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LARVICIDAL POTENCY OF *Andrographis paniculata* PLANT EXTRACT ON *Anopheles gambiae* LARVAE

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Abstract

Control of mosquito vectors has relied heavily on chemicals which has disadvantages such as resistance development, and environmental hazards. This study investigated the phytochemical constituents and larvicidal potency of *Andrographis paniculata* against *Anopheles gambiae* larvae. Soxhlet and Maceration techniques were utilized, with ethanol and water as solvents respectively. Ten fourth instar larvae of *An. gambiae* were exposed to three replicates of different concentrations (20%, 15%, 10%, and 5%) of the extracts, using a Completely Randomised Design with ethanol and water in soxhlet and maceration extraction respectively. Monitoring was conducted at intervals of 0, 1, 3, 24, and 48-hours post-treatment. The data gathered were examined through log-probit regression and analysis of variance. Findings indicated increased mortality with higher levels of concentration and exposure time to Soxhlet extract, displaying a statistically significant difference (p = 0.035) (p < 0.05). Result showed *A. paniculata* had larvicidal effect on *An. gambiae* larvae. There was concentration dependent mortality as mortality increases with increase in concentration of both extracts. For Soxhlet extract, the highest concentration of 20% caused 86.7% mortality while the lowest concentration of 5% caused 49.0% mortality. Similarly, for maceration extract, 20% concentration caused 76.7% mortality while 5% caused 40.0% mortality. The lethal concentration LC_{50} and LC_{90} were 6.9% and 36.58% respectively for soxhlet extract while they were 9.1% and 56.1% respectively for maceration extract. Also, the LT_{50} and LT_{90} for Soxhlet extract was lower (23hrs and 53hrs) than the maceration extract (48.7hrs and 60hrs). Result also showed that Soxhlet extraction produced more bioactive compounds than the maceration. This study showed that *A. paniculata* is a potential larvicidal agents in the control of *Anopheles gambiae*. These findings suggest that *A. paniculata* could serve as an eco-friendly alternative for controlling malaria vectors.

Keywords: *Andrographis paniculata*, Extracts, Larvicidal, *Anopheles gambiae*, Control

Introduction

Several Public health issues are caused by mosquitoes and include life-threatening diseases such as malaria, chikungunya fever, filariasis, yellow fever, encephalitis, dengue fever, West Nile Virus infection and other conditions (Grigoraki *et al*., 2016). These are mainly diseases that impact individuals living in tropical regions and are therefore classified as tropical diseases (Rahuman *et al*., 2009). *Culex*, *Aedes*, and Anopheles play a crucial role as the main vector in human disease transmission. About 229 million cases and 409 thousand deaths were reported due to malaria incidence, while 56 million cases for dengue fever have been reported globally (WHO, 2020). Also, over ten million people were reported dead annually by other mosquito-borne diseases, such as filariasis, yellow fever, and encephalitis (WHO, 2021). There are 140 *Anopheles* species, 20 of these species are known to transmit malaria to human (Sinka *et al*., 2012).

Mosquito vectors' resistance to synthetic insecticides is a significant public health concern worldwide, as it hinders efforts to control and eliminate mosquito-borne diseases globally. The reason for this has been associated with the excessive use of insecticides (Grigoraki *et al*., 2016). This excessive use leads to easy evasion of the insecticides' deadly impact on mosquitoes through antigenic shifting and other resistance strategies. Hence, there is a necessity for utilizing botanicals, which are typically safe for humans and domestic animals, as they are natural plant products (Ejeta *et al*., 2021). These plants contain bioactive organic chemicals and have benefits compared to synthetic insecticides due to low toxicity to humans, less likely to develop resistance, and biodegradable (Ajaegbu *et al*., 2016).

Several plant species have been known to possess insecticidal qualities (Vilvest *et al*., 2023; Imakwu *et al*., 2024). *Andrographis paniculata*, also called green chiretta, is a yearly herb in the Acanthaceae family that originates from India and Sri Lanka (Kumar *et al*., 2012). In Southern and Southeastern Asia, it is extensively grown and thought to have medicinal properties against bacterial infections and certain illnesses. The leaves and roots are mainly utilized for these purposes; occasionally, the entire plant is also used (National Institute of Industrial Research, 2003).

During the COVID-19 outbreak in Thailand in December 2020, the health ministry approved using the plant extract for a pilot alternative treatment program (Bangprapa, 2021). In Nigeria, *A. paniculata* have been used in alternative medicine in the treatment of febrile illnesses and conditions such as hypertension (Adaramola *et al.,* 2018).

Jelin *et al*., (2023) reported the larvicidal, pupicidal and adulticidal activity of *Andrographis paniculata* on *Aedes aegypti* and *Culex quinquefasciatus* third instar larvae using methanol Soxhlet extraction and they observed that the leaves are highly effective against *Aedes aegypti* and *Culex quinquefasciatus* larvae, pupae and eggs with LC50 values of 86.74ppm and 84.41ppm for larvicidal activity and 95.27ppm and 92.91ppm for pupal activity respectively.

This study aimed at determining the larvicidal effect of *A. paniculata* on *An. gambiae* and to also conduct a comparative analysis of the Soxhlet and maceration extraction methods.



Andrographis paniculata

Materials And Methods

Experimental Design

The study was laid out in a Completely Randomized Design (RBD). Ten fourth instar larvae of *An. gambiae* were exposed to three replicates of different concentrations (20%, 15%, 10%, and 5%) of the extracts.

Collection and Processing of *Andrographis paniculata*

The leaves of *A. paniculata* (Green Chiretta) were obtained from Nnewi, Anambra State, Nigeria. The leaves were air dried and ground using a grinder (Electric blender).

Collection of Mosquito Larvae used in the experiment.

The *An. gambiae* larvae were collected from the Insectary of Parasitology and Entomology Laboratory, Nnamdi Azikiwe University Awka, Nigeria.

Leaf Extract Preparation using Maceration method

The finely ground 100 g of *A. paniculata* was put into a Winchester bottle and 200 ml of Ethanol added, before it was allowed to stand. To ensure proper soaking of the ground leaves, the bottle was properly closed with its' cover and lightly shaken at intervals. After three days of soaking, filter paper was used to filter the soaked ground leaves. The crude extract was produced by weighing the filtrate and evaporating it in a water bath at 40°C (Eneh *et al*., 2016).

Leaf Extract Preparation using Soxhlet method

Soxhlet extraction apparatus with 50 g of dried plant powder was placed into the paper thimble. Following that, cotton wool was placed at the top of the extraction flask to prevent the sample from overflowing into other components of the apparatus. In a round-bottom flask with the heating mantle underneath, one litter of ethanol solvent was poured. Along with the finely ground plant materials, the solvent was repeatedly refluxed and heated. It was done so that the desired plant compound may be extracted into the round-bottom flask. The extraction in the Soxhlet apparatus was carried out for around three (3) hours at boiling point 60°C until the solvent in the syphon arm became clear, indicating that the sample had been extracted completely. The extracts were concentrated in the water bath (Atanasova *et al*., 2018).

Formulation of Extracts

Different concentrations of the plant extracts were constituted from the stock solution. Ethanol was used as a medium to permeate the crude extracts into the larvae (Atanasova *et al*., 2018). The extracts were taken as

100% concentration and diluted into various concentrations viz: 25%, 15%, 10% and 5%.

Phytochemical Analysis

The phytochemical screening of the extracts was carried out following standard methods for Gas Chromatography as described by Trease and Evans (1989)**.**

Larval Bioassay

The standard procedure used in the study was adopted from WHO (2005).

Statistical Analysis

The data was analyzed using one-way Analysis of variance (ANOVA) for mean and Standard error (SE) at 5%. Log-probit regression analysis was carried out (Finney, 1971) for determining LC_{50} and LC_{90}. Log-probit analysis was also done to compare lethal time (LT_{50}) for Soxhlet and Maceration extracts. The means of mortality count were separated using Duncan's test at 0.05% level of significance using SPSS in IBM version 25.

Results

Larvicidal Effects of Maceration Leaf Extract of *A. paniculata* on *An. gambiae* larvae

Table 1 shows difference in mortality responses of *A. gambiae* larvae exposed to residual applications of *A. paniculata* at hourly intervals. There was a concentration-dependent mortality response as mortality increases with an increase in concentration of the extract. The highest concentration of 20% caused 76.7% mortality while the lowest concentration of 5% caused 40.0% mortality. Statistical analysis using ANOVA revealed that the mortality due to different concentrations showed significant differences (p=0.047) ($p \leq 0.05$).

Table 1: Hourly mortality response of *An. gambiae* larvae exposed to residual application of *A. paniculata* maceration extract

Conc (%)	0hrs	1hrs	3hrs	24hrs	48hrs	Mean±s.e	% Mortality	Probit
20	0.67	3.33	4.00	6.33	7.67	4.40±1.21	76.7	5.66
15	0.33	3.00	3.33	4.00	6.33	3.39±0.96	63.3	5.39
10	0.33	2.33	3.00	3.67	5.00	2.86±0.77	50.0	5.14
5	0.00	2.00	2.33	2.67	4.00	2.20±0.64	40.0	4.90
Mean±s.e	0.33±0.13	2.67±0.30	3.16±0.34	4.16±0.77	5.75±0.79		-	
% Mortality	3.3	26.7	31.6	41.6	57.5		-	
Control	0.0	0.5	1	0.5	1		5	
Probit	4.21	4.75	5.26	4.75	5.26			

Mean of the three replicates (±s.e), Pv=0.047; Pv=0.000

Larvicidal Effects of Soxhlet Leaf Extract of *Andrographis paniculata An. gambiae* larvae

Table 2 shows the difference in mortality responses of *An. gambiae* larvae exposed to residual applications of *A. paniculata* at hourly intervals. There was concentration-dependent mortality response as mortality increases with an increase in concentration of the extract. The highest concentration of 20% caused 86.7% mortality while the lowest concentration of 5% caused 49.0% mortality. Statistical analysis using ANOVA revealed that the mortality due to the different concentrations showed significant difference (p = 0.035) (P<0.05).

Table 2: Mortality response of *An. gambiae* larvae exposed to *A. paniculata* soxhlet extract

Conc (%)	0hr	1hr	3hrs	24hrs	48hrs	Mean±s.e	% Mortality	Probit
20	1.00	3.67	4.67	6.67	8.67	4.93±1.30	86.7	5.66
15	0.67	3.00	3.67	5.33	6.67	3.86±1.02	66.7	5.39
10	0.33	2.67	3.33	3.67	5.67	3.13±0.86	56.7	5.14
5	0.00	2.00	2.67	2.67	4.90	2.44±0.78	49.0	4.90
Mean±s.e	0.50±0.21	2.83±0.34	3.58±0.41	4.58±0.88	6.47±0.81		-	
% Mortality	5.0	28.3	35.8	45.8	64.7		-	
Control	0.0	0.5	1	0.5	1		5	
Probit	4.21	4.75	5.26	4.75	5.26			

Mean of the three replicates (±s.e), Pv=0.035; Pv=0.000

Probit against log concentration of *A. paniculata* Soxhlet and maceration extracts

The lethal concentration LC_{50}, that is, the concentrations that caused 50% and 90% mortality was obtained as 6.9% and 36.58% respectively for the Soxhlet extractor and the concentrations that caused 50% and 90% mortality were obtained as 9.1% and 56.1% respectively for maceration extract (Fig. 1).

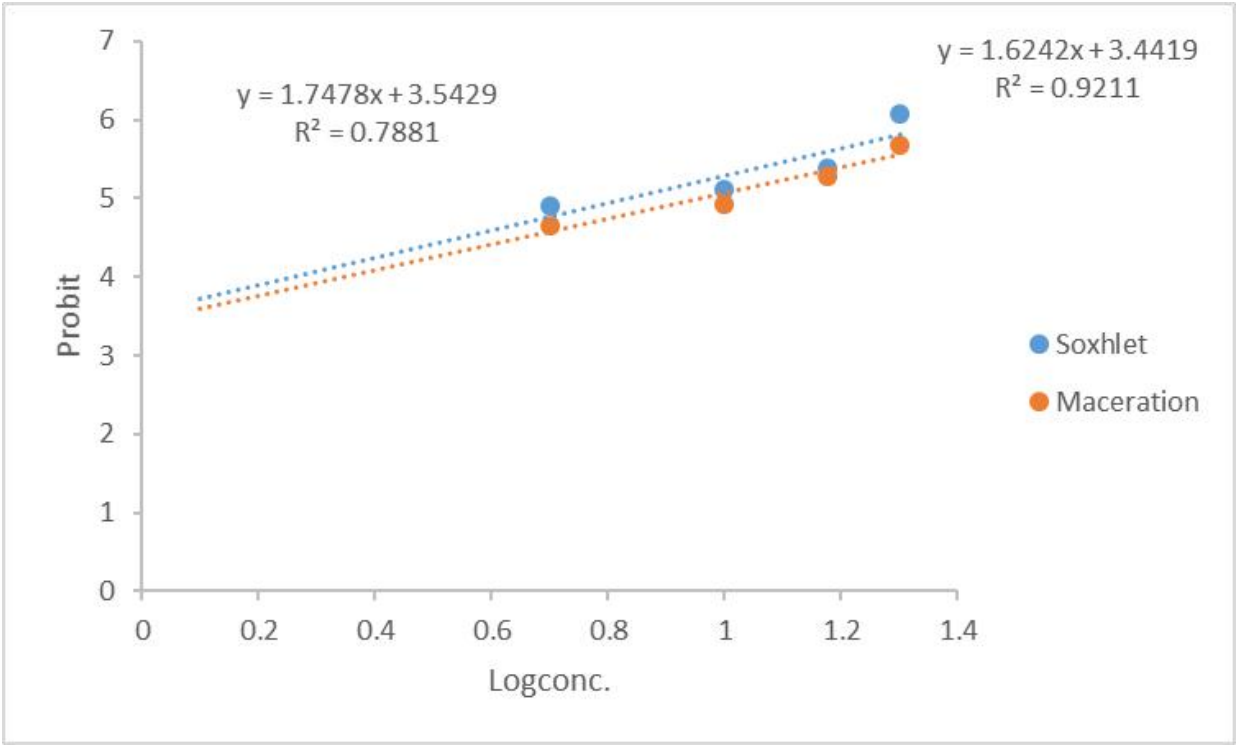


Fig 1: Plot of Probit against Log-concentration of *Andrographis paniculata* oil extract from Soxhlet and maceration methods

Soxhlet

Y= 1.7478x + 3.5429
R^2= 0.7881
LC_{50}=6.9%
LC_{90}=36.8%
Maceration
Y= 1.6242x + 3.4419
R^2= 0.9211
LC_{50}=9.10%
LC_{90}=56.01%

Probit against log time of *Andrographis paniculata* soxhlet and maceration extracts

The lethal time LT_{50} that is the concentrations that caused 50% and 90% mortality were obtained at 23hrs and 53hrs respectively for Soxhlet extract and the concentrations that caused 50% and 90% mortality were obtained at 48.7hrs and 60hrs respectively for maceration extract (Fig 2).

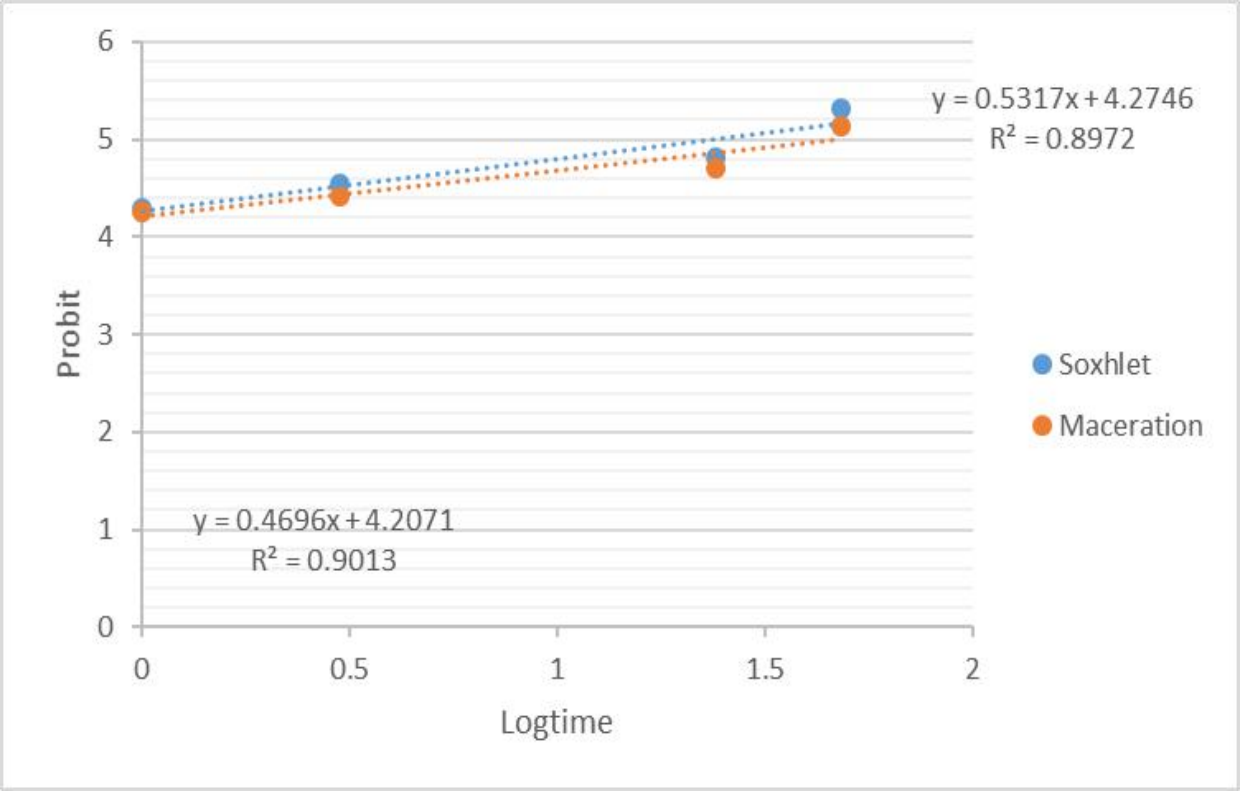


Fig 2: Plot of Probit against Logtime of *Andrographis paniculata* oil extract from Soxhlet and maceration methods

Soxhlet
Y= 0.5317x + 4.2746
R^2= 0.8972
LT_{50}=23hrs
LT_{90}=59hrs
Maceration
Y= 0.4696x + 4.2071
R^2= 0.9013
LT_{50}=48.7hrs
LT_{90}=60hrs

Phytochemical composition of Soxhlet and maceration extracts of *Andrographis paniculata*

The concentrations of various phytochemicals are compared between the two types of extracts, measured in milligrams per 100 grams. Soxhlet extracted oil yielded comparatively higher compositions of the phytochemicals in mg/100g of *A. paniculata* with tannins, saponins, phenols, and phytates while maceration extracted oil yielded higher with alkaloids and oxalates (Table 3).

Table 3: Quantitative phytochemical composition of *Andrographis paniculata* leaf extract

Phytochemicals	Soxhlet extract (mg/100g)	Maceration extract (mg/100g)
Tannins	7.4	7.3
Alkaloids	5.2	5.7
Saponins	7.4	6.5
Total phenols	12.3	12.2
Flavonoids	6.0	5.3
Oxalates	1.1	1.2
Phytates	1.4	1.02

Discussion

The result of this study reveals that the ethanolic extract of *A. paniculata* leaves using Soxhlet extraction has more larvicidal effect against *An. gambiae* larvae compared to the maceration method. This is similar to a comparative study between soxhlet and maceration extraction of *Orthosiphon stamineus* by Othman *et al.,* (2024) who recorded that soxhlet extraction yielded an overall higher yield (12.38%) as compared to maceration (10.8%). Soxhlet extraction was also the best method in terms of shorter extraction time and lesser organic solvent usage. Result from the study also showed that leave extracts from Soxhlet yielded more bioactive compounds than the maceration extraction. It contrasts Hasham *et al.,* (2021), who reported that leaves extract achieved from the cold maceration method revealed significantly better bio-active compounds presence than the extract obtained from soxhlet extraction.

A higher mortality rate was recorded in the Soxhlet extract of *Andrographis paniculata* compared to the maceration extract. The result of the study indicates that the ethanol extract from the plant leaves is highly effective against *An. gambiae* larvae with lower LC_{50} value while maceration has a higher LC_{50} value for larvicidal activity. This study also reveals that Soxhlet extract had higher phytochemicals compared to the maceration extract. This is because Soxhlet extraction efficiently extracts phytochemicals from plant material by ensuring complete extraction through removal of phytochemicals from plant matrix, while in maceration extraction, the extraction may be incomplete due to the leaf's phytochemicals trapped in plant matrix. (Kumar *et al*., 2021).

The result of the phytochemical analysis shows that the plant extract contained at least one of the paramount phytochemicals or bioactive constituents. This agreed with Edeoga *et al.* (2005) who reported some bioactive constituents such as saponins, tannins, flavonoids and alkaloids. The screening of the leaf powder extract of *A. paniculata* Soxhlet and maceration method revealed the presence of alkaloids, tannins, saponins, total phenols, flavonoids, oxalates, phylates. Similarly, Imakwu *et al*., (2024) in their study on the use of neem leaves extract in the control of *Aedes aegypti* larvae recorded bioactive constituents like Alkaloid, Saponin, Tannin and Flavonoids.

The findings from this study showed that *A. paniculata* is a mosquito-control agent due to its larvicidal activity on *An. gambiae* larvae. This is similar to a study by Vilvest

et al., (2023) that showed the larvicidal, pupicidal and ovicidal activity of *Andrographis paniculata* on *Aedes aegypti* and *Culex quinquefasciatus* third instar larvae.

Soxhlet extraction is a continuous extraction technique that requires less time and solvent consumption than maceration, although very high temperature in the Soxhlet extraction will increase the possibilities of thermal degradation (Mariappan and Wilkie, 2014). However, maceration method is well simple, easy to conduct and relatively cheap since it does not require advanced tools.

This study showed that extracts of *A. paniculata* would serve as potential larvicidal agents in the control of *An. gambiae* due to the bioactive compounds present which can be developed further for the control of mosquito vectors.

Conclusion

Soxhlet extract was more effective against *An. gambiae* 4th instar larvae with higher mortality rate and lower LC_{50} value. Soxhlet extraction also yielded more phytochemicals. However, maceration was more cost-effective and can be used in rural areas where there is no much access to electricity. Both extracts can be exploited and developed more to be used as control methods against mosquito vectors.

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