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LABORATORY EVALUATION OF ETHANOLIC EXTRACTS OF CITRUS SINENSIS PEELS AND PIPER GUINEENSE (SEEDS AND LEAVES) ON MOSQUITO LARVAE

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Abstract:
Ethanolic extracts of Citrus sinensis peels, seeds and leaves of Piper guineense were evaluated for larvicidal activity against mosquito larvae in the laboratory. Phytochemical analysis of *C. sinensis* peel has been shown to contain reducing sugars, saponins, piperines, tannins and flavonoids while the leaves and seeds of *P. guineense* have been shown to contain glycocides, saponins, polyphenol, alkaloids, piperanine and dihydrowasanine. Mosquito larvae were exposed to different test concentrations, ranging from 10mg/ml 100mg/ml for 12 hours. Mosquito larvae were susceptible to the extracts. Ethanolic extracts of *C. sinensis* peel showed 100% mortality rate after 12 hours at 100mg/ml while that of *P. guineese* seeds and leaves were 90% and 80% respectively after 12 hours at 100mg/ml. Ethanolic extracts of *P. guineense* seeds were more active with LC50 of 25.12mg/ml than the ethanolic extracts of *P. guineense* leaves and peels of *C. sinensis* which were 39.82mg/ml and 50.12mg/ml respectively. These botanical larvicides can be used in place of the synthetic ones as they are cheap, eco-friendly, readily available, biodegradable and safe.

Keywords:
Citrus Sinensis; Piper Guineense; Ethanolic Extract; Larvicidal Activity; Mosquito Larvae

1. INTRODUCTION

Mosquitoes are the most important group of insects, both medically and in ecological perspectives. Mosquito transmitted pathogens infect more than 700 million people annually and worldwide through diseases like malaria, filariasis, dengue fever, yellow fever, encephalitis, [1]. Malaria alone kills 3 million people each year, including one child every 30 seconds [2, 3]. Even though mosquito borne diseases represent a greater health problems in tropical and subtropical regions, no part of the world is left out of the risk due to climate change [4, 5].

Control of mosquito-borne diseases is becoming more difficult because their insect vectors are becoming increasingly resistant to insecticides [6, 7]. Different methods of protection from mosquitoes are practiced widely, e.g. use of insecticide treated nets, mosquito repellents, indoor spraying of insecticides, (etc). These synthetic chemicals are effective at controlling mosquitoes but their environmental consequences are unpredictable. Several reports reveal that sleeping under mosquito repellents impairs proper development of brain in infants. Such chemicals promote pollution and also open avenues for emergence of resistant strains [8]. There is need to evaluate the larvicidal activities of ethanolic extracts of C. sinensis peels, P. guineense (seeds and leaves) on mosquito larvae. These plants are safe, less expensive and contain eco-friendly phytochemicals.

The phytochemical analysis of Citrus sinensis peel has revealed the presence of reducing sugars, saponins, deoxysugars, cardiac glycosides, tannin and flavonoids [9]. Piper guineense seeds and leaves have also been shown to contain glycocides, alkaloids, saponins, polyphenol, phelobatnins, piperanine, dihydrowasanine [10, 11].

2. MATERIALS AND METHODS

The study was conducted at the microbiology laboratory, Abia State Polytechnic, Aba, Abia State.

3. PLANT COLLECTION AND EXTRATION

Fully developed sweet orange (C. sinensis) fruits, leaves and seeds of Piper guineense were collected in the month of March, 2013 and identified in the Biology/Microbiology Laboratory, Abia State Polytechnic, Aba. The orange fruits were washed and the peels were separated manually after which they were dried and blended with the Blender. The African black pepper (P. guineense) seeds were washed and dried in the oven at 60^{o}C while the leaves were washed, dried under shade and blended separately using the blender. Ethanolic extracts were obtained by soaking 100 grams of finely ground plant materials in 500ml of 75% ethanol for 24 hours with periodic shaking.

The content of each set up was filtered using Whatmanns No. 1 filter paper and the residue evaporated to dryness using the soxhlet apparatus. After this, the extracts were allowed to cool and then, each was put into a container and labelled accordingly. 10mg, 20mg, 50mg and 100mg of each of these were mixed with 100ml of ethanol to get the test concentration to be used.

4. EFFECTS OF PLANT EXTRACTS ON MOSQUITO LARVAE

Ten active larvae were collected from stagnant water bodies and put into 500ml transparent container which have 249ml of dechlorinated water, fed with glucose and allowed to acclimatize. 1ml of each test concentration was added to each container and labeled accordingly. Each test concentration ranging from 10mg/ml, 50mg/ml, 100mg/ml and a control (made up of 249ml of dechlorinated water and 1ml of ethanol) were set up and observed under room temperature of 30^{o}C $\pm$2^{o}C. Mortality of larvae was observed after 12 hours and percentage mortality calculated thus: no. of dead larvae $\times$ 100 / total no. of larvae tested.

Table 1. **Mortality Rate of Ethanolic Leaf Extract of Piper guineense on Mosquito Larvae after 12 hours**

Concentration (mg/ml)	Total No. of Larvae Tested	No.of Dead Larvae	% Mortality	$10g_{10}$ Conc.	Probt.
100	10	8	80	2.00	5.84
50	10	4	40	1.70	4.75
20	10	3	30	1.30	4.48
10	10	4	40	1.00	4.47
Control	10	0	0	0.00	0.00

Table 2. **Mortality Rate of Ethanolic Seed Extract of Piper guineense on Mosquito Larvae after 12 hours**

Concentration (mg/ml)	Total No. of Larvae Tested	No. of Dead Larvae	% Mortality	$10g_{10}$ Conc.	Probt.
100	10	9	90	2.00	6.26
50	10	6	60	1.70	5.25
20	10	4	40	1.30	4.75
10	10	0	0	1.00	0.00
Control	10	0	0	0.00	0.00

5. RESULTS

Ethanolic extracts of Citrus sinensis peel, Piper guineense seeds and leaves showed some larvicidal activities on mosquito larvae tested at concentrations ranging from 10mg/ml to 100mg/ml for P. guineense leaf extract and C. sinensis peel extract and 20-100mg/ml for P. guineense seed extract. The results are shown on Table 1 , Table 2 , Table 3 . The effects of the plant extracts increased with increasing test concentration. No larvicidal effect was observed in the control.

6. DISCUSSION/CONCLUSION

The Study has shown that ethanolic extracts of Citrus sinensis peel, Piper guineense seeds and leaves have larvicidal effects at high concentrations on mosquito larvae. The study revealed that C. sinensis peel, P. guineense seeds and leaves have larvicidal effects on mosquitoes with 100%, 90% and 80% mortality at 100mg/ml respectively. This finding agrees with the observations of Aina et al., 2009 who observed a high mortality rate with P. guineense seed extract. Citrus peel extract has also been reported to have strong larvicidal activities against mosquito larvae [9]. Mortality could not be linked to the solvent, ethanol as no death was recorded in the control containers. The larvicidal effects could be linked to phytochemicals present in the plants used. The presence of alkaloids in plants may be associated with their

Table 3. **Mortality Rate of Ethanolic Peel Extract of Citrus sinensis on Mosquito Larvael after 12 hours**

Concentration (mg/ml)	Total No. of Larvae Tested	No.of Dead Larvae	% Mortality	$10g_{10}$ Conc.	Probt.
100	10	10	100	2.00	8.09
50	10	5	50	1.70	5.00
20	10	2	20	1.30	4.16
10	10	2	20	1.00	4.16
Control	10	0	0	0.00	0.00

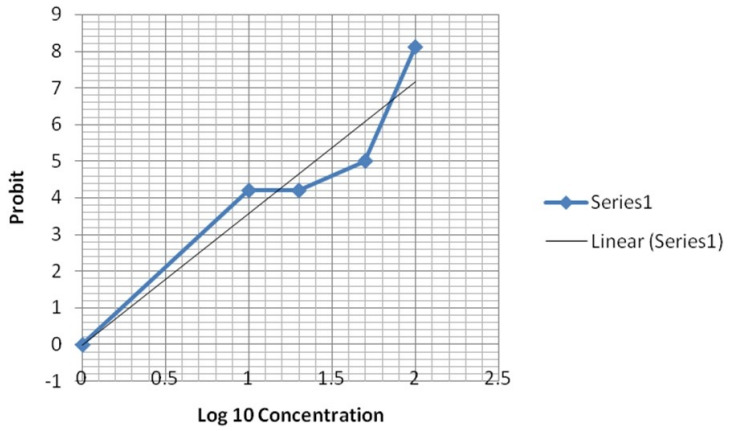

Figure 1. **for Citrus sinensis leaf extract.**

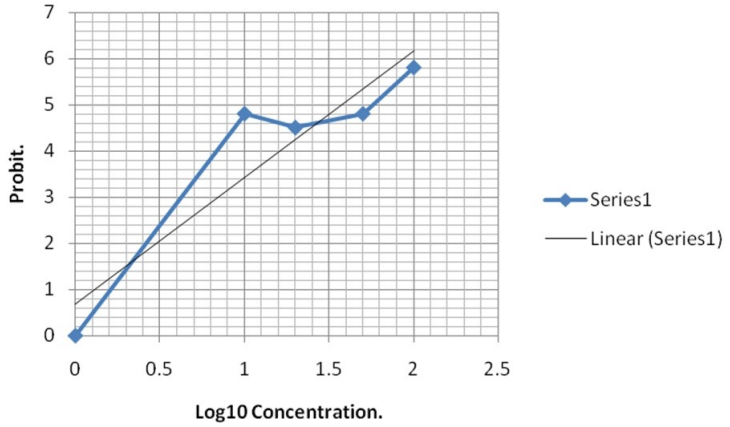

Figure 2. **for Piper guineense leaf extract.**

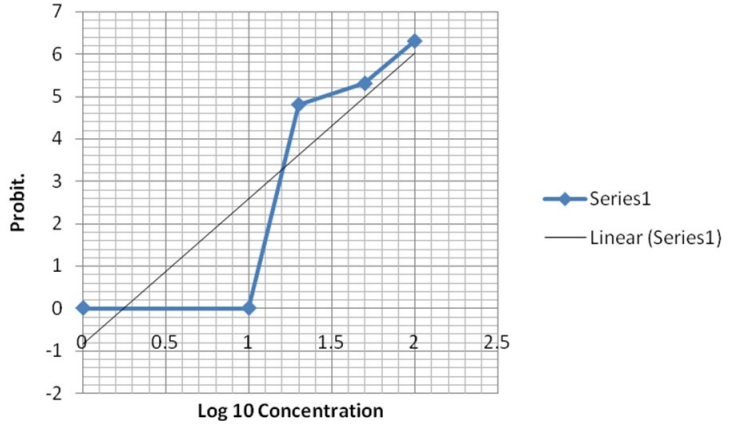

Figure 3. **Piper guineense seed extract.**

biological activities [12]. Ethanol is a good solvent, capable of extracting phytochemical components. The more the concentration of the plant extracts, the more the larvicidal effects. The LC50 for ethanolic extracts for C. sinensis peel, P. guineense seed and leaf were 50.12mg/ml, 25.12mg/ml and 39.82mg/ml respectively. These can be compared with LC50 of 55-65mg/ml for some Neem extracts as reported by Asher and Meisner, (1989) and 60.9mg/ml of ethanolic extract of Ocimum gratissimum [13]. Resistance of mosquito vectors to synthetic insecticides, high cost of these insecticides and their adverse effects on the environment have been a major challenge in vector control. Botanical insecticides/larvicides may be used as alternatives to synthetic ones as they are cheap, eco-friendly, readily available, biodegradable and sage [14–17].

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