals infected but treated with single extract of *P. nitida* and *G. latifolium*. This implies that extracts of *P. nitida* and *G. latifolium* could not prevent hypoproteinemia in the animals. Hypoproteinemia might occur due to increased motility of intestine which makes the proteins to get lost from the bowel (Deka & Borah, 2008).

The histopathology of the intestine of mice treated with extract of *P. nitida* at 500mg/kg showed moderate villi atrophy when compared to *G. latifolium* at the same dosage. This could be due to bioactive substances in the plant as the highest chemo suppression and deparasitisation rate was observed when 500mg/kg of *P. nitida* was administered. This is also suggests that *P. nitida* could prevent cryptal hyperplasia in the mice and improve the pathology induced by the parasite. The intestine of mice that received 500mg/kg of *G. latifolium* showed moderate villi atrophy and cryptal hyperplasia. This could be due to higher worm burden recorded in mice treated with *G. latifolium* compared to *P. nitida* which prevents proliferation of new absorptive cells at Crypt of Lieberkühn in the intestinal mucosa. Furthermore, severe villi atrophy and intra-luminal parasites were observed in the intestine of the untreated mice when compared to intestine of mice that received extracts of *G. latifolium* and *P. nitida.* This suggests that the extracts of *G. latifolium* and *P. nitida* could ameliorate the severity of damage caused by parasite in the mice.

Conclusion

The study showed that extracts of *P. nitida* and *G. latifolium* had anthelmintic activity that was dose-dependent, and this may offer potential drug lead for the development of safe, effective and affordable anthelmintics. The preliminary phytochemical screening of *P. nitida* and *G. latifolium* showed that these plants contain flavonoids, polyphenols, alkaloids, saponins and tannins which might be responsible for the anthelmintic activity of the plant.

Conflict of Interest

The authors declare that they have no conflict of interest

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