ANTHELMINTIC ACTIVITIES OF *POLYALTHIA LONGIFOLIA* LEAF AND STEM BARK EXTRACTS IN *HELIGMOSIMOIDES BAKERI* INFECTED MICE

[1]LOUIS, Vera Chisara, [1]OKOLIE, Nnaemeka Jireh Cosmas, [1]AMAH, Henry Chidozie, [2]UROKO, Robert Ikechukwu, [3]AMADI, Anthonia Nnenna Chris, [4]MADUBUIKE, Kelechi Gideon, [2]IJIOMA, Solomon Nnah

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Imo State, Nigeria.

[2]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[3]Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[4]Department of Veterinary Physiology and Pharmacology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Corresponding Author: Ijioma, S. N. Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. **Email:** ijiomasolo@yahoo.co.uk **Phone:** +234 8062395552

Received *May 18, 2021;* ***Revised*** *June 30, 2021;* ***Accepted*** *July 23, 2021*

ABSTRACT

Polyalthia longifolia is used traditionally to manage intestinal worm infections. In this study, the anthelmintic activities of the leaf and stem bark extracts were evaluated in Heligmosimoides bakeri infected mice. Extracts were first subjected to phytochemical and acute toxicity (LD_{50}) tests. For the anthelmintic study, in vivo and in vitro models were adopted. In the in vivo study, groups 3 – 6 of infected mice were assigned specific extract treatments, while groups 1, 2 and 7 were the normal, negative and standard (albendazole treated) groups respectively. Egg count was determined every 3 days during treatment. In the in vitro model, the extracts were applied to the worms in Petri dishes before larvae counts. Results obtained showed the presence of significant amounts of alkaloids, flavonoids, tannins, steroids, terpenes, saponins, cardiac glycosides and phenols in both extracts with LD_{50} values >5000 mg/kg body weight for both extracts. Result of the in vivo anthelmintic study showed significant fall in egg/larval count in all groups treated with the extracts ($p<0.05$), as 400 and 800 mg/kg of the leaf extract lowered egg count to 0.60 ± 0.24 and 0.20 ± 0.20 respectively while same doses of the stem bark extract lowered egg count to 0.40 ± 0.24 and 0.20 ± 0.20 respectively, when compared with the negative control group which had a count of 297.80 ± 13.18. Results obtained in the in vitro model also indicated significant vermicidal effect for both extracts. We therefore conclude that P. longifolia may be potential sources of vermicidal agent.

Keywords: *Heligmosimoides bakeri*, *Polyalthia longifolia*, Roundworm, Helminthiasis

INTRODUCTION

Intestinal worm infection (helminthiasis) currently contributes greatly to the world's disease burden, with about one billion people said to be infected in 2014, especially in the poorly developed and developing countries (WHO, 2020). Varying health challenges including anaemia, malnutrition, eosinophilia, and pneumonia have also been associated with

helminthic infections (WHO, 2020). Out of the over 350 known species of intestinal worms; roundworm, whipworm, hookworm, thread worm and tapeworm appear to be the main agents of soil-transmitted helminthiasis and have the highest prevalence rates in developing regions of Asia, Africa and Latin America (Hotez *et al*., 2008). *Heligmosimoides bakeri* is a roundworm which affects millions of people globally, especially children of all ages and have over the years been identified as major precipitator of impaired growth and development in children (Bethony *et al*., 2006; Roepstorff *et al*., 2011; Chanda *et al*., 2012). Complications due to roundworm infection have always resulted from the migration of roundworm to the biliary tract (Sanai and Al-Karawi, 2007), bronchus (Prakash *et al*., 2014) or appendix (Engin *et al*., 2010).

Although significant progress has been made in terms of drug interventions for the control of intestinal worms, desired outcomes are yet to be achieved for reasons like the high cost of these drugs and global poverty indices, especially across underdeveloped and the developing nations (Chanda *et al*., 2012), hence, the need for an effective, cheap and readily available alternative. Today, it is a common practice across all countries of the world to use medicinal plants as alternative treatment agents. In fact, it is now fully established that over 80 % of the world's population is relying on herbal medicine (Ekor, 2014). This renewed interest in plant based medicines is also supported by the fact that the development of numerous orthodox medicines usually begins with findings from medicinal plant research (Yuan *et al*., 2016). The side effects usually associated with the use of current orthodox antihelminthic agents also speak in favour of the on-going search for a cheap and effective natural alternative (Mali and Mehta, 2008). It is now well known that in developing countries of the world, plants and plant derived agents are currently used to treat parasitic infections, including intestinal worms (Sofowora, 1993a).

Polyalthia longifolia (Sonn.) is one of the many indigenous plants believed to be of value in the treatment of intestinal worms infections (Kirtikar and Basu, 1995). *P. longifolia* belongs to family *Annonacae* and has been used in many countries as a horticultural and ornamental commodity and for the alleviation of noise pollution (Chanda *et al*., 2011). Wood from the tree has also been employed in the manufacturing of small articles like pencils, boxes, and matchsticks, while the leaves are mainly used for ornamental decoration. Decoctions from the leaves are used traditionally to kill and expel worms from the gastrointestinal tract (Kirtikar and Basu, 1995; Chanda *et al*., 2012). Other traditional uses of the plant include; treatment of fever, skin disorder, diabetes, hypertension, cold and cough (Rahmatullah *et al*., 2010; Chanda *et al*., 2011).

H. bakeri is a known parasitic helminth seen mainly in the duodenum and small intestine of wood mice as well as other rodents (Cable *et al*., 2000). This study was therefore designed to evaluate the antihelminthic effects of *P. longifolia* in *H. bakeri* infected mice.

MATERIALS AND METHODS

Collection of Plant Materials: Fresh leaves and stem bark of *P. longifolia* (Figure 1) were collected from a horticulture farm in Aba, Osisioma Ngwa LGA of Abia State, Nigeria.

Figure 1: *Polyalthia longifolia* plant, photographed from a home in Aba, Abia State, Nigeria

The plant sample was identified using a field guide by Hawthorne and Jongkind (2006), and a voucher number MOUAU/FOREM/21/008 was assigned to the specimen sample which was deposited in the herbarium of the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara

University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of Extracts: The collected leaves and stem bark were dried under shade for 21 days on a laboratory bench and were pulverized separately into powder using an electric blender (Model CB-82314, Century Electrical Products Limited, China). The method of extraction used by Abah and Egwari (2011) was adopted for the extraction. Briefly, for each plant material and round of extraction, 80 g of the grounded sample was introduced into the extraction chamber of the soxhlet extractor and ethanol was used as the extraction solvent. The extraction temperature was maintained at 65^{o}C through-out the period (48 hours) of extraction. Thereafter, the extract in solution was concentrated in a hot air oven at low temperature (40^{o}C), to obtain for the leaf, a dark green extract which weighed 9.30 g and represented a yield of 11.63 %. For the stem bark, a yellow brown extract which weighed 5.93 g was obtained and represented a yield of 7.41 %. The extracts were preserved in the refrigerator until needed and are hereafter referred to as *P. longifolia* leaf extract (PLLE) and *P. longifolia* stem bark extract (PLSE).

Animals: A total of seventy-two (72) adult albino mice were used for the study. Fourty-two (42) were used for LD_{50}, determination of the extracts while the other thirty (30) were used for the antihelmintic study. The mice were obtained from the Animal House of the College of Veterinary Sciences, Michael Okpara University of Agriculture Umudike and were transferred in well-ventilated aluminum cages to the Animal House of the Department of Zoology and Environmental Biology (of the same University) where the study was carried out. The animals were allowed access to food (Vita Feed Finisher containing 18 % crude protein and 3000 kcal/kg metabolizable energy) and water *ad libitum* and to acclimatize for a period of two weeks before use. All animal experiments were carried out in accordance with international guidelines for care and use of laboratory animals (Kirkland, 1998).

Acute Toxicity Assay: The Lorke's method of acute toxicity testing involving 2 stages of test was adopted (Lorke, 1983). Briefly, in the first phase, 9 albino mice were divided into 3 groups of 3 mice each and were administered 10, 100 and 1000 mg/kg of extract respectively. With no mortality observed, the study proceeded to the second phase involving the use of another set of 9 mice assigned to 3 groups with groups 1, 2 and 3 treated with 1600, 2900 and 5000 mg/kg of the extract respectively. The various groups were observed for mortalities within 24 hours to 7 days. With zero mortality still recorded, the highest dose (5000 mg/kg) was repeated on another set of 3 mice as a confirmatory test. These procedures were carried out separately on both the leaf and stem bark extracts. LD_{50} value for each extract was determined using Lorke's formula: $LD_{50} = \sqrt{AxB}$, where A - maximum dose that did not produce mortality and B - minimum dose that produce 100 % mortality in a group. These procedures were carried out separately on both the leaf and stem bark extracts.

Quantitative Phytochemical Assay: Phytochemical analysis of the leaf extract was carried out qualitatively and quantitatively in accordance with standard procedures (Harborne, 1973; Sofowora, 1993b; Trease and Evans, 2002).

Preparation of Helminth Material and Infection of the Mice: A mouse adapted strain of *H. bakeri* larva (3rd stage larva) obtained from the Department of Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike was used. The droppings of mice infected with the parasites were collected in water for 4 hours before decanting excess water. The faeces crushed in a glass mortar was transferred into plastic containers in lots of 5.0 g and shaken with glass beads before being mixed with 1litre of water and strained through several layers of gauze. The filtrate was transferred to centrifuge tubes and centrifuged at 2000 rpm. The supernatant solution was then discarded and the sediment mixed with vermiculite in labelled plastic petri dishes and incubated at 4°C for 7 to 14 days. The infective 3rd stage larvae (L_3) were

recovered from 7 to 14 days old vermiculite faecal cultures using modified Baermann's method (Baermann, 1917). The third stage (L_3) obtained were washed several times with distilled water and their number determined by dilution counting. The volume was adjusted to give 200 L_3 in 0.2 ml. The mice were infected orally with the parasites (*H. bakeri* larva) by a single oral administration of 0.1 ml of the already prepared helminth parasite.

***In vivo* Antihelmintic Activities of PLLE and PLSE:** These were carried out in stages which included preparation of helminth material, infection of the mice, treatment and parasite/egg load counts. The establishment of infection happened over a period of 13 days and infection was confirmed via parasitic egg counts across the groups. The experimental mice were grouped and treated thus: Group 1: Normal uninfected mice, Group 2: Infected mice only with no treatment, Group 3: Infected mice treated with 400 mg/kg of leaf extract (PLLE), Group 4: Infected mice treated with 800 mg/kg of leaf extract (PLLE), Group 5: Infected mice treated with 400 mg/kg of stem bark extract (PLSE) and Group 6: Infected mice treated with 800 mg/kg of stem bark extract (PLSE). Treatments were oral and daily while samples were collected every 3 days for egg/parasite counts to access the effectiveness of treatment.

Parasite Egg Counts: Parasitic load/egg count was carried out on 1 g of faeces collected from each group on the 13th day following infection of the animals and every 3 days from day of commencement of treatment. The helminth eggs that were recovered were examined qualitatively by flotation using saturated NaCl solution and egg output per gram of faeces were calculated. For each sample, two grids of a McMaster slide were counted, the average taken and was used as the eggs per gram of faeces for the parasite (Annan *et al*., 2015). The percentage fall in faecal egg count fall was calculated using this formula: % Fall = Post induction egg count – Egg count at the end of treatment / Post induction egg count x 100.

***In vitro* Antihelmintic Activities of PLLE and PLSE:** The method used by Gogoi and Yadav (2016) was adopted with slight modifications. Six Petri dishes were set up and labeled A – F according to the treatments. Petri dish A serve as control while Petri dishes B – F serve as the test groups treated with the extracts and standard drugs. One millilitre of the solution containing the parasites was introduced into each of Petri dishes (B – F) containing 9 ml of normal saline and mixed appropriately.
An initial worm count was carried out to ascertain the concentration of worms in each Petri dish before treatment according to the order below: Petri dish A (no treatment), Petri dish B (2 drops of 400 mg/ml PLLE), Petri dish C (2 drops of 800 mg/ml PLLE), Petri dish D (2 drops of 400 mg/ml PLSE), Petri dish E(2 drops of 800 mg/ml PLSE) and Petri dish F (2 drops of 8 mg/ml of abendazole). Each treatment was replicated thrice and allowed to stand for 24 hours. Live and dead worm count was done at the end of one hour and at 24 hours.

Statistical Analysis: Analyses were performed using one way analysis of variance (ANOVA) and P values less than 0.05 ($p<0.05$) for test versus control were considered statistically significant. The means were compared using Duncan's multiple comparison tests, while paired sample t-test was used to compare the phytochemical constituents of the two plant extract. The results were presented as mean ± standard error of mean (SEM).

RESULTS

Phytochemical Composition of PLLE and PLSE: Quantitative phytochemical test carried out on PLLE and PLSE extracts revealed the presence of terpenoids, tannins, alkaloids, flavonoids, cardiac glycosides, steroids, saponins and phenolic compounds as major secondary metabolites. When quantified, these phytochemical agents were found to be in higher amounts in the stem bark extract (PLSE) than the leaf extract (PLLE) (Table 1).

Table 1: Quantitative compositions of *Polyalthia longifolia* leaf and stem bark extracts

Parameters	Leaf Extract (PLLE)	Stem bark Extract (PLSE)
Alkaloids (mg/100g)	8.23 ± 0.14^{g}	8.68 ± 0.10^{h}
Cardiac glycosides (mg/100g)	0.72 ± 0.01^{a}	0.80 ± 0.02^{b*}
Flavonoids (mg/100g)	6.36 ± 0.07^{f}	6.75 ± 0.15^{f}
Phenolic compounds (mg/100g)	1.25 ± 0.05^{c}	1.45 ± 0.02^{c*}
Saponins (mg/100g)	6.76 ± 0.11^{f}	7.24 ± 0.13^{g*}
Steroids (mg/100g)	0.84 ± 0.04^{b*}	0.72 ± 0.05^{a}
Tannins (mg/100g)	2.43 ± 0.07^{d}	3.54 ± 0.06^{e*}
Terpenes (mg/100g)	3.80 ± 0.06^{e*}	2.23 ± 0.13^{d}

Means on the same column with different letter superscripts are significantly different ($p<0.05$), mean on the same row with asterisk is significantly different ($p<0.05$), PLLE = Polyalthia longifolia leaf extract while PLSE = Polyalthia longifolia stem bark extract

Acute Toxicity (LD_{50}) of PLLE and PLSE: The administration of single oral doses of *P. longifolia* leaf and stem bark extracts produced no mortality at all doses within the 24 hour of acute toxicity evaluation and a further 7 days, even at the highest dose of 5000 mg/kg body weight. The animals instead remained active, healthy and had normal dispositions and did not present any apparent symptoms of toxicity. Hence, the acute toxicity values of both extract were put at a value of $\geq$ 5000 mg/kg body weight.

***In vivo* Antihelminthic Activities of PLLE and PLSE:** Treatment with both PLLE and PLSE significantly lowered ($p<0.05$) egg and larval counts in the test animals when compared with control and compared favorably ($p>0.05$) with the standard drug used. At the end of the treatment period, egg count in the control group was 0.00 ± 0.00 while in the groups treated with 400 and 800 mg/kg of PLLE egg counts were 0.60 ± 0.20 and 0.20 ± 0.20 respectively.

For the groups treated with 400 and 800 mg/kg of PLSE, the counts fell from 99.20 ± 4.26 and 77.60 ± 6.31 on day 0 to 0.40 ± 0.24 and 0.20 ± 0.20 by the end of treatment (Table 2). The relative activities of the treatment agents were also compared based on percentage of fall in egg count by the end of treatment but no significant difference ($p>0.05$) was found between the activities of the leaf and stem extracts. However, steady rise in egg count was observed in the untreated group 2 (Table 3).

***In vitro* Antihelminthic Activities of the Extracts:** Results of *in vitro* antihelminthic study showed that both PLLE and PLSE exhibited significant antihelmintic ($p<0.05$) activity when compared with control, such that by the end of 24 hours, percentage fall in worm count was 43.76 ± 2.37 %, while Petri dishes B and C treated with 400 and 800 mg/kg of PLLE had 99.34 ± 0.33 % and 100.00 ± 0.00 % fall in worm count respectively. Petri dishes D and E treated with 400 and 800 mg/kg had 99.59 ± 0.41 % and 100.00 ± 0.00 % fall respectively (Table 4).

DISCUSSION

The presence of secondary metabolites like flavonoids, steroids, terpenes, alkaloids, saponins, tannins and cardiac glycoside in significant proportions in both leaf and stem bark extracts of *P. longifolia* is indicative of the potential medicinal values of the plant. Phytochemical agents in plant materials are known to be responsible for the bioactivities such plants' parts and usually define their usefulness in the management of diseases (Mendoza and Silva, 2018). The specific activities of flavoniods, alkaloids, tannins, steroids, terpenes, saponins and glycosides are well documented (Kumar *etal.*, 2013). Findings in this study therefore corroborate with available data on the healing potentials of *P. longifolia*.

Zero mortality observed following acute toxicity (LD_{50}) evaluation of leaf and stem bark extracts of *P. longifolia,* even at 5000 mg/kg suggests that *P. longifolia* leaves and stem bark may be completely free from any form of acute toxicity when consumed and could be well tolerated at low to moderate doses, agreeing with the OECD guideline for acute toxicity study which stipulates that mortality is the expected

Table 2: Egg counts with time following treatment with *Polyalthia longifolia* leaf and stem bark extracts

Groups	Treatment	Day 0	Day 3	Day 6	Day 9	Day 12
1	Normal control	0.00 ± 0.00^{1a}	0.00 ± 0.00^{1a}	0.00 ± 0.00^{1a}	0.00 ± 0.00^{1a}	0.00 ± 0.00^{1a}
2	Negative control	140.40 ± 4.77^{4a}	188.00 ± 24.75^{2b}	210.20 ± 7.91^{3b}	270.40 ± 11.07^{2c}	297.80 ± 13.18^{2d}
3	400 mg/kg PLLE	137.80 ± 3.28^{2d}	14.20 ± 1.28^{1c}	6.80 ± 1.02^{2b}	1.20 ± 0.37^{1a}	0.60 ± 0.24^{1a}
4	800 mg/kg PLLE	80.80 ± 3.76^{2d}	10.80 ± 1.02^{1c}	6.20 ± 0.66^{2b}	0.60 ± 0.24^{1a}	0.20 ± 0.20^{1a}
5	400 mg/kg PLSE	99.20 ± 4.26^{3d}	7.60 ± 1.33^{1c}	$4.40 \pm 0.75^{1,2b,c}$	$1.40 \pm 0.40^{1a,b}$	0.40 ± 0.24^{1a}
6	800 mg/kg PLSE	77.60 ± 6.31^{2b}	5.60 ± 0.93^{1a}	$2.80 \pm 0.86^{1,2a}$	0.40 ± 0.24^{1a}	0.20 ± 0.20^{1a}
7	50 mg/kg Albendazole	277.80 ± 10.12^{5b}	6.60 ± 0.87^{1a}	$1.00 \pm 0.45^{1,2a}$	0.20 ± 0.20^{1a}	0.00 ± 0.00^{1a}

Means on the same row with different letter superscripts are significantly different ($p<0.05$), Means on the same column with different number superscripts are significantly different ($p<0.05$), PLLE = Polyalthia longifolia leaf extract while PLSE = Polyalthia longifolia stem bark extract

Table 3: Percentage fall in egg counts across the groups by the end of treatment with *Polyalthia longifolia* leaf and stem bark extracts

Groups	Treatments	% Fall in egg count
1	Normal control	0.00 ± 0.00^{a}
2	Negative control	110.08 ± 12.26^{c}
3	400 mg/kg PLLE	99.52 ± 0.20^{b}
4	800 mg/kg PLLE	99.74 ± 0.26^{b}
5	400 mg/kg PLSE	99.60 ± 0.25^{b}
6	800 mg/kg PLSE	99.68 ± 0.32^{b}
7	50 mg/kg Albendazole	100.00 ± 0.00^{b}

Means on the same column with different superscripts are significantly different ($p<0.05$), PLLE = Polyalthia longifolia leaf extract while PLSE = Polyalthia longifolia stem bark extract

Table 4: *In vitro* antihelminthic activities of *Polyalthia longifolia* leaf and stem bark extracts

Petri dish	Treatments	Pre-treatment worm count	1 hour post treatment	24 hours post treatment	% fall in worm count
A	Normal control	135.67 ± 2.91^{1c}	125.33 ± 5.84^{3b}	76.33 ± 3.84^{2a}	43.76 ± 2.37^{1}
B	400 mg/kg PLLE	107.67 ± 6.69^{2c}	12.33 ± 0.88^{2b}	0.67 ± 0.33^{1a}	99.34 ± 0.33^{2}
C	800 mg/kg PLLE	93.00 ± 2.31^{1c}	6.33 ± 1.76^{1b}	0.00 ± 0.00^{1a}	100.00 ± 0.00^{2}
D	400 mg/kg PLSE	86.00 ± 3.61^{1c}	4.67 ± 0.88^{1b}	0.33 ± 0.33^{1a}	99.59 ± 0.41^{2}
E	800 mg/kg PLSE	84.00 ± 8.19^{1c}	3.00 ± 0.58^{1b}	0.00 ± 0.00^{1a}	100.00 ± 0.00^{2}
F	50 mg/kg Albendazole	92.00 ± 5.13^{1c}	6.33 ± 0.88^{1b}	0.00 ± 0.00^{1a}	100.00 ± 0.00^{2}

Means on the same row with different letter superscripts are significantly different ($p<0.05$), Means on the same column with different number superscripts are significantly different ($p<0.05$), PLLE = Polyalthia longifolia leaf extract while PLSE = Polyalthia longifolia stem bark extract

end result of acute toxicity evaluation and that where no such is observed, then the agent been tested may be considered safe for oral use (OECD, 2008). Toxicity signs including mortality of animals following toxicity tests for plant extracts have been linked to the consumption/administration of intolerable amounts of bioactive substances in plants

(Akomas *et al*., 2014) but zero mortality in the case of *P. longifolia* suggests that the plant is safe for oral acute use. In this study, the observed antihelminthic effects of *P. longifolia* leaf and stem bark extracts following *in vivo* and *in vitro* trials suggest that the extracts may contain active ingredients with antihelminthic properties. Phenolic compounds in plants reportedly accounts for the antihelminthic effects of plants based treatment agents (Udoha *et al*., 2015) and findings in this study reveal how amount of substance in *P. longifolia*. The observed antihelminthic activities of both extracts may also be due to their alkaloids and tannins contents. These phytochemical agents have been found to exhibit significant anthelminthic activities following in vitro experimental trials (Pratap *et al*., 2018). There is also documented evidence that alkaloids act specifically on the central nervous system of helminths to cause their paralysis. Tannins are also known to cause death of helminthes by interfering with their energy generation pathway and uncoupling oxidative phosphorylation in the worms. These biochemical and physiological changes in worm may cause damage to its mucopolysaccharide membrane, exposing its outer layer to chemical attacks and causing its death (Jain *et al*., 2011; Pratap *et al*., 2018). Also, each extract, like albendazole, may have been able to inhibit eggs hatching and larval development in the worms due to its ability to inhibit the formation and development of vital structures in the parasites and by that also inhibit the polymerization of the parasitic transformation from tubulin into microtubules. High affinity of albendazole to the tubulin inhibits cytoplasmic microtubules development in worms and prevents the movement of glucose into the larval and adult stages of the worms. This inactivates the worms and leads to their death (Udoha *et al*., 2015). The fact that the extracts compared favourably with albendazole suggests that extracts from *P. longifolia* leaves and stem bark may be good alternative agents for the treatment of intestinal worms and may be used as such to avoid side effects like fever, nausea, abdominal pain, vomiting and headache usually associated with the use of orthodox agents like albendazole (Udoha *et al*., 2015; Ferreira *et al*., 2013).

Conclusion: Findings from this study have shown that *P. longifolia* leaves and stem bark are potential sources of anthelminthic agents having shown significant positive effects in *H. bakeri* infected mice following *in vivo* and *in vitro* trials. These effects of the extracts may be due to their alkaloids, tannins and phenolic compounds contents. The extracts may therefore be of value in the management of intestinal worms affecting man and other animals and may be used as such. Further studies may be carried out to fully establish these findings.

ACKNOWLEDGEMENTS

Authors wish to acknowledge and appreciate the Head of Department, Zoology and Environmental Biology, Dr. N. Ukpai, for allowing them access to the laboratory facilities and equipment used for the study. The authors also appreciate the laboratory technologists for their various roles during the study.

REFERENCES

ABAH, S. E. and EGWARI, L. O. (2011). Methods of extraction and antimicrobial susceptibility testing of plant extracts. *African Journal of Basic and Applied Sciences*, 3(5): 205 – 209.

AKOMAS, S. C., OKAFOR, A. I. and IJIOMA, S. N. (2014). Hypoglycemic, hematologic and hypolipidemic activity of *Mucuna pruriens* ethanol leaf extract in alloxan induced diabetic rats. *Annual Research and Review in Biology*, 4(24): 4284 – 4292.

ANNAN, K., EKUADZI, E., ASARE, C., SARPONG, K., PISTORIUS, D., OBERER, L., GYAN, B. A. and OFORI, M. (2015). Antiplasmodial constituents from the stem bark of *Polyalthia longifolia* var *pendula*. *Phytochemistry Letters*, 11: 28 – 31.

BAERMANN, G. (1917). Eine einfache methode zur auffindung von Ancylostomum

(Nematoden) larven in erdproben. *Geneeskundig Tijdschrift Voor Nederlandsch-Indie,* 57: 131 – 137.

BETHONY, J., BROOKER, S., ALBONICO, M., GEIGER, S. M., LOUKAS, A., DIEMERT, D. and HOTEZ, P. J. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet*, 367(9521): 1521 – 1532.

CABLE, J., HARRIS, P. D., LEWIS, J. W. and BEHNKE, J. M. (2006). Molecular evidence that *Heligmosomoides polygyrus* from laboratory mice and wood mice are separate species. *Parasitology*, 133: 111 – 122.

CHANDA, S., BARAVALIA, Y. and KANERIA, M. (2011). Protective effect of *Polyalthia longifolia* var. *pendula* leaves on ethanol and ethanol/HCl induced ulcer in rats and its antimicrobial potency. *Asian Pacific Journal of Tropical Medicine*, 4(9): 673 – 679.

CHANDA, S., DAVE, R., KANERIA, M. and SHUKLA, V. (2012). Acute oral toxicity of *Polyalthia longifolia* var. *pendula* leaf extract in Wistar albino rats. *Pharmaceutical Biology*, 50(11): 1408 – 1415.

EKOR, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4: 177. https://dx.doi.org/10.3389%2Ffphar.2013.00177

ENGIN, O., CALIK, S., CALIK, B., YILDIRIM, M. and COSKUN, G. (2010). Parasitic appendicitis from past to present in Turkey. *Iranian Journal of Parasitology*, 5(3): 57 – 63.

FERREIRA, L. E., CASTRO, P. M. N., CHAGAS, A. C. S., FRANÇA, S. C. and BELEBONI, R. O. (2013). In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. *Experimental Parasitology*, 134(3): 327 – 332.

GOGOI, S. and YADAV, A. K. (2016). In vitro and in vivo anthelmintic effects of *Caesalpinia bonducella* (L.) Roxb. leaf extract on *Hymenolepis diminuta* (Cestoda) and *Syphacia obvelata* (Nematoda). *Journal of Intercultural Ethnopharmacology*, 5(4): 427 – 433.

HARBORNE, J. B. (1973). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall, London.

HAWTHORNE, W. D. and JONGKIND, C. C. (2006). *Woody Plants of Western African Forests, A Guide to the Forest Trees, Shrubs and Lianes from Senegal to Ghana*. Royal Botanic Gardens, Kew.

HOTEZ, P. J., BRINDLEY, P. J., BETHONY, J. M., KING, C. H., PEARCE, E. J. and JACOBSON, J. (2008). Helminth infections: the great neglected tropical diseases. *Journal of Clinical Investigation,* 118(4): 1311 – 1321.

JAIN, P., SINGH, S., SINGH, S. K., VERMA, S. K., KHARYA, M. D. and SOLANKI, S. (2013). Anthelmintic potential of herbal drugs. *International Journal of Research and Development in Pharmacy and Life Sciences*, 2(3): 412 – 427.

KIRKLAND, G. L. (1998). Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy*, 79(4): 1416 – 1431.

KIRTIKAR, K. R. and BASU, B. D. (1995). *Indian Medicinal Plants.* International Book Distributors, Dehradun, India.

KUMAR, M., GAUTAM, M. K., SINGH, A. and GOEL, R. K. (2013). Healing effects of *Musa sapientum* var*. paradisiaca* in diabetic rats with co-occurring gastric ulcer: cytokines and growth factor by PCR amplification. *BMC Complementary and Alternative Medicine*, 13: 305. https://doi.org/10.1186/1472-6882-13-305

LORKE, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4): 275 – 287.

MALI, R. G. and MEHTA, A. A. (2008).A review on anthelmintic plants. *Natural Product Radiance*, 7(5):466 – 475.

MENDOZA, N. and SILVA, E. M. E. (2018). Introduction to phytochemicals: secondary metabolites from plants with active

principles for pharmacological importance. Pages 11 – 28. *In*: ASAO, T. and ASADUZZAMAN, M. (Eds.). *Phytochemicals: Source of Antioxidants and Role in Disease Prevention*. IntechOpen, London. https://dx.doi.org/10.5772/intechopen.78226

OECD (2008).*Acute Oral Toxicity - Up-and-Down-Procedure (UDP)*. Guidelines for the Testing of Chemicals (OECD 425), Organisation for Economic Co-operation and Development, Paris, France.

PRAKASH, S., NARAYANAN SITALAKSHMI, J. S., DAYAL, M. and GOGIA, A. R. (2014). Ascaris: an unusual cause of airway obstruction during general anesthesia with ProSeal laryngeal mask airway. *Journal of Anaesthesiology, Clinical Pharmacology*, 30(2): 298 – 300.

PRATAP CHANDRAN, R., DEEPAK, V., KRISHNA, S., FATHIMA, S., AMEENA, T. and RAJ, J. (2018). Allelopathic activity of leaf extracts of *Mimosa pudica* on growth parameters of *Brassica juncea* seeds. *BAOJ Biotechnology*, 4(1): 1 – 7.

RAHMATULLAH, M., KHATUN, M. A., MORSHED, N., NEOGI, P. K., KHAN, S. U. A., HOSSAN, M. S., MAHAL, M. J. and JAHAN, R. (2010). A randomized survey of medicinal plants used by folk medicinal healers of Sylhet Division, Bangladesh. *Advances in Natural and Applied Sciences*, 4(1): 52 – 62.

ROEPSTORFF, A., MEJER, H., NEJSUM, P. and THAMSBORG, S. M. (2011). Helminth parasites in pigs: new challenges in pig production and current research highlights. *Veterinary Parasitology*, 180 (1-2): 72 – 81.

SANAI, F. M. and AL-KARAWI, M. A. (2007). Biliary ascariasis: report of a complicated case and literature review. *Saudi Journal of Gastroenterology*, 13(1): 25 – 32.

SOFOWORA, A. (1993a). Recent trends in research into African medicinal plants. *Journal of Ethnopharmacology*, 38(2-3): 197 – 208.

SOFOWORA, A. (1993b). *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edition, Spectrum Books Limited, Ibadan, Nigeria.

TREASE, G. E. and EVANS, W. C. (2002). *Pharmacognosy.* 15th Edition, Saunders, Edinburgh.

UDOHA, M. U., OKOLIE, N. J. and IJIOMA, S. N. (2015). Antihelmintic activity of *Ocimum gratissimum* leaf extract in *Heligmosomoides bakeri* infested experimental mice. *Annals of Biological Sciences,* 3(4): 1 – 7.

WHO (2020). *Soil-Transmitted Helminth Infections*. World Health Organization, Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections Accessed 28th April, 2021.

YUAN, H., MA, Q., YE, L. and PIAO, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules (Basel, Switzerland)*, 21(5): 559. https://doi.org/10.3390/molecules21050559