CYTOTOXICITY OF FRACTIONS OF *Pistia stratiotes* L. ON LARVAE OF *Culex* MOSQUITO AND *A. salina*

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ABSTRACT

Crude chloroform and aqueous extracts of the duckweed, Pistia stratiotes L., were bioassayed at various concentrations for larvicidal activities against larvae of Culex mosquito and Artemia salina (brine shrimp). The crude (LC_{50} = 159.50$^{\mu}$ g/ml) and chloroform extracts (LC_{50} = 0.0909$^{\mu}$ g/ml) exerted mortality at 40$^{\mu}$ g/ml of 16.67 % and 90.0 % respectively on Culex mosquito larvae, while the aqueous extract (LC_{50} = >1000$^{\mu}$ g/ml) at 200$^{\mu}$ g/ml, resulted in 3.33 % mortality. The crude (LC_{50} = 2524.22$^{\mu}$ g/ml) was moderately toxic on A. salina larvae at 1000$^{\mu}$ g/ml which killed 30.00 % of the test organisms. Whereas the chloroform extract showed lower activity on brine shrimp larvae (3.3 % mortality, LC_{50} > 1000$^{\mu}$ g/ml). The aqueous extract demonstrated no activity on brine shrimp at all concentrations tested. The study showed that the chloroform extract of P. stratiotes selectively exerts cytotoxic effect on Culex mosquito larvae resulting in high mortality with LC_{50} = 0.0909$^{\mu}$ g/ml than on the brine shrimp larvae at LC_{50} >1000$^{\mu}$ g/ml. It is therefore recommended that these extracts of P. stratiotes L. should be tested for adulticidal and/or mosquitocidal activity as well as toxicity in higher animals up to man. This may yield a more base line data valuable for use in the development of a microbially active chloroform fraction of the plant for possible use in modern medicine.

Keywords: Toxicity, *Pistia stratiotes,* Chloroform fractions, Aqueous fractions*, Artemia salina, Culex* mosquito.

INRODUCTION

Phytochemical constituents analysis of indigenous medicinal plants can assist in identifying those components that have positive therapeutic values. Important also will be the identification of any toxic element or injurious fractions whose use should be actively discouraged (Mahjuba, 1995). The bioassay of plants used as medicine covered a large number of plants (Mchlaughlin, 1996) but still, there are numerous, other plants including *P. stratiotes* that need to be microbiologically and chemically investigated and evaluated thoroughly.

Cytotoxicity is the ability of a compound to kill a cell. Guided by the brine shrimp lethality test, the cytotoxicity values of several plants extracts such as *Xylopia aromatica, Euphobia poisnii, Lantana camara, Fusarium proliferatum* e.t.c. have been evaluated (Colman and Mclaughing, 1994).

Test for cytotoxic effect of plant extracts on mosquito larvae is also currently accepted as additional pharmacognostic step towards elucidating the lethality and/or safety aspects of a candidate plant extract being investigated (Fatope, 1995). Presently however, there has been paucity of literature on cytotoxicity of *P. stratiotes* on insect larvae.

P. stratiotes, referred to as the tropical duck weed, is one of the most dominant aquatic weeds in fresh water, polluted water and streams of Nigeria. The plant is raised in fishponds as a shelter for certain edible shrimp species. Its usage as salad in swine feeds as well as its preference as foliage by buffalos have been reported. However, it has been reported to exert a poisonous effect on rabbits (Mukhtar and Hafiz, 2001).

Herbalist recommends its use as concoction for relieving nervous disorders and fever. It was also reported to have antagonistic effect on intestinal bacteria. While its leaves are

used locally for the treatment of lice (Mukhtar and Huda, 2003).

The various uses of *P. stratiotes* in the treatment of stomach disorder, throat, and mouth inflammation have been documented. The tissues of *P. stratiotes* when in contact with the mucous membrane are exceedingly irritating (Mukhtar and Tukur, 2000). It was reported that ethanol and hot water fractions of the plant exert antimicrobial action on a few pathogenic bacteria. It was also observed that chloroform fraction of the same plant possess antifungal and antibacterial activities on some pathogens (Mukhtar and Huda, 2003).

The objectives of the present study were: (1) to determine whether extract(s) of *P. stratiotes* can be cytotoxic to larvae of mosquitoes and brine shrimps. (2) to assess what extracts of *P. stratiotes* can be reputed as pesticides/insecticides and as safe potential source of antimicrobial agents.

MATERIAL AND METHODS

The Plant Material: Whole plant of *P. stratiotes* L. was collected by hand-picking on 12th August, 2003 at Kofar Naisa ponds along BUK Road and identified in the Department of Biological Sciences, Bayero University, Kano using the keys after Arber (1972).

Extraction: The leaves of the plant material were air dried and ground into powder using pestle and mortar. 200g of the powder was percolated with 4 litres of 95% ethanol for 2 weeks. The percolate was filtered and the solvent evaporated using a rotary evaporator at 40^0C (Fatope *et al,* 1993). The residue ($F0_1$) was utilized as the crude extract for the subsequent steps.

Fractionation of the Crude Extract ($F0_1$): The crude extract was dissolved in 200ml water and Trichloroform $CHCL_3$ in the ratio of 1:1 mixture, shaken for about 15 minutes, and left to stand overnight. The mixture was partitioned into distinct water – soluble layer, which was drained and labeled ($F0_2$), and the chloroform soluble layer ($F0_3$). These were all evaporated and preserved (Fatope *et al,* 1993).

Brine Shrimp Lethality Bioassay: A portion of instant sea water was poured into a hatching chamber and charged with about 250 *A. salina* shrimp eggs. These were allowed to hatch and mature for two days (Fatope *et al,* 1993).

The vials for testing were prepared and tested initially at 10,000 μ g/ml. Subsequently, a concentration of 1000 μ g/ml and 100 μ g/ml were prepared for each fraction respectively. Three vials for each concentration were prepared for a total of 6 vials per fraction plus a control. The solvent was evaporated at room temperature, overnight. The vials for the control contain non-of the plant extracts. 2 drops of DMSO (Dimethyl Sulphuroxide) were added to each vial plus 4 ml of the sea water followed by addition of 10 shrimps. The volume of the liquid in each vial was adjusted to 5ml with instant sea water. After 24 hours, the numbers of survivors were counted and the LC_{50} was determined at 95% confidence interval using regression analysis (Arias and Mulla, 1975).

Collection and Rearing of Culex Mosquitoes: The eggs of *Culex* mosquitoes were identified by their appearance as they always fastened together vertically in batches of about 100 – 300 forming raft like structure which can float (Sarosini, *et al* 1979). These were collected by scooping from gutters around the Bayero University, Kano (Old campus). The eggs were placed in a container of sterile water to which 0.3g/L of ascorbic acid have previously been added in order to create a low oxygen tension required to facilitate rapid and simultaneous egg hatching. The larvae were harvested and transferred to several beakers of sterile water to which a few grains of baker's yeast were daily added. Every 2 - 3 days a Pasteur pipette was used to suck, so as to remove the feacal and dead matter as well as changing the water (Arias and Mulla, 1975).

As the larvae turn to pupae, it was removed and placed in fresh beakers of sterile tap water and transferred into 'mosquitories', which is in a laboratory fume chamber covered with net to prevent flying adults from escaping or stray mosquitoes entering. The mosquitoes, prior to pupal introduction were sterilized by subjection to perpetual ultra-violet radiation for 48 hours, in addition to thorough cleansing with 'Dettol' disinfectant. Within a day or two, the pupae hatch out into imagoes that were fed with glucose solution.

A mouse (for blood meal) was placed in the mosquitory and left to stand overnight. After successful mating some females proceeded to lay eggs in containers of sterile water.

The containers were daily examined and any batch of eggs laid were immediately transferred to fresh beakers of water containing a little amount of ascorbic acid to stimulate egg

hatching. Emergent larvae were extracted, placed in fresh beakers of water and fed with baker's yeast. The larvae were daily examined and the first instar larvae were harvested for bioassay (Gerberg, 1970).

Preparation of Concentrations of the Plants Extracts for the Test: Four solutions for each of the different fractions were prepared such that a final concentration of 400, 200, 40 and 0 μg/ml in distilled water was obtained. 10 larvae were then added in each case. 0.0 μg/ml of test sample served as control. Test at each dosage was carried out in triplicates at room temperature. After a period of 24 hours, the survivors were counted and the percentage mortality at each dosage was determined. The LC_{50} was determined at 95% confidence interval (Fatope *et al,* 1993).

RESULTS

Some physical parameters (weight, pH, colour and transparency) of crude, chloroform and aqueous extracts of *P. stratiotes* are presented in Table 1.

Table 1: Some physical parameters of residues recovered from chloroform and aqueous extracts of *P. stratiotes*

S/N	Fraction	Weight (g)	pH	Colour
1	Crude	5.11	6.85	Dirty green
2	Chloroform	4.10	7.0	Dirty green
3	Aqueous	3.90	5.6	orange

Toxicity of the crude, chloroform and aqueous extracts on Culex larvae at various concentrations respectively were shown in Tables 2 - 4. The crude extract (LC_{50}, 159.5 μg/ml) exerted larvicidal activity on Culex which is directly proportional to the concentration of the extract. For example, mortality rate was 16.6% at concentration 40 μg/ml. 50% died after exposure to 200 μg/ml and it was 80% mortality when the larvae were exposed to 400 μg/ml (Table 2).

The larvicidal effect of the chloroform extract on the culex larvae was high with 96.6% mortality rate at 400 μg/ml. At 200 μg/ml the death rate was 93.3% and at 40 μg/ml, the mortality rate was up to 90% (Table 3). The aqueous extract shows a low activity with mortality rate at of 13.3% at 400 μg/ml, 3.3% at 200 μg/ml and no death at 40 μg/ml. the LC_{50} was too large thus cannot be defined within the limit of this work (Table 4).

The activity of the extracts on *A. salina* (brine shrimp) is presented in Tables 5 - 7. There was 30% mortality even at 1000 μg/ml concentration of the crude extract, corresponding to LC_{50} of 2524.22 μg/ml (Table 5). The chloroform extract (FO_2) LC_{50} > 1000 μg/ml killed 3.3% at 1000 μg/ml. There was no observed death at 100 μg/ml concentration (Table 6). The aqueous extract did not exert lethal effect on brine shrimp at all concentrations (Table 7).

DISCUSSION

The bioassay showed that chloroform soluble extract of *P. stratiotes* LC_{50} = 0.0909 μg/ml exerted highest lethal activity even at lower concentrations on Culex mosquito larvae. This was followed by the crude extract (LC_{50}: 159.5 μg/ml). The lowest activity was found in the aqueous extract at much higher dose of 400 μg/ml, the LC_{50} of which proves to be too large. Thus, the most mosquitocidal component of the plant could be said to be carried in chloroform soluble compartment.

The extracts of *P. stratiotes* have low activity on Brine shrimp larvae. The crude fraction was moderate at 1000 μg/ml. this confirmed some few reported works, which stated that the crude fraction of *P. stratiotes* L. was moderately toxic to brine shrimp (Adoum *et al,* 1997). Comparatively, the chloroform extract of the plant showed a very low toxicity at 1000 μg/ml, while the aqueous extract did not show any activity on brine shrimp at all. Perhaps, this may partly be the reason why some shrimps prefer to associate with the plant in their sea environment (Mukhtar and Hafiz, 2001).

Effects such as delayed mortality, reduced survivorship of mature insects, reduction in the production of viable eggs and reduction in fecundity, example in mosquitoes were however, outside the scope of the current study. An interesting observation was that the plant extracts that have shown high activity on Culex mosquito larvae have shown low activity on brine shrimp larvae, despite the brine shrimp being more primitive than the mosquitoes. Additionally, an investigation carried out on the toxicity of extracts of *P. stratiotes* in rats has

Table 2: Larvicidal effect of crude extracts (FO_1) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	400	10	7	9	8	3	1	2	80	
2	200	10	4	5	6	6	5	4	50	159.50
3	40	10	2	0	3	8	10	7	16.67	
Control	0	10	0	0	0	10	10	10	0	

Table 3: Larvicidal effect of chloroform extracts (FO_3) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	400	10	9	10	10	1	0	0	96.6	
2	200	10	4	10	8	0	0	2	93.3	0.0909
3	40	10	9	8	10	1	2	2	90.0	
Control	0	10	0	0	0	10	10	10	0	

Table 4: Larvicidal effect of aqueous extracts (FO_2) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	400	10	2	1	1	8	9	9	13.33	
2	200	10	0	0	1	10	10	2	3.33	> 1000
3	40	10	0	0	0	10	10	10	0	
Control	0	10	0	0	0	10	10	10	0	

Table 5: Larvicidal effect of crude extracts (FO_1) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial N0. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	1000	10	4	4	1	6	6	9	30	2524.22
2	100	10	0	0	1	10	10	9	3.3	or
Control	0	10	0	0	0	10	10	10	0	>1000

Table 6: Larvicidal effect of chloroform extracts (FO_3) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	1000	10	1	0	0	9	10	10	3.3	
2	100	10	0	0	0	10	10	10	0	>1000
Control	0	10	0	0	0	10	10	0	0	

Table 7: Larvicidal effect of aqueous extracts (FO_2) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial N0. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC_{50} μ g/ml
			1	2	3	1	2	3		
1	1000	10	0	0	0	10	10	10		
2	100	10	0	0	0	10	10	10	0	>1000
Control	0	10	0	0	0	10	10	10	0	

shown adverse effects but no lethality was recorded (Mukhtar and Hafiz, 2001).

CONCLUSION

The crude extract of *P. stratiotes* was cytotoxic on both brine shrimp and Culex mosquito larvae. Where but, the chloroform and aqueous extracts were active on Culex mosquito larvae only. The chloroform fraction appeared with highest potential of acting as anti-mosquito larvae because of its high cytotoxicity with LC_{50} of 0.0909 μg/ml than all the other tested fractions of the plant. It can be recommended that the extracts be further assayed to ascertain their potential use as antibacterial, antifungal or as insecticides, so that its economic values can be harnessed.

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