Larvicidal activities of crude and purified lectins from the stem bark of Ordeal Tree *(Erythrophleum suaveolens) on Culex quinquefasciatus* larvae.

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ABSTRACT

Lectins from the saline extract of *Erythrophleum suaveolens* stem bark were partially purified and evaluated in terms of their larvicidal properties against *Culex quinquefasciatus.* A series of nine concentrations of the extracts ranging from 0.0001 mg/L, 0.001 mg/L, 0.01 mg/L, 0.1 mg/L, 1mg/L, 5mg/L, 10 mg/L, 50 mg/L and 100mg/L were tested against the third instar larvae of C*ulex quinquefasciatus* and their percentage mortalities and LC_{50} values were obtained. The crude extract of *Erythrophleum suaveolens* showed very high larvicidal activity with 96% mortality achieved at a concentration of 100 mg/L and had an LC_{50} of 4.39 mg/L. The gel peaks of *Erythrophleum suaveolens* showed larvicidal activities to C*ulex quinquefasciatus* larvae with 74.67% and 69.33% mortality achieved at a concentration of 100 mg/L and had LC_{50} of 43.73mg/L and 43.65mg/L respectively. The ion exchange purified extracts of *Erythrophleum suaveolens* showed low larvicidal activity to C*ulex quinquefasciatus* larvae with 10.67% and 9.33% mortality achieved at a concentration of 100 mg/L respectively. These results suggest that the saline stem bark extract of *Erythrophleum suaveolens* is promising as a larvicide against mosquito larvae and can be used directly in small volumes in aquatic habitats and in mosquito population management programme.

KEY WORDS: *Erythrophleum suaveolens*, *Culex quinquefasciatus*, Concentrations larvicidal activities, Lectins,

1.0 INTRODUCTION

Insect transmitted diseases are important health problems in the world. These vectors are insects such as mosquitoes (Roberts, 2002). Mosquitoes are among the most important group of insects in terms of their medical importance to both humans and animals. Most insecticides are non-selective, not biodegradable and can be harmful to other organisms and to the environment. An approach to obtain new efficient, safe and selective insecticides is the study of natural models such as the defensive mechanisms of plants (Ciccia *et al.*, 2000). Bioactive organic compounds produced by plants can act as repellant, food deterrents, growth inhibitors, and toxins (Ezeonu *et al.*, 2001; Carlini and Grossi-de-Sá, 2002). Thus, crude plant extracts have been screened as natural and biodegradable forms to control pests and vectors of infectious diseases (Omena *et al.*, 2007). Lectins play an important role in plants defense against insect pests, and have been found to be toxic to viruses, bacteria, fungi, insects and higher animals (Sauvion *et al*., 2004.This carbohydrate recognition property is involved in the lectin entomotoxic activity on larvae, developing stages and mature forms of insects (Bandyopadhyay *et al.*, 2001; Macedo *et al.*, 2002; Macedo *et al.*, 2004; Sauvion *et al*., 2004; Leite *et al.*, 2005; Kaur *et al.*, 2006). The larvicidal activity was related to lectin binding to chitin components in the insect gut, interaction with glycoconjugates on the surface of epithelial cells along the digestive tract, binding to the sugar moiety of glycosylated digestive enzymes or assimilatory proteins and resistance to insect digestive proteases (Coelho *et al.*, 2007). *Erythrophleum suaveolens* commonly called “ordeal tree” is known as *Erun obo* in Yoruba, *Gwaska* in Hausa and *Inyi* in Ibo (Lawal, 2010). It is a poisonous plant that is widespread in tropical Africa, belonging to the *Fabaceae* family. It is a large tree with a smooth bark which is pale grey, with large irregularly shaped thin pieces peeling off the lower trunk. The leaves are 8 to 20 alternate and terminal leaflets (Burkill, 1995).

Mosquitoes are flying, biting insects that develop in water during their immature stages. Some of the many species found in the world are considered pests and can transmit diseases to humans. The three most important mosquito groups are the *Anopheles* (carrier of malaria), *Culex* (carrier of viral encephalitis), and *Aedes* (carrier of yellow fever, dengue, and encephalitis) (Anonymous, 2009. The use of synthetic insecticides in the control of insects is expensive (Franzen, 1993). There are

problems of pathogen resistance and negative effects on non-target organisms including humans and the environment in terms of pollution (F.A.O.1992, Franzen, 1993, Rembold, 1984). There is a need, therefore, to identify botanical insecticides which are toxic to insects but at the same time do not exhibit toxicity to mammals and humans (Jbilou *et al*., 2006). Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Jbilou *et al.*, 2006). The application of natural insecticides and fungicides could provide a cheap and environmentally friendly alternative (Macedo *et al*., 2000). Therefore, there is a need to curtail the presence of chemicals in the environment as a means of guarding against their adverse effect on the environment, by employing environmentally safe method of insect and pest control, using lectins from *Erythrophleum suaveolens.* The wood of *Erythrophleum suaveolens* is resistant to termite and fungi biodegradation (Okeyo, 2006). Although this information has been available for hundreds of years and hence the popularity of *Erythrophleum suaveolens* for construction purposes, there is no scientific investigation to determine the toxic activity against insects or the mode of action of the active substances. Based on the foregoing, this study sort to scientifically evaluate the larvicidal activity of the *Erythrophleum suaveolens* on the *Culex quinquefasciatus* larvae and its mode of action and application.

2.0 MATERIALS AND METHOD

2.1 Plant Material

The plant stem bark and leaves were collected from Kagarko in Kaduna State. The plant was identified to be E*rythrophleum suaveolens* with voucher number 242 at the Herbarium, Botany Unit, Ahmadu Bello University, Zaria, Kaduna state. The *Erythrophleum suaveolens* bark was dried to a constant weight at 45°C for four days. The dried bark was pulverized into powdered form with a wooden mortar and pestle. A 25g portion of *Erythrophleum suaveolens* powder was extracted in 500mls of 0.02M phosphate buffer pH 7.2 at 4°C at room temperature for 24 hours. The suspension was filtered through muslin cloth and then the filtrate was centrifuged at 8000g for 15 min at 4°C (Shangary *et al.,* 1995). The clear supernatant was collected and assayed for haemagglutination activity and total protein content. Purification was carried out by Gel filtration, ion exchange respectively.

2.1.1 Larval Collection and Authentication

The mosquito larvae were harvested from their natural breeding sites within the campus of Ahmadu Bello University, Zaria and identified at the Entomology Laboratory of the Biological Science Department, Ahmadu Bello University (A.B.U), Zaria, to be *Culex quinquefasciatus*.

3.0 Larvicidal Bioassay

3.1 Breeding of *Culex quinquefasciatus* Larvae in the Laboratory

The collected *Culex quinquefasciatus* larvae were reared according to (WHO, 1996) standard of breeding mosquitoes in the laboratory.

3.2 Bioassay of *Culex quinquefasciatus* Larvae with Crude and Purified *Erythrophleum suaveolens* Lectin.

A stock solution each of the crude and purified *Erythrophleum suaveolens* lectins were made and kept separately in a screwed cap vials. First, the stock solutions were serially diluted; A control dish was setup alongside the experimental ones. The larvae in all the bowls were fed every twenty four hours on a pinch of ground fish meal which was spread evenly across the water surface. The larval mortalities were recorded after 24hrs and 48hrs exposure as percent corrected mortalities. The experiment was carried out in triplicate values. The LC_{50} and LC_{90}values of the crude and purified bark extract of *Erythrophleum suaveolens* for *Culex quinquefasciatus* were obtained separately by calculating the regression line employing probit analysis of Finney, (1964) as described by Busvin, (1971).

3.3 STATISTICAL ANALYSIS

The corrected percentage mortality was expressed as mean ± SEM and analyzed by ANOVA.

4.0 RESULTS AND DISCUSSION

The relationship between larvae mortality and the concentration of the crude and purified fractions after 24 hours was as represented in Figure 1. The corrected percentage mortality for the larvicidal activity of the crude extract of *Erythrophleum suaveolens* bark produced 88% death at 100mg/L

concentration. At 50 mg/L concentration the mortality recorded was 86% and at 10mg/L concentrations the percentage mortality recorded was 80%. The same relationship monitored after 48 hours is as represented in Figure 2. Increase of exposure time to 48hrs raised the percentage mortality of the crude *Erythrophleum suaveolens* lectin to 96% at 100mg/L concentration. At 50 mg/L and 10mg/L concentrations the mortalities recorded were similar (93.33%). The Gel peak I fraction produced, 68% and 74.67% mortalities at 100mg/L concentration after 24 and 48 hours exposure respectively. While at 50 mg/L concentration the mortalities recorded were 64% and 68% after 24 and 48 hours exposure respectively. At 10mg/L concentrations the percentage mortalities recorded were 58.67% and 62.67 % after 24 and 48 hours exposure respectively. Gel peak II fraction resulted in 65.33% and 69.33% mortalities at 100mg/L concentration after 24 and 48 hours exposure respectively. At 50 mg/L concentration the mortalities recorded were 60% and 65% after 24 and 48 hours exposure respectively. At 10mg/L concentration the mortalities recorded were 49.33% and 60% after 24 and 48 hours exposure respectively. Ion peak I fraction produced similar mortalities (10.67%) at 100mg/L concentration after 24 and 48 hours exposure respectively. At 50 mg/L concentration the mortalities recorded were 0% and 1.33 % after 24 hours and 48 hours exposure respectively. At 10mg/L concentrations there was no mortality recorded after 24 and 48 hours exposure. The Ion peak II fraction resulted in 4 % and 9.33% mortalities at 100mg/L concentration after 24 hours and 48 hours exposure respectively. At 50 mg/L concentration the mortalities recorded were 0% and 9.33% after 24 and 48 hours exposure respectively. At 10mg/L concentrations there was 1.33% mortality was recorded after 24 hours exposure while 4% mortality was recorded after 48 hours exposure. Based on probit analysis the crude extract had LC_{50} of 4.49 mg/L and LC_{90} of 16.20mg/L; while the gel peaks I and II fractions had LC_{50} and LC_{90} of 10mg/L and 43.73 mg/L respectively.

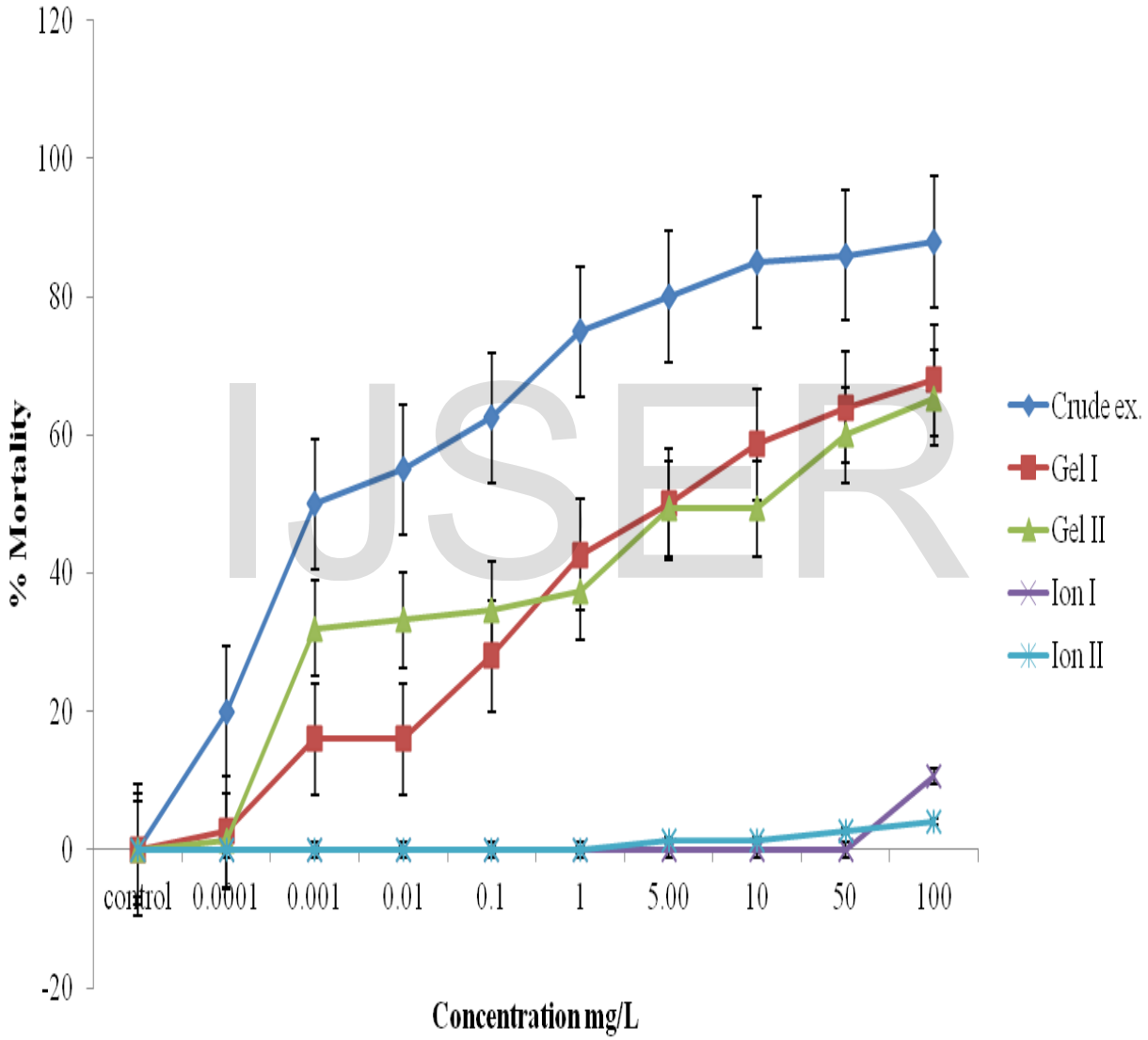

Figure 1: The Corrected Percentage Mortality (± SEM) of Third Instar Larvae of *Culex quinquefasciatus* Exposed to Different Concentrations of Crude and Purified *Erythrophleum suaveolens* Lectin After 24hrs Exposure

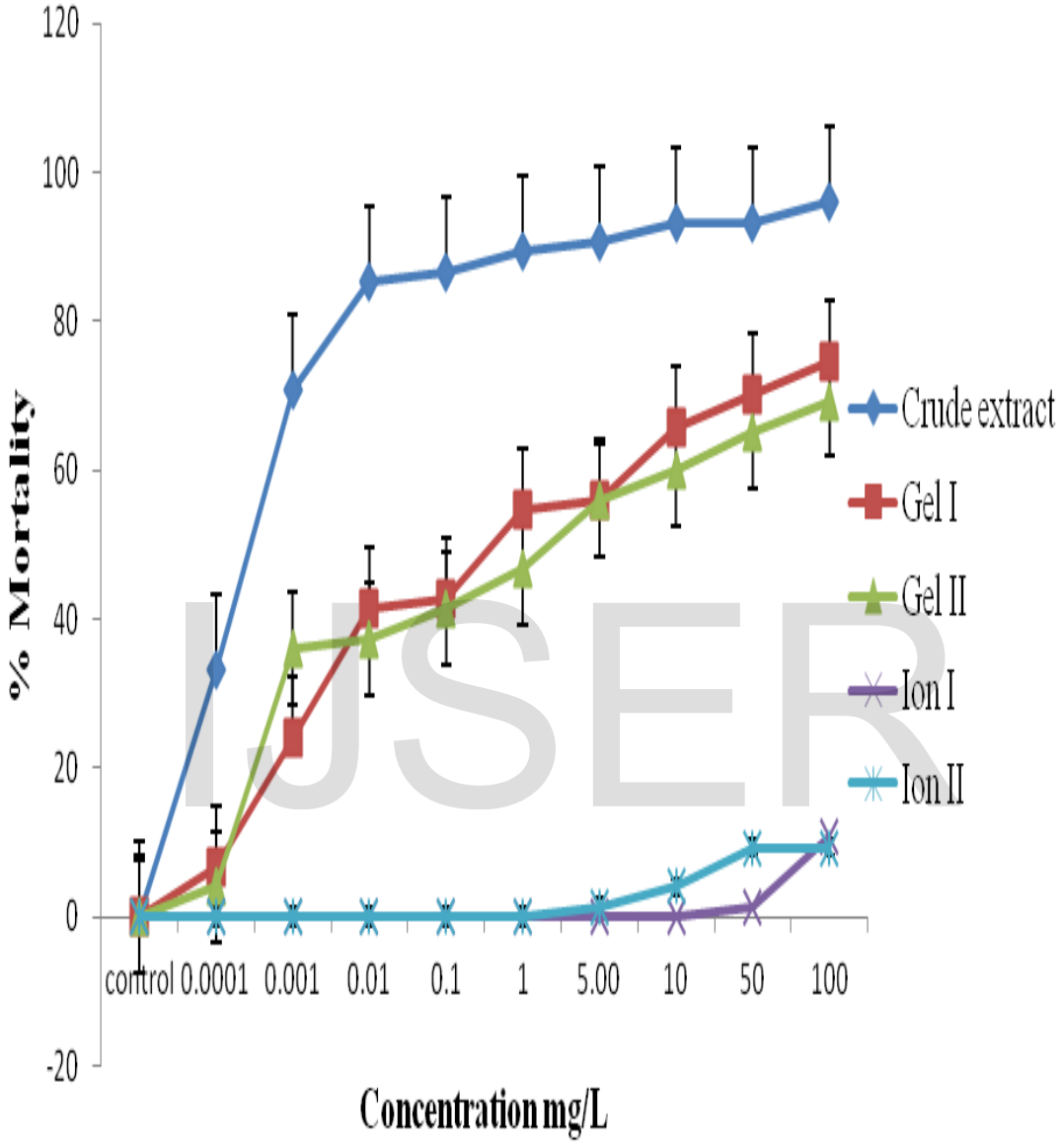

Figure 2: The Corrected Percentage Mortality (± SEM) of Third Instar Larvae of *Culex quinquefasciatus* exposed to Different Concentrations of Crude and Purified *Erythrophleum suaveolens* Lectin After 48hrs exposure.

Table 4.4: Log probit Regression Analysis of the Mortality of 3rd Instar Larvae of *Culex quinquefasciatus* to Crude and Purified *Erythrophleum suaveolens* Lectin

Samples	Regression Equation[c]	Chi-Square[b] (DF)	[a]LC_{50} (Fiducial Limit)	[a]LC_{90} (Fiducial Limit)
Crude ex.				
100mg/l	y= 2.33x+3.5	0.42	4.49	16.20
		(4)	-	-
50mg/l	y= 2.33x+3.5	2.02	4.49	16.20
		(4)	(-14.90-2.23)	(-56.782-10.354)
10mg/l	y= 2x+3	0.26	10	43.73
		(4)	(-1.90-4.33)	(-61.16-7.35)
Gel1				
100mg/l	y= 2x+3	0.36	10	43.73
		(3)	(-1.80 -0.48)	(-17.76-4.40)
50mg/l	y= 2x+3	2.71	10	43.73
		(2)	(-0.50-1.28)	(-2.926-0.857)
10mg/l	y= 1.67x+2.5	2.02	29.55	167.68
		(1)	(0.56-3.54)	(-18.41-1.05)
Gel2				
100mg/l	y= 2x+3	4.73	10	43.73
		(4)	(-2.00-0.73)	(-25.88-6.21)
50mg/l	y= 2x+3	2.02	10	43.73
		(1)	(0.556-3.54)	(-18.41-1.05)
10mg/l	y= 1.67x+2.5	59.91	29.55	167.68
		(3)	-	-
Ion1	y= 0	0	0	0
Ion2	y= 0	0	0	0

[a] Lethal concentrations of proteins required to kill 50% (LC50), 90% (LC90) the 3rd instar larvae of *Culex quinquefasciatus* after 48hours exposure. [b] chi-square values. [c] Simple linear regression and regression coefficient values (R2) established using by probit analysis. y: mortality rate (%); x: protein concentration (mg/mL).

The crude and purified *Erythrophleum suaveolens* lectin preparations were able to kill the 3rd instar larvae of *Culex quinquefasciatus* after 24 and 48hours exposure. The LC_{50} and LC_{90} revealed that purification steps yielded preparations with higher values than the initial crude preparations. The crude fraction gave LC_{50} and LC_{90} values lower than the values obtained for the purified fractions revealing that the lectin activity was lost with further purification. Roberto *et al.,* (2009) reported that the evaluation of larvicidal activities of lectins from *Myracrodruon urundeuva* on *Aedes aegypti* revealed that purification protocols yielded preparations with lower LC values than initial crude preparations. Since LC_{50} is a standard measure of the toxicity of a surrounding medium that is required to kill half of a sample population, it does account for chronic effects. A lower LC_{50} means that a substance is more toxic and would require less of the substance to kill the organism ingesting it. The varying susceptibility observed here is in line with reports from previous findings that mosquito species showed differential susceptibility to plant extracts (Pathak *et al.,* 2000). The binding of lectins specific to N-acetylglucosamine to the peritrophic matrix interferes with the digestion and absorption of nutrients (Peumans and Van Damme, 1995; Zhu Salzman *et al.,* 1998; Zhu-Salzman and Salzman, 2001; Carlini and Grossi-de-Sá, 2002; Macedo *et al.,* 2004; Macedo *et al*., 2007). The deleterious effect of lectin on the metabolic processes in the larvae leads to death of the larvae by nutritional deprivation (Fitches and Gatehouse, 1998). The integrity of peritrophic matrix, which has important roles in the digestive processes of insects as well as protection of the insect from invasion by micro-organisms and parasites, is essential for larvae survival (Tellam *et al.,* 1999). Latex of *Calotropis procera* promoted mortality (100%) of third instar larvae of *Aedes aegypti* and it has been suggested that the toxic effect is at least due to latex proteins (Ramos *et al.,* 2006). Ethanolic extract of *Melia azedarach* leaves showed a strong larvicidal activity with LC_{50} of 0.76 g/L determined after 96 hours of bioassay (Coria *et al.,* 2008). Studies with saponins from *Balanites aegyptiaca* callus produced from *in vitro* cultures of roots revealed that concentrations of 500 ppm or greater killed 100% of the larvae population. Therefore the plant could be used as a larvicidal agent against mosquito (Chapagain *et al.,* 2008). Essential oils from plants have investigated for their insecticidal activity. Heartwoods from *Cryptomeria japonica, Cunninghamia lanceolata, Taiwania cryptomerioide* and *Calocedrus formosana* were sources of larvicidal essential oils with LC50 (µg/mL) of 72.0, 106.4, 79.8 and 75.2, respectively (Cheng *et*

al., 2003). From the above study *Erythrophleum suaveolens* lectin (LC_{50} 4.49 mg/mL or 449 ppm) is more efficient than these heartwood oils mentioned above, and it is lower than that obtained for essential oils from *Hyptis pectinata* (502 ppm) revealing its more strong larvicidal activity (Silva *et al.*, 2008). Cavalcanti *et al*., (2004) reported LC_{50} values between 60 and 538 ppm for 9 essential oils from branches, leaves or fruits of 9 plants of the *Graminae (Poaceae), Labiateae (Lamiaceae), Myrtaceae, Rutaceae, Verbenaceae and Zingiberaceae* families.

The crude extract of *Erythrophleum suaveolens* bark was found to be more lethal to the larvae, followed by gel peaks I and II, while Ion peaks I and II produced the lowest mortality. This might be as a result of loss of activity due to the purification processes employed, resulting is low protein content and hence low activity of the lectin.

CONCLUSION

The results from the study showed that the crude extract and Gel peak I and II fraction exhibited good larvicidal activities on exposure to *Culex quinquefasciatus* mosquito larvae. The lowest mortality was observed in the Ion exchange peaks I and II fraction.

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