In-Vitro Studies on Nauclea Latifolia (Uvuru-Ilu) for Vermicidal Activities in Heligmosomodies Bakeri Infected Mice.

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ABSTRACT

Nauclea latifolia has been used locally as herbal remedies for treatment of helminthic infections and other some other ill conditions. In this study, the phytochemical profile and the vermicidal activities of *N. latifolia* leaf extracts, Stem bark extracts and combination of the two extracts were evaluated in *Heligmosomoides bakeri* infected mice. Extracts prepared from the plant were subjected to phytochemical and acute toxicity evaluations. For the vermicidal trials, in-vitro study model were employed. The in-vitro study was carried out by applying the extracts to the worms in Petri dishes before larval counts in accordance with standard protocols. Results obtained from the phytochemical tests on the leaves and stem extracts revealed the presence of significant amounts of Saponins, Flavonoids, Terpenoids, Tannins, Alkaloids, Phenolics and Cardiac glycosides. Steroids were obtained from the stem bark extract. Acute toxicity of 3,807.87mg/kg and 2,828.43mg/kg body weight were obtained for the leaf and stem bark extracts. The result obtained in the in-vitro model did not show significant fall in larval count in all the extracts when compared with Albendazole. Therefore, *Nauclea latifolia* may not be a safe and effective alternative vermicidal agent pending in-vivo trials of the plant.

Keywords: In-vitro, *Nauclea latifolia,* phytochemical, vermicidal, *Heligmosomodies bakeri*

INTRODUCTION

Parasitic infections are the most common infections worldwide, causing substantial challenges to public health, the economy and physical and cognitive development in individuals in developing countries like Nigeria (Agbalaka *et al.,* 2019). Parasites are present throughout the world in varying degrees of prevalence and are particularly relevant because they affect the poorest and most deprived areas in the tropical and subtropical regions (Faria *et al.,* 2017). Kaminsky and Kaminsky and Mäser (2025) reported that parasitic organisms such as worms, ticks, mites, fleas, biting flies, mosquitoes, and pathogenic protozoa affect humans and their pets as well as their livestock globally, both in terms of severity and numbers. The poor personal hygiene and poor health system commonly observed in developing countries make the prevalence high among the population (Agbalaka *et al.,* 2019). Ravichandran *et al.* (2023) reported that humans have depended on nature for their simple requirements; as source for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers, and means of transportation.

Humans have used medicinal plants for several thousand years to treat illness or health disorder. A good number of medicinal plants, such as those known from Asia, Africa or America have been employed to treat infections (VanWyk and Wink, 2004). Parwiz and Abdul (2024) in their paper on the role of plants in traditional and modern medicine stated that diverse tribal communities living in remote areas rely on plants for sustenance, including edible and medicinal parts.

Jit ˇareanu *et al*. (2023) in their narative review on Current Trends in Toxicity Assessment of Herbal Medicines stated that herbal medicine has always represented an important component of primary health care with approximately 80% of the world's population using herbal medicinal products for their therapeutic virtues. Balogun *et al. (*2016) reported that plants have provided the basis for traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agents.

According to World Health Organization (WHO), an approximately 80% of world population relies on herbal preparations as their primary source of healthcare (Balogun *et al.,* 2016). *Nauclea latifolia* is a valuable medicinal plant that is widespread in the humid tropical rainforest zone or in Savannah woodlands of west and central Africa. It is a spreading evergreen multi-stemmed shrub or small tree native to tropical Africa and Asia (Gidado *et al.,* 2005). In Nigeria, it is found in Niger, Abuja, Akwa ibom, Cross River, Enugu, Abakaliki and other states of the country (Balogun *et al.,* 2016). Its generic name is derived from the Greek word "Sarco" (freshly) and "Cephalus" (headed) in reference to the flowers. The specific name is from the Latin words "lati" (broad) and "folius" (leaved) (Arbonnier, 2000). *N. latifolia* is commonly known as "Uvuru-inu" or Ubulu ini among Igbos in Nigeria; the Hausas called it "Tafashiya", "Marga", "Tabashiya" or Tuwonbiri"; in Yoruba it is called "Egbesi" while the Ibibios know it as "Mbom-Ubom". In English it is called "Pin Cushion Tree" or "African Peach" (Arise *et al.,* 2012).

The plant is known to have various medical uses, particularly in folk medicine (Gidado *et al.,* 2008). Gidado *et al. (*2021) reported that wounds, cough, gonorrhea, malaria and hypertension have been treated using *N. latifolia.* Ezurike and Prieto (2014) reported that *N. latifolia* formulation and decoction preparations were used in ethnomedicine to treat hyperglycemia and diabetes by different ethnic groups in Nigeria. It can also serve as a chewing stick to treat stomach ache and tuberculosis at its initial stage (Gidado *et al.,* 2005).

Heligmosomoides polygyrus, is a naturally occurring intestinal roundworm of rodents (Gregory *et al.,* 1990). It measures 5-20mm in length and due to the pigmentation of its tissue, it appears bright red in colour. The worm is coily in nature, with the female having 12-15 coils and the male 8-12 coils (al-Bassel *et al*., 2000). *Heligmosomoides bakeri* is commonly referred to as *H. polygyrus* in the literature though their genomes show levels of divergence that are consistent with at least a million years of independent evolution. The genomes of both species contain hyper-divergent haplotypes that are enriched for proteins that interact with the host immune response. This nematode is widely used as a gastrointestinal parasitic model in immunological, pharmacological, and toxicological studies (Stevens *et al*., 2023). This research thus sought to determine the in-vitro vermicidal effects of *N. latifolia* in *Heligmosimoides bakeri* infected mice with specific the following objectives

- To ascertain the quantitative and qualitative phytochemical properties of *N. latifolia*
- To determine the acute toxicity level (LD50) of *N. latifolia*
- To evaluate the vermicidal effects of *N. latifolia* in-vitro

MATERIALS AND METHOD

Study Area: The study was done in Michael Okpara University of Agriculture, Umudike, Ikwuano L.G.A., Abia State, Nigeria. Ikwuano has an area of 281km^2 and a population of 137, 993 as at the 2006 census. It is made up of about 52 villages and communities. Ikwuano lies between the latitudes 5º.25´N and 5.433ºN and longitudes 7º.34´N and longitudes 7.º34´E and 7.56ºE (Chidiebere-Mark and Nneka; 2018). It is within the tropical rainforest zone of Nigeria and has a marked difference in annual season. There are eight months of rainy seasons, from April to November and four months of dry season with short period of harmathan from December to January (Ironemenefu; 2006). They are predominantly farmers with relatively few traders and civil servants.

Experimental Site: The study was carried out in Zoology and Environmental Biology Laboratory, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State.

Collection And Identification Of Plant Materials: Fresh leaves and stem bark of ***Nauclea latifolia*** **were collected from the forest in MOUAU, Ikwuano L.G.A of Abia State and were identified by a Botanist from Plant Science and Biotechnology, College of Natural Sciences, MOUAU**

Preparation of the Extracts: Both the leaves and stem bark were air dried for about 21 days on the Laboratory bench and dried samples were ground separately using a grinding machine. The method of extraction used by Ijioma *et al.,* (2014) was adopted for the extraction. 80g of each ground sample was introduced into the extraction chamber of the soxhlet extractor and absolute ethanol was used as the extraction solvent. Thereafter, the extract in solution was concentrated in a laboratory oven at 40ºC to obtain dried extracts.

Phytochemical Analysis: The extracts were analyzed both qualitatively and quantitatively using standard method.

Collection Of Animals: A total of 36 adult albino mice was used for the study. The mice were obtained from the Laboratory animal unit of College of Veterinary Sciences, MOUAU and were housed in well-ventilated cages in the animal house of College of Natural Sciences MOUAU. They were allowed to acclimatize for a period of two weeks for proper adaptation to the environment and living condition. The animals were given free access to feed (growers guinea feed) and clean water. Experiment animals were handled according to the guidelines for care and use of laboratory animal (Ijioma *et al.,* 2014).

Acute Toxicity Studies (Ld_{50} Determination): The new Lorke's method as reported by Orieke *et al.* (2019) involving 3 stages was used. The 36 albino mice wre used for the acute toxicity studies, 18 mice for each extract. In the first phase, 9 albino mice were divided into 3 groups of 3 mice each and were administered 10mg/kg, 100mg/kg and 1000mg/kg respectively for each extract. With no mortality observed after 24hours, the study proceeded to the second phase. Here, other 9 albino mice were assigned to 3 groups of 3 mice each and were treated with 1600mg/kg, 2900mg/kg and 5000mg/kg respectively for each extracts. After 24 hours, observation of no mortality marked the end of the phases. Thereafter, the LD_{50} value for each extract was determined using Lorke's formula stated as:

$LD_{50} = (A \times B)^{1/2}$; Where:

A - Maximum dose that did not produce mortality

B - Minimum dose that produced 100% mortality in a group

In-Vitro Anthelmintic Activities of the two Extracts: The method used by Sneda *et al*. (2022), Suresh *et al.* (2016) and Aleme et al. (2015) with modifications was adopted. Five Petri dishes were set up and labeled A-E according to the design for treatment. 20mls of normal saline was added in each of the dishes. All the dishes A-E were added 1ml of parasite suspension each and mixed to make it homogeneous. The parasite load was determined for each dish. Thereafter, the Petri dishes were treated according to the design for the treatment as follows:

Petri dish A – Parasites (1ml) only, no treatment (Negative control)

Petri dish B – 1ml of parasites + 5mg/ml standard drug (Albendazole)

Petri dish C – 1ml of Parasites with 5mg/ml of leaf extract

Petri dish C – 1ml of 1ml of Parasites with 5mg/ml of stem bark extract

Petri dish E – 1ml Parasites with 5mg/ml of the combination of both extracts (leaf and stem).

Thereafter, the content of each Petri dish was examined microscopically to ascertain the mortality of the parasite larvae in 1 hour interval. For 3 hours duration.

Statistical Analysis: The data obtained for the study were subjected to one way analysis of variance using a Duncan multiple range comparison test with level of statistical significance established at 95% interval. The statistical analysis was performed using software. Statistical products and service solutions (SPSS) version 23.

RESULTS AND DISCUSSION

Phytochemical Analysis of Nl Leaf Extract: The qualitative and quantitative phytochemical test of NL leaf extract carried out revealed the presence of saponins, flavonoids, terpenoids, tannis, and alkaloids, phenolics, and cardiac glycosides compounds as major secondary metabolites. Qualitatively, alkaloid recorded the highest content (+++) followed by Saponins, Tannins and the Phenolics (++) while the flavonoids, Terpenoids and cardiac glycocides recorded the lowest (+). Quantitatively, the Alkaloids recorded the highest value followed by the Phenolics with 30.74 ± 0.41^{h} and 14.15 ± 0.22^{g} while the least value was recorded for the cardiac glycosides

with 4.36±0.03[b]. There was no steroids found. These results are presented in Table 1. Figure 1 shows the concentration in 100g/mg of the phytochemical content of the leaf extract.

TABLE 1: Phytochemical Analysis Of Nl Leaf Extract

Phytochemical agent	Qualitative results	Quantitative results
Saponins	++	**9.32±0.03[e]**
Flavonoids	+	**6.60±0.09[c]**
Terpenoids	+	**7.77±0.07[d]**
Tannins	++	**11.98±0.16[f]**
Alkaloids	+++	**30.74±0.41[h]**
Phenolics	++	**14.15±0.22[g]**
Steroids	-	**0.00[a]**
Cardiac glycosides	+	**4.36±0.03[b]**

Values are presented as mean ± standard deviation of replicated determination (n = 3).Means in the same column bearing different letter superscripts are statistically significantly different.

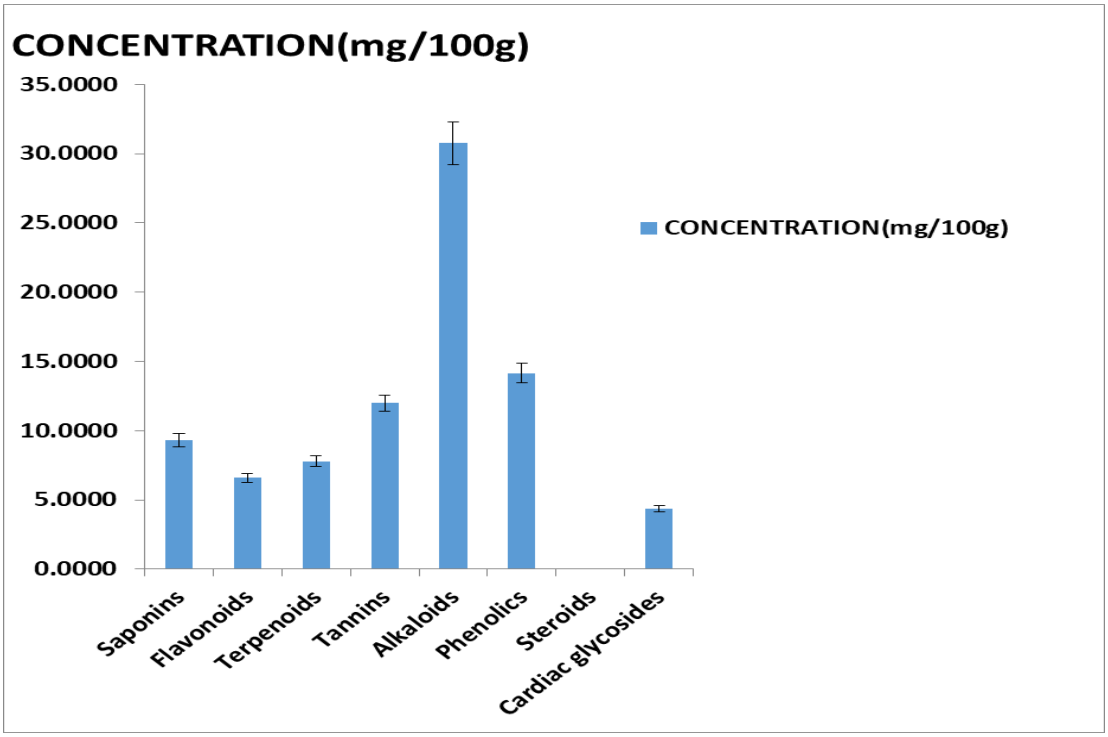

FIGURE 1: Bar chart showing concentration of phytochemical NL leaf extract

Phytochemical Analysis of Nl Stem Bark Extract:

The phytochemical tests of NL stem bark extract carried out, revealed the presence of saponins, flavonoids, terpenoids, tannins, alkaloids, phenolics, steroids and cardiac glycosides compound as major secondary metabolites. Qualitatively, alkaloid recorded the highest content (+++) followed by Saponins, Tannins and the Phenolics (++) while the flavonoids, Terpenoids, steroids and cardiac glycocides recorded the lowest (+). Quantitatively, the Alkaloids recorded the highest value followed by the Phenolics with 33.06±0.16[h] and 17.68±0.10[g]. However, there were steroids found in the stem extract recording the least value of 1.65±0.05[a]. These results are presented in Table 2. Figure 2 shows the concentration in 100g/mg of the phytochemical content of the stem extract.

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TABLE 2: Phytochemical Analysis of Nl Stem Bark Extract

Phytochemical agent	Qualitative results	Quantitative results
Saponins	++	7.41 ± 0.09^{c}
Flavonoids	+	9.48 ± 0.26^{e}
Terpenoids	+	8.68 ± 0.07^{d}
Tannins	++	10.58 ± 0.04^{f}
Alkaloids	+++	33.06 ± 0.16^{h}
Phenolics	++	17.68 ± 0.10^{g}
Steroids	+	1.65 ± 0.05^{a}
Cardiac glycosides	+	5.66 ± 0.11^{b}

Values are presented as mean ± standard deviation of replicated determination (n = 3). Means in the same column bearing different letter superscripts are statistically significantly different.

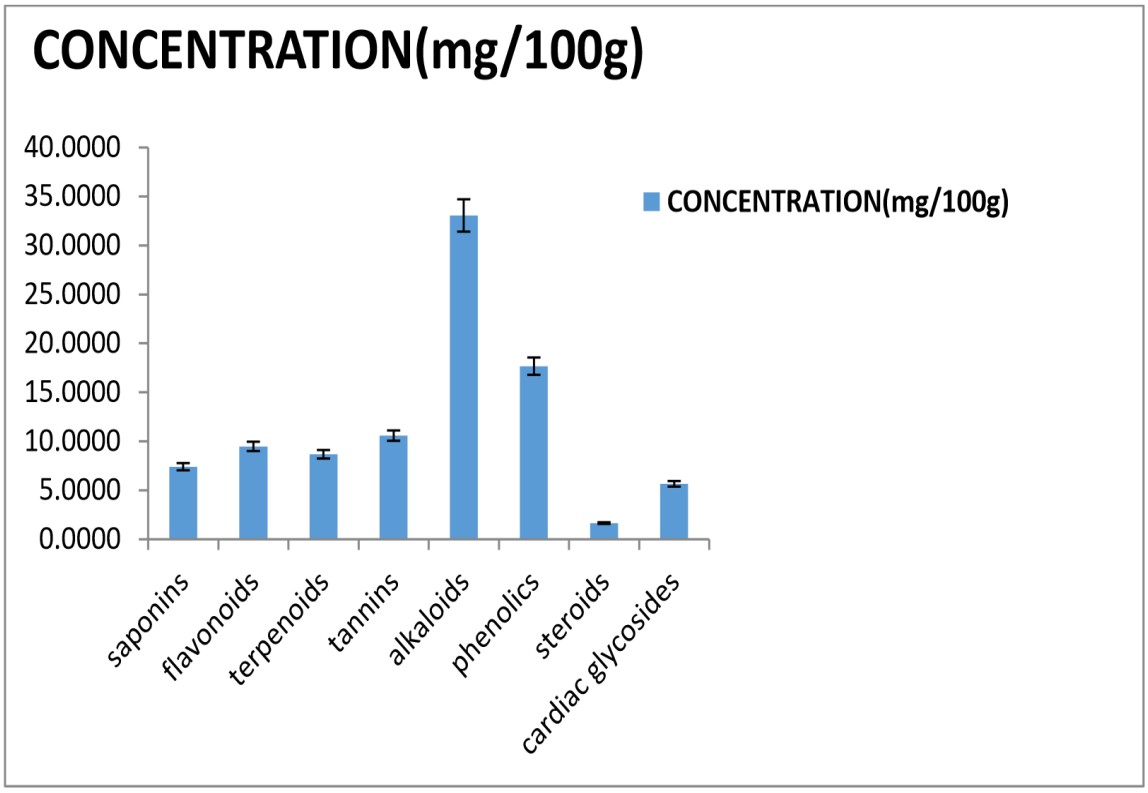

FIGURE 2: Bar chart showing concentration of phytochemical NL stem extract Acute Toxicity (Ld50) Evaluation of Nl Leaf Extract

The LD_{50} evaluation was carried out in two different phases. In the phase 1 of the LD50 evaluation, administration of 10mg/kg, 100mg/kg and 1000mg/kg doses of NL leaf extract produced no mortality. Administration of 2900mg/kg produce calmness and physical in-activeness for about 25minutes before the animal regained physical activeness. However at the highest dose of 5000mg/kg, 100% mortality was recorded at 66.67 percent mortality. Signs of toxicity like agitation and writhing reflexes were observed before death.

Therefore, the acute toxicity of NL leaf extracts, was evaluated as follow; $LD_{50} = (2900 \text{ X } 5000)^{1/2} =$ 3,807.87mg/kg body weight. The results are presented in tables 3 and 4 respectively

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TABLE 3: Phase 1 Ld_{50} Results for Nl Leaf Extract

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

TABLE 4: Phase 2 Ld_{50} Results for Nl Leaf Extract

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	0/3	Animals were calm and physically inactive for about 25 minutes but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5000	3/3	66.67 percent mortality recorded. Signs of toxicity like agitations and writhing reflexes were observed before death.

$LD_{50} = (2900 \times 5000)^{1/2} = 3{,}807.87$ mg/kg body weight

LD_{50} Evaluation of Nl Stem Bark

The acute toxicity of NL stem bark also involved two phases. In phase 1, administration of 10mg/kg, 100mg/kg and 1000mg/kg doses of NL stem bark extract produced no death nor physical in-activeness. Observations from the 2900mg/kg and 5000mg/kg doses were signs of toxicity like agitation and writhing reflexes with observed mortality rates of 33.33 percent and 100 percent at 66.67 percent mortality respectively. The LD_{50} of NL stem bark therefore was evaluated as follow

$LD_{50} = (1600 \times 5000)^{1/2} = 2{,}828.43$mg/kg body weight

The results are presented in tables 5 and 6 respectively

TABLE 5: PHASE 1 LD_{50} Results for Nl Stem Bark Extract

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

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3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

TABLE 6: Phase 2 Ld_{50} Results for Nl Stem Bark Extract

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were calm for about I hour before gradually recovering their physical activities. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	1/3	33.33 percent mortality recorded. Signs of toxicity like agitations and writhing reflexes were observed before death.
3	5000	3/3	66.67 percent mortality recorded. Signs of toxicity like agitations and writhing reflexes were observed before death.

$LD_{50} = (1600 \times 5000)^{1/2} = 2{,}828.43$ mg/kg body weight

Result of in-Vitro Vermicial Study

In the in-vitro study with the NL leaf, stem bark and combination of the extracts in Petri dishes C, D and E, there was no show significant fall in larval count when compared with the Petri dishes B treated with the standard drug (Albendazole). The initial parasite load records were 334.33 ± 59.48^{b}, 341.00 ± 16.78^{b}, 334.00 ± 35.79^{b}, 334.33 ± 51.16^{b}, and 370.00 ± 15.72^{b} for Petri dishes treated with albendazole, leaf extract, stem bark extract, combination of the extracts and negative control respectively. The final parasite loads obtained after 3 hours of administering the treatments were 8.00 ± 3.61^{a}, 281.67 ± 17.79^{b} 309.33 ± 52.17b and 331.67 ± 53.20^{bc} for the dishes with albendazole, leaf extract, stem bark extract and the combined extracts respectively.

There was a reduction in the parasite load across the treatments but the negative control recorded an increase; 377.00 ± 23.58^{c} as against the initial 370.00 ± 15.72^{b}. The results are presented in the table 8

TABLE 8 : **Antehelminthic Activity (*In Vitro*)**

TREATMENT	INITIAL PARASITE LOAD	1H	2H	3H
Normal control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Negative control	370.00 ± 15.72^{b}	380.67 ± 20.55^{b}	381.67 ± 10.69^{a}	377.00 ± 23.58^{c}
Albendazole 5mg/ml	334.33 ± 59.48^{b}	93.67 ± 11.37^{c}	64.00 ± 12.12^{b}	8.00 ± 3.61^{a}
Stem Bark Extract 5mg/ml	341.00 ± 63.78^{b}	306.33 ± 78.14^{c}	326.33 ± 71.06^{b}	281.67 ± 17.79^{b}
Leaf and Stem Extract 5mg/ml	334.00 ± 35.79^{b}	334.33 ± 39.31^{c}	323.67 ± 50.06^{b}	309.33 ± 52.17^{b}
Leaf extract 5mg/ml	334.33 ± 51.16^{b}	347.00 ± 59.36^{c}	339.00 ± 54.81^{b}	331.67 ± 53.20^{bc}

Values are presented as mean ± standard deviation of replicated determination (n = 3).Means in the same column bearing different letter superscripts are statistically significantly different.

DISCUSSION

The presence of secondary metabolites such as Saponins, Flavonoids, Terpenoids, Tannis, Alkaloids, Phenolics, Cardiac glycosides in significant proportion from both the leaf and stem bark extracts of *Nauclea latifolia* indicates the potential medicinal values of the plant. This is in line with the report of Oshilonyah *et al.* (2016) that the phytochemical agents in plant materials are responsible for such plant's bio-activities and as well define their usefulness in disease management.

In the LD_{50} evaluation, records of calmness was observed following the administration of 2900mg/kg and 1600mg/kg of NL leaf and stem bark extracts respectively. However with 5000mg/kg of the leaf and 2900mg/kg of the stem bark extracts administered, death occurred suggesting that the plant contains some levels of toxicity. This result agrees with Akomas, *et al* (2014) and Madubunyi *et al* (2012) who recorded toxicity signs including mortality of animals following consumption of plants materials. More studies are recommended to ascertain levels of consumption or administration of plant bioactive substances.

Pratap *et al*., 2018 and Jain *et a*l.; 2011 reported that Tannis are known to cause death of worms by interfering with their energy generation pathway and uncoupling oxidative posphorilations 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. The fall in the larval and egg counts recorded in the groups treated with stem bark and combined extracts than in the group treated with the leaf extract indicates that stem bark extract has more vermicidal effect. This may be so as stem bark extracts had higher amounts of the secondary metabolites than the leaf extract. Steroids were absent in the leaf extracts but present in the stem recording the value of $1.65\pm0.05^{a..}$ There was no significant difference in the quantitative values recorded for the Alkaloids and Tannins the leaf and stem bark extracts (see table 1 and 2). However, the Phenolics recorded significant values for the leaf and stem bark extracts (14.15 ± 0.22^{g}; 17.68 ± 0.10^{h})

Udoha *et al.* (2015) and Castanendae- Ramirez *et al.* (2017) stated that albendazole have the ability to inhibit eggs hatching and larval development in worms due to it's ability to inhibit the formation and development of vital structures in these parasites. It inhibits the polymerization of the parasitic transformation from tubulin into microtubules. High affinity of the albendazole to the tubulin inhibits cytoplasmic microtubules development in worms and hinders the movement of glucose into the larval and adult stages of the worms. This process makes the worms inactive and leads to their death. This may be the reason the albendazole caused the reduction in the parasite load as recorded in this experiment.

In this in-vitro studies, the extracts did not compare favorably with albendazole. There was a slight reduction in the parasite count though not significant. The agrees with the work done by Castanendae- Ramirez *et al* (2017) which revealed significant anthelmintic effect of *N latifolia* following in-vitro trials. Of the extracts used in this study, the stem extract showed more anthelminthic effect recording highest reduction in the parasite load from the onset of the experiment to the 3hour duration it lasted (341.00 ± 63.78^{b}, 306.33 ± 78.14^{c},326.33 ± 71.06^{b}, 281.67 ± 17.79^{b})(see table 8). This study may suggests that *N latifolia* leaf and stem bark extracts from this locality are not effective anthelmintic agents in in-vitro trials..

CONCLUSION AND RECOMMENDATIONS

For safe and effective control of intestinal worms using plant materials such as *N. latifolia* following the findings in this study, these recommendations are therefore made:

1. The use of any plant materials such as N. latifolia should be done with scientific guide. This will prevent consumption of intolerable amount of bioactive substances from the plant.

2. The Stem bark and Leaf of N. latifolia may not serve as alternative vermicidal agents as the dosage used proved not effective; more work on using another dosage is recommended.

3. In-vivo studies of N. latifolia for vermicidal activities in H. bakeri infected mice is suggested and should be done to ascertain if the plant may prove the anthelminthic effects of the plant.

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