British Journal of Pharmaceutical Research
13(3): 1-9, 2016, Article no.BJPR.28577
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

A Lectin with Larvicidal Potential from the Fresh Leaves of *Agelanthus brunneus* (Engl.) Van Tiegh Loranthaceae

Imoh I. Johnny[1], Adenike Kuku[2*], Oludele O. Odekanyin[2] and Simeon K. Adesina[3]

[1]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[2]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.
[3]Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

All the authors contributed to the work. Authors AK and SKA supervised the author IIJ who carried out the bench work and wrote the first draft of the manuscript. Author AK provided the facilities for the analysis while the biological work was carried out in author SKA's department. Author OOO contributed to literature search, collation of data and referencing while author AK perfected the final manuscript which was approved by all for publication.

Article Information

DOI: 10.9734/BJPR/2016/28577
Editor(s):
(1) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
(2) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) George W. Francis, University of Bergen, Norway.
(2) Khalid Shawky Hamadah, Al-Azhar University, Egypt.
(3) Ludmila Nakamura Rapado, Universidade de Sao Paulo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/16316

Original Research Article

Received 26th July 2016
Accepted 9th September 2016
Published 24th September 2016

ABSTRACT

Aims: This study reports the physicochemical properties and larvicidal activities of a lectin isolated from the leaves of *Agelanthus brunneus* (Engl.) Van Tiegh with a view to exploring its potential application and usage.
Methods: The fresh leaves were homogenized with PBS and partial purification of the lectin was carried out by gel filtration on Sephadex G-75; physicochemical and biological properties of the

**Corresponding author: E-mail: adenikekuku@yahoo.com, adenikekuku@gmail.com;*

purified lectin were also determined.
Results: The results revealed that lectin from *A. brunneus* leaves agglutinated non-specifically the red blood cells of the human ABO system as well as the rabbit erythrocytes and the haemagglutinating activity was inhibited by lactose. The lectin was stable over a broad temperature range (up to 70℃) and maximum activity was observed in the 3 - 7 pH range. EDTA had no inhibitory effect on its haemagglutinating activity. Lectin activity was found to be maximal when photosynthesis was at its peak. The lectin was found to be toxic on *Culex quinquefasciatus* (Say) with LC_{50} of 0.24 mg/ml.
Conclusion: The findings of the present study clearly revealed that the lactose-specific lectin from *A. brunneus* was an effective larvicidal agent against *C. quinquefasciatus* mosquito larvae and could be developed as biological control agent.

Keywords: Agelanthus brunneus; lectin; larvicidal; Culex quinquefasciatus; leaves.

1. INTRODUCTION

Mosquitoes are not only the most important vectors for the transmission of malaria, filariasis and viral diseases but are also an important pest of humans, causing allergic responses that include local skin reaction and other biological reactions. The vector-borne diseases (VBDs) are increasing and have been spreading to newer areas recently due to the increased risk of transmission fuelled by development activities, demographic changes and introduction of new products. Control of the mosquito larvae is frequently dependent on continuous application of organophosphates (chlorpyrifos, temephos, and fenthion) and insect growth regulators (diflubenzuron and methoprene) [1].

Mosquitoes have become highly resistant to these products and beside this the synthetic insecticides have been shown to be harmful to human health. Hence there is a constant need to develop biologically - active plant based products as larvicides, A larvicide is an insecticide that is specifically targeted against the larva life stage of an insect. They may be contact or stomach poisons, growth regulators or increasingly biological control agents [2] Use of plant-based larvicide will be an effective alternative on combating the larval stage of the mosquito, since it is more biodegradable, nonpollutant and shows selective toxicity towards invertebrates.

Plant lectins are proteins with the ability to interact with carbohydrates and thus combine with glycocomponents on the cell surface, leading to their biological properties such as agglutination, mitogenicity, insecticidal, anti-inflammatory, anti-cancer e.t.c. [2-4]. The anti-insect/ larvicidal activities of this class of proteins have been widely investigated. The most likely mechanisms underlying their entomotoxic activity is the interaction of the proteins with different glycoproteins or glycan structures in the phytophagous insects which may interfere with some physiological processes such as digestion, detoxification, development and reproduction in these organisms [5,6]. Ingestion of plant lectins have been reported to affect several biological parameters in insects, such as larval weight, fecundity, pupation, survival, as well as, delay in larval development and adult emergence [6].

Seven genera of the Loranthaceae - *Helixanthera, Berhautia, Englerina, Globimetula, Agelanthus*, *Tapinanthus* and *Phragmanthera* – with about five dozen or more species are recognized in West Africa [7]. *A. brunneus* is found on many tree crops of economic importance including the citrus species, especially sweet orange (*Citrus sinensis* L.), tangerine (*C. reticulata* Blanco) and grape (*C. paradisi* L.), cocoa (*Theobroma cacao* L.) and rubber (*Hevea brasiliensis* Muell Arg) [7].

We report herein the isolation and partial characterization of a lectin from the leaves of *A. brunneus* and investigation of the larvicidal activity of the lectin against the mosquito, *C. quinquefasciatus*.

2. MATERIALS AND METHODS

2.1 Materials

Mistletoe (*A. brunneus*) was obtained in March-April (dry season) and June-July (rainy season) from the host *C. reticulata* Blanco at the Agricultural Research Farm of the Obafemi Awolowo University, Ile Ife, Nigeria and was taxonomically identified in the IFE Herbarium at the Department of Botany of the University. Human red blood cells from the ABO system were obtained from the blood of healthy donors

in the Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife and rabbit blood was obtained from rabbits purchased from the Agricultural Research Farm of the Obafemi Awolowo University, Ile Ife, Nigeria.

2.2 Methods

2.2.1 Extraction and purification of lectin

A. brunneus leaves were washed and sliced into small pieces. Sliced leaves (100 g) were homogenized with phosphate - buffered saline (PBS, 500 ml), pH 7.2 containing phenylmethylsulfonylflouride (PMSF, protease inhibitor). The homogenate was centrifuged at 4500 rpm for 15 mins and the supernatant was collected, filtered, and stored as crude extract at −20℃. The crude extract was applied on a Sephadex G-75 column (2.5 X 40 cm), previously equilibrated with PBS, pH 7.2 and eluted with the same buffer. Fractions (4 ml) were collected at a flow rate of 24 ml/hr, monitored spectrophotometrically at 280 nm and assayed for hemagglutinating activity. The elution profile of the gel filtration on Sephadex G-75 is shown in Fig. 1. The fractions in the hemagglutinating activity active peak were pooled and dialyzed exhaustively against double distilled water. The dialysate was lyophilized into powder and resuspended in PBS.

2.2.2 Sample protein concentration determination

The protein concentration was determined according to the Lowry method [8] using Bovine serum albumin as standard.

2.2.3 Assay of hemagglutinating activity and sugar specificity

Agglutination of red blood cells by the lectin was carried out as described by Kuku and Eretan [9]. PBS (100 µl) was delivered sequentially into wells arranged in rows (each row contained 12 wells) in a U – shaped microtitre plate. Lectin (100 µl) was added into the first well to obtain a two-fold dilution. A serial dilution was made by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS. Aliquots (50 µl) of the 2% erythrocyte suspension were added to each well and the microtitre plates were left undisturbed for 1 h. The hemagglutination titre was taken as the reciprocal value of the highest dilution of the lectin causing visible hemagglutination of the erythrocytes. Specific activity of the lectin is the number of hemagglutination units per mg protein expressed as hemagglutination units (HU) / mg.

The inhibitory effect of various sugars on the hemagglutinating activity of the lectin was carried out according to the method of [10]. Each sugar (0.2 M) was prepared in PBS and a serial dilution of the lectin sample was made until the end-point causing hemagglutination was obtained. Sugar solution (50 µl) was added to each well and allowed to stand for 30 minutes at room temperature and then mixed with erythrocyte suspension (50 µl, 2%). The hemagglutination titres obtained were compared with a non-sugar containing blank.

2.2.4 Effect of Temperature, pH, and EDTA on hemagglutinating activity of *A. brunneus* lectin

The heat stability test was performed as described by Sampaio et al. [11]. Aliquots of the lectin solution were incubated in a water bath at different temperatures (30 – 90℃) for 30 min, then rapidly cooled in ice and assayed for hemagglutinating activity. The agglutinating activity of lectin sample kept at 20℃ for 30 min was used as control.

The effect of pH on hemagglutinating activity of the lectin was determined by incubating the lectin sample in buffer solutions of different pH values at room temperature for 1 hr. The buffers used were 0.2 M citrate buffer (pH 2 – 6); 0.2 M Tris-HCl buffer (pH 7 – 8) and 0.2 M glycine-NaOH buffer (pH 9 – 11). The hemagglutinating activity of the lectin was determined. The hemagglutination titre of the lectin sample incubated in PBS, pH 7.2, served as the control.

To examine the divalent cations requirement of *A. brunneus* lectin **(**ABL) for hemagglutinating activity, demetallization of the purified lectin was carried out according to the method of Wang et al. [12] using 10 mM EDTA. The lectin sample was dialyzed against 10 mM EDTA for 24 h and the hemagglutinating activity of the lectin was determined. The treated lectin was then incubated with 50 µl each of the following salts: 10 mM $ZnSO_4$, $CaCl_2$, $MgCl_2$, $MnCl_2$, $HgCl_2$, $NiCl_2$, $CoCl_2$ and $SnCl_2$ for 2 hours followed by hemagglutination assay.

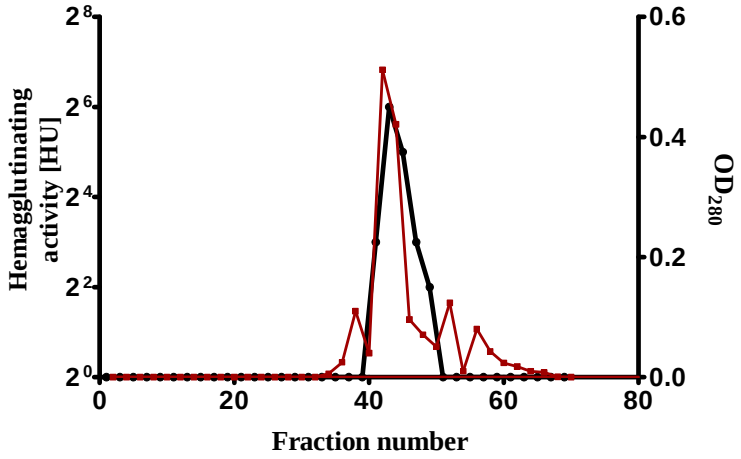


Fig. 1. Elution profile for *A. brunneus* leaf crude extract
Eluant was PBS, pH 7.2, Column size was 2.5 × 40 cm; Flow rate was 24 ml/hr while the fraction size was 4 ml. (------■-----) OD_{280} nm, (------●-----) Hemagglutinating activity

2.2.5 Larvicidal activity studies

2.2.5.1 Origin and rearing of the mosquitoes

Mosquitoes used in this study were *Culex quinquefasciatus* Say. They were collected from stagnant water at the Agricultural Research Farm of the Obafemi Awolowo University, Ile Ife, and were reared for several generations in the insectarium of the Drug Research and Production Unit Laboratory, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, under controlled conditions at temperature of 27 ± 2℃, relative humidity 70 ± 10% and 12 - 12 light-dark regime. Adult mosquitoes were kept in (30 × 30 × 30 cm) wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3 - 4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (anautogeny). Plastic cup oviposition (15 × 15 cm) containing dechlorinated tap water was placed in the cage. The obtained egg rafts were picked up from the plastic dish and transferred into plastic pans (25 × 30 × 15 cm) containing 3 liters of tap water and left for 24 hours. The hatching larvae were provided daily with fish food as a diet. This diet was found to be the most preferable food for the larval development and female fecundity [13].

2.2.5.2 Larvicidal bioassay

Fourth instar larvae of *Culex quinquifasciatus* were tested with different concentrations (0.05 - 0.80 mg/ml) of *A. brunneus* lectin according to the standard WHO procedure [14]. A total of 10 fourth instar larvae were introduced into 50 ml plastic cups containing various concentrations of the lectin. The treatments were carried out in three replicates, and each replicate set contained one control. Mortalities were recorded after 24 hours of the exposure period. Laboratory room temperature was maintained at 27 ± 2℃ during the experiment period. The moribund and dead larvae in three replicates were combined and average expressed as percentage mortality for each concentration. Dead larvae were identified when they failed to move after probing with a needle. Moribund larvae were those unable to rise to the surface within a reasonable period of time. The percentage mortality was calculated and analysis of data was carried out using probit analysis [15].

3. RESULTS AND DISCUSSION

In the last two decades, researchers in the field of mosquito control have focused their attention on searching for environmental friendly insecticide molecules from plant materials. This has revealed the presence and efficacy of different phytochemicals from various plants against different species of mosquitoes. Plant lectins have been isolated from bark, flowers, leaves, rhizomes, roots and seeds. They differ from each other with respect to their molecular structures, carbohydrate-binding specificities and biological activities [16]. Many plant lectins were shown to have harmful effects on larvae, developing stages and mature forms of insects from orders such as Lepidoptera, Coleoptera,

Diptera (where the mosquito belong), Hemiptera among others [17-19]. This necessitated further search for more lectins with larvicidal activity which could be developed to mosquito control agents with practical utility.

The present study revealed that phosphate-buffered saline was able to isolate lectin from *A. brunneus* leaves in measurable amount. Partial purification of the lectin was done by subjecting the crude extract to gel filtration technique on a Sephadex G-75 column. A total of four protein peaks were obtained, only one peak was active. *A. brunneus* leaves lectin was a strong hemagglutinin when tested against the human red blood cells of the ABO system. The lectin agglutinated all human and rabbit blood erythrocytes to the same magnitude (Titre: 2^8). The lectin from *A. brunneus* agglutinated red blood cells non-specifically and thus belongs to the group of lectins called panalectins. A similar result was reported by Kuku and Eretan [9] for the lectin from *Kalanchoe crenata* (Andr.) Haw leaves. It was also reported that purified *Erythrina speciosa* lectin agglutinated all the human blood group erythrocytes with slight preference toward the O blood group erythrocytes [20].

Lectins agglutinate erythrocytes by binding to carbohydrate residues of glycoproteins on their surfaces. Thus, the interaction of lectins with cells can sometimes be inhibited specifically by simple sugars. A number of monosaccharide, disaccharides and derived sugars were tested for their ability to inhibit the hemagglutinating activity of the lectin. The hemagglutinating activity of the lectin was slightly inhibited by the disaccharide, lactose. Galactose, arabinose and dulcitol also reduced the activity to some extent (Table 1). The *A. brunneus* leaf lectin can therefore be classified as a galactose-specific lectin because of its affinity towards galactose and galactose derived sugars (lactose). Lectins isolated from *E. speciosa* seed and *E. indica* leaves were shown to have carbohydrate specificity directed towards D-galactose and its derivatives with distinct preference for lactose [20,21].

It is well known that the concentration of biologically active constituents varies with time and season [22]. Table 2 shows the variability of hemagglutinating activity of *A. brunneus* leaf lectin investigated based on time and seasons of collection. The results of this study suggested that the best harvesting time and season was when photosynthesis was at its peak in the two seasons. Sahoo et al. [23] studied the variations in the total amount of secondary metabolites of some plants during different season and discovered that the most favourable season for the production of secondary metabolites is summer season. Recently, Yadav et al. [22] also observed that the plants which were collected in summer season were noticeably more active than the plants collected in winter and rainy seasons. Such effects on biochemical and bioactive ingredients have been observed in a number of studies in different species such as *Mentha spicata* [24], *Toona sinensis* [25], and *Adiantum capillus-vineris* [26]. These studies reported that quantity and quality of secondary metabolites are associated with weather conditions that favour higher concentration of biologically active chemicals.

Table 1. Sugar inhibition of hemagglutinating activity of the *A. brunneus* leaf lectin

Sugar	Hemagglutinating titre
Control	2^{8*}
Glucose	2^7
Rhamnose	2^7
Galactose	2^6
Arabinose	2^6
Sucrose	2^8
Fructose	2^8
Maltose	2^8
Lactose	2^4
Cellobiose	2^8
Sorbose	2^8
Mannitol	2^7
N-acetyl-glucosamine	2^7
D (+)-glucosamine hydrochloride	2^7
1-O-methyl-α-D-glucopyranoside	2^9
Mannose	2^7
Dulcitol	2^6
D-galactosamine hydrochloride	2^7
D-sorbitol	2^7

Each experiment consisted of 100 µl lectin serially diluted in U-shaped microtitre wells. Sugar solution (50 µl of 0.2 M in PBS) was added to each well followed by 50 µl of 4% suspension of type B red blood cells. Heamagglutinating Titre (HU) was taken as the reciprocal of the last dilution showing agglutination of cells

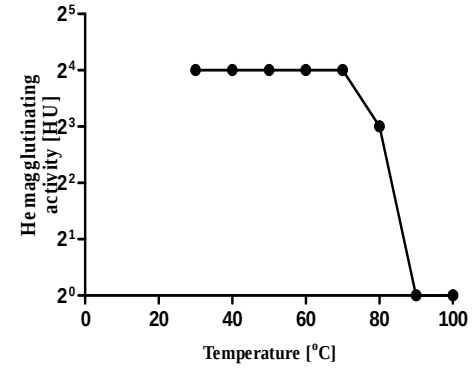
The thermostability and pH stability characteristics of lectins are known to differ from one lectin to the other [12]. *A. brunneus* lectin was found to be heat-stable below 70℃, where it

maintained 100% of its hemagglutinating activity. Increasing the temperature to 80℃ led to 50% loss in its activity. The activity was totally inactivated when heated at 90℃ (Fig. 2A). Leaf lectin of *E. indica* heat stability was over a wide range of temperature (25 – 50℃) but at 60℃ the lectin lost almost all of its activity [20]. Another leaf lectin from *Manihot esculenta* showed heat stability that fell in line with the result of the current study. *Manihot esculenta* leaf lectin was found stable up to 70℃ [27]. The thermal stability of ABL is comparable to what was reported for the *E. speciosa* seed lectin which was found stable up to 65℃ for 90 minutes above which there was a gradual loss of activity [20]. A highly thermostable lectin from *Ganoderma capense* was reported to be stable at 100℃ for 60 minutes [28]. Stability over a broad range of temperature is characteristic of plant lectins and can be attributed to their function in plant defense mechanisms against environmental stress [2]. *A. brunneus* lectin was insensitive to acidic pH, where it maintained 100% maximal activity between pH 3 – 7, but was markedly affected by basic pH with the hemagglutinating activity decreasing to 50%, 25%, 12.5% and 0% at pH values of 8 – 11 respectively (Fig. 2B). The ABL pH stability fell within the range at which *Ganoderma capense* lectin was found stable (pH 4 - 11) [28]. Also, a comparable result of pH sensitivity was reported in 2011 by Charungchitrak et al. [29] for *Archidendron jiringa* seed lectin.

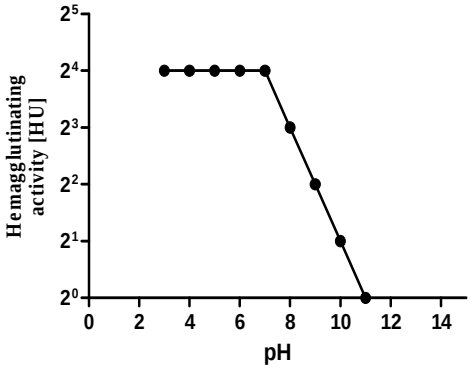
Table 2. Seasonal changes in hemagglutinating activity in the leaves of *A. brunneus* during the dry season and rainy season

Time of collection	Hemagglutinating activity titre	
	Dry season (March-April)	Rainy season (June-July)
8.45 am	2^1	2^0
10.45 am	2^4	2^0
12.45 pm	2^8	2^2
2.45 pm	2^4	2^8
4.45 pm	2^2	2^2

Previously, the importance of metal ions to the hemagglutinating activity of lectin has been established [20,30]. Kuku et al. [30] showed that *E. senegalensis* lectin required the presence of Ca^{2+} and Mn^{2+} for its maximum hemagglutinating activity. In the present studies, extended dialysis of ABL against 10 mM EDTA did not cause any change in its hemagglutinating activity, even when the EDTA concentration was increased to 50 mM. This could mean that either the lectin activity was not dependent on the metal ions or that the ions present were too strongly held in the lectin structure and could not be removed by dialysis against the chelating agent (EDTA).



A



B

Figs. 2 A & B. Effect of temperature (A) and pH (B) on haemagglutinating activity of *A. brunneus* leaf lectin

Worldwide use of insecticides has dramatically increased during the last two decades and this is known to cause a lot of problems to both the environment and human health. It is, therefore, necessary to balance environmental and health issues when using insecticides. So far, insecticides of plant origin have been the focus of many researchers. Secondary metabolites like tannins, phenols, flavones, favonols, saponin and alkaloids [31,32] and proteins such as lectins, toxins, arcelins, protease inhibitors [33,34] have been mentioned as possible compounds with insecticidal activity. Our investigations revealed that *A. brunneus* lectin, although partially purified, showed larvicidal activity against fourth-stage larvae in a concentration-dependent

Table 3. Larvicidal activity of partially purified lectin from the leaf of *A. brunneus*

S/N	Lectin conc. (mg/ml)	No. of larvae	Recorded death	% mortality
1	0.050	10	1.00±0.00	10.00±0.00
2	0.100	10	3.00±0.82	33.00±4.50
3	0.200	10	5.00±0.50	50.00±5.80
4	0.400	10	6.00±0.84	60.00±6.25
5	0.800	10	8.00±1.43	80.00±7.50
Control	Distilled water	10	0	0

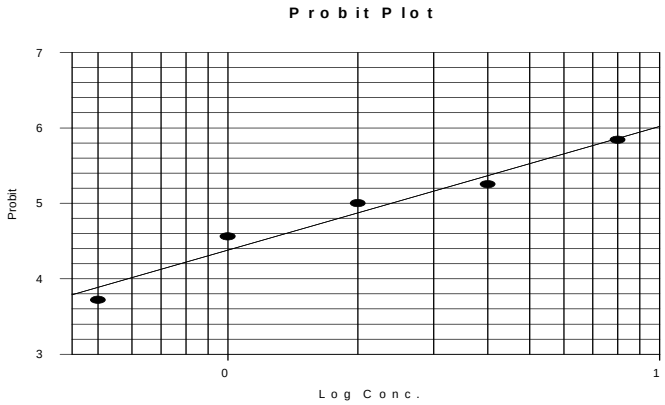


Fig. 3. Probit graph for determination of LC_{50}

manner. The lectin promoted larvae mortality with LC_{50} of 0.24 mg/ml (Table 3, Fig. 3 above). Similar findings of increased mortality with increased dose rate were also reported by Ferreira et al. [35], 99.2 percent larval mortality within 24 hours at 5,200 µg/ml of water extract of *Moringa oleifera* seeds at the highest application rate. The hemagglutinating activity of ABL was not affected by exposure to sunlight, indicating that the lectin was resistant to environmental radiation, an important characteristic used in *C. quinquifasciatus* control. Sa et al. [18] reported that *Myracrondruon urundeuva* bark and heartwood lectin (MuBL and MuHL) showed larvicidal activity against fourth-stage larvae in a concentration-dependent manner with LC_{50} of 0.125 mg/ml and 0.04 mg/ml respectively. Lectin activity detected from the seed water extract of *Dioclea megacarpa* and *Enterolobium contortisiliquum* were demonstrated to be likely cause of the larvicidal activity shown against *Aedes aegypti* [32].

4. CONCLUSION

Our investigation demonstrated the larvicidal potential of partially purified lectin from *Agelanthus brunneus* leaf. This lectin elicited physicochemical characteristics similar to the lectin from *Manihot esculenta* leaf and other plant lectins with good larvicidal property. The results may help in developing an effective larvicidal agent that is eco-friendly and a better alternative to chemical insecticides. Further study is therefore needed to elucidate the mechanism of the lectin larvicidal activity and also to establish its potential against other developmental stages of the mosquito.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to acknowledge Mr. F.B. Adewoyin of the Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, for his assistance in the rearing

of the mosquito larvae in the Insectarium of the Unit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yang YC, Lee HK, Kim MK, Lee SH, Lee HS. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agricul. Chem. Biotechnol. 2002;50:3765-3767.
2. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J. Med Res. 2012;135:581–598.
3. Weis WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. Ann. Rev. Biochem. 1996;65:441-473.
4. Vandenborre G, Smagge G, Van Damme EJM. Plant lectins as defense proteins against phytophagous insects. Phytochem. 2011;72:1538–1550.
5. Michiels K, Van Damme EJM, Smagge G. Plant- Insect interactions: What can we learn from plant lectins? Arch. Insect Biochem. Physiol. 2010;73:193–212.
6. Kaur M, Singh K, Rup PJ, Kamboj SS, Singh J. Anti-insect potential of lectins from *Arisaema species* towards *Bactrocera cucurbitae*. J. Environ. Biol. 2009;36: 1263-1268.
7. Adesina SK, Illoh HC, Johnny II Jacobs IE. African mistletoes (Loranthaceae): Ethnopharmacology, chemistry and medicinal values: An update. Afri. J. Trad., Compl. Alter. Med. 2013;10(3):161-70.
8. Lowry OH, Rosenbrough NJ, Farr AL, Rondall RJ. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951; 193:265-273.
9. Kuku A, Eretan OB. Purification and partial characterization of a lectin from the fresh leaves of *Kalanchoe crenata* (Andr. Haw). J. Biochem. Molec. Biol. 2004;37(20):229-233.
10. Eghianruwa Q, Odekanyin O, Kuku A. Physicochemical properties and acute toxicity studies of a lectin from the saline extract of the fruiting bodies of the shiitake mushroom, *Lentinula edodes* (Berk). Inter. J. Biochem. Mol. Biol. 2011;2(4):309–317.
11. Sampaio AH, Rogers DSJ, Barwell CJ. A galactose-specific lectin from the red marine algae, *Ptilota filicina*. Phytochem. 1998;48:765-769.
12. Wang H, Liu WK, Ng TB, Ooi VEC, Chang ST. The immunomodulatory and antitumor activities of lectins from the mushroom *Tricholoma mongolicum*. Immuno-pharmacol. 1996;31:205-211.
13. Kasap M, Demirhan L. The effect of various larval foods on the rate of adult emergence and fecundity of mosquitoes. Turkiye Parazitologi Dergisi. 1992;161:87-97.
14. World Health Organizations. Instruction for determining the susceptibility or resistance of mosquito larvae to insect development inhibitors. WHO, Geneva; 1981. (WHO/VBC/81.812).
15. Finney DJ. Probit analysis. 3rd ed. Cambridge University Press, New York; 1971.
16. Paiva PMG, Napoleão TH, Sá RA, Coelho LCBB. Insecticide activity of lectins and secondary metabolites, insecticides - advances in integrated pest management, Dr. Farzana Perveen (Ed.). InTech; 2012. DOI: 10.5772/29785
Available:http://www.intechopen.com/books/insecticides-advances-in-integrated-pest-management/insecticide-activity-of-lectins-and-secondary-metabolites
17. Kaur M, Singh K, Rup PJ, Saxena AK, Khan RH, Ashraf MT, Kamboj SS, Singh J. A tuber lectin from *Arisaema helleborifolium* schott with anti-insect activity against melon fruit fly *Bactrocera cucurbitae* (Coquillett) and anti-cancer effect on human cancer cell lines. Arch. Biochem. Biophy. 2006;445:156-165.
18. Sá RA, Santos NDL, da Silva CSB, Napoleão TH, Gomes FS, Cavada BS, Coelho LCBB, Navarro DMAF, Bieber LW, Paiva PMG. Larvicidal activity of lectins from *Myracrodruon urundeuva* on *Aedes aegypti*. Comp. Biochem. Physiol. C, 2009; 149(3):300-306.
19. Souza JD, Silva MBR, Argolo ACC, Napoleão TH, Sá RA, Correia MTS, Paiva PMG, Silva MDC, Coelho LCBB. A new *Bauhinia monandra* galactose-specific lectin purified in milligram quantities from secondary roots with antifungal and termiticidal activities. Inter. Biodeter. Biodegra. 2011;65(5):696-702.
20. Konozy EHE, Bernardes ES, Rosa C, Faca V, Greene LJ, Ward RJ. Isolation, purification and physicochemical characterization of a d-galactose-binding

lectin from seeds of *Erythrina speciosa.* Arch. Biochem. Biophy. 2003;410:222-229.
21. Konozy EHE, Mulay R, Faca V, Ward RJ, Greene LJ, Roque-Barriera MC, Sabharwal S, Bhide SV. Purification, some properties of a d-galactose-binding leaf lectin from *Erythrina indica* and further characterization of seed lectin. Biochimie. 2002;84:1035-1043.
22. Yadav R, Tikar SN, Sharma AK, Tyagi V, Sukumaran D, Jain AK, Veer V. Screening of some weeds of larvicidal activity against, *Aedes albopictus*, a vector of dengue and chikungunya. J. Vector Borne Dis. 2015;52:88–94.
23. Sahoo KP, Kasera PK, Mohammed S. Secondary metabolites produced during different seasons in some arid medicinal plants. Asian J. Plant Sci. Res. 2012;2(6): 650–652.
24. Kofidis G, Bosabalidis A, Kokkini S. Seasonal variation of essential oils from a linalool-rich chemotype of *Mentha spicata* Grown Wild in Greece. J. Essent. Oil Res. 2004;16:469-472.
25. Wang W, Geng C, Zhang Y, Shi X, Ye J. CE-ED separation and determination of seasonal content variations of some active ingredients in *Toona sinensis* (A. Juss.) Roem. leaves. Chromatographia. 2007; 66:697-701.
26. Ahmad I, Hussain M, Ahmad MSA, Ashraf MY, Ahmad R, Ali A. Spatio temporal variations in physiochemical attributes of *Adiantum capillus-veneris* from Soone Valley of salt range (Pakistan). Pak. J. Botany. 2008;40:1387-1398.
27. Silva MC, Correa AD, dos-Santos CD, Marcos FCA, de-Abreu CMP. Partial purification of leaf lectin from *Manihot esculenta* and screening its fungicidal activity. J. Agric. Biotechnol. Sustain. Develop. 2010;2(8):136–141.
28. Ngai PHK, Ng TB. A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes and antiproliferative activity toward tumor cells. Biochem. Biophy. Res. Comm. 2004;314: 988-993.
29. Charungchitrak S, Petson A, Sangvannich P, Karnchanatat A. Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. Food Chem. 2011;126:1025–1032.
30. Kuku A, Odekanyin OO, Okonji RE. Physicochemical properties of a Lactose-specific lectin from the seeds of *Erythrina senegalensis* DC. Ife Journal of Science 2012;14(1):143-154.
31. Luna JS, Santos AF, Lima MRF, Omena MC, Mendocan FAC, Bieber LW, Sant'ana AEG. A study of the larvicidal and mollusicidal activities of some medicinal plants from Northeast Brazil. J. Ethnopharmacol. 2005;97:199–206.
32. Farias DF, Cavalheiro MG, Viana MP, Queiroz VA, Rocha-Bezerra LCB, Vasconcelos IM, Morais SM, Carvalho AFU. Water extracts of Brazilian leguminous seeds as rich sources of larvicidal compounds against *Aedes aegypti* L. An. Acad. Bras. Cienc. 2010; 82:585–594.
33. Carlini CR, Grossi-de-Sa MF. Plant toxic protein with insecticidal properties: A review on their potentialities as bioinsecticides. Toxicon. 2002;20:1519–1539.
34. Whetstone PA, Hammock BD. Delivery methods for peptide and protein toxin in insect control. Toxicon. 2007;49:576–590.
35. Ferreira PMP, Carvalho AFU, Farias DF, Cariolano NG, Melo VMM, Queiroz MGR, Martins AMC, Machado-Neto JG. Larvicidal activity of water extract of *Moringa oleifera* seeds against *Aedes egypti* and its toxicity upon laboratory animals. Anais da Academia Bras Cien. 2009;81(2):207-216.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16316