

LETHALITY OF THE AQUEOUS EXTRACTS OF *ACACIA NILOTICA*, *GUIERA SENEGALENSIS*, *KIGELIA AFRICANA* AND *SECURIDACA LONGEPEDUNCULATA* ON *CULEX* MOSQUITO LARVA

D.W. TAURA¹⁺, M.D. MUKHTAR¹ and O.A. ADOUM²

1. Department of Biological Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria.

2. Department of Chemistry, Bayero University, P.M.B. 3011, Kano, Nigeria.

(Submitted: 26 August 2004; Accepted: 30 November 2004)

Abstract

Cytotoxic activity indicated by lethal effects (that is mortality rate) of aqueous extracts of fruits of *Acacia nilotica*, stem bark of *Kigelia africana*, roots of *Securidaca longepedunculata*, and leaves of *Guiera senegalensis* on the culex mosquito larva was investigated by direct contact method. Larvicidal effects were observed with dose concentration ranging from 400 µg/ml down to 40 µg/ml of all the plant extracts tested except for *G. senegalensis* where no death was observed at the dose of 40 µg/ml. Distilled water with 0 µg/ml concentration served as the control. *S. longepedunculata* was found to be most active with average percentage mortalities of (80%), (50%) and (13.3%) at 400 µg/ml, 200 µg/ml and 40 µg/ml respectively. *K. Africana* caused 70%, 40% and 10% of average percentage mortalities accordingly. *A. nilotica* and *G. senegalensis* seemed to show similar larvicidal activity but their activities differed in one respect. At 400 µg/ml *G. senegalensis* killed 53.3%, 200 µg/ml killed 30% and at 40 µg/ml there was no larvicidal activity, whereas *A. nilotica* killed about 50%, 20% and 7% respectively. The study revealed that all the four plant species could impart a dose dependent lethality effect on *Culex* larvae. For this reason, it is therefore recommended that the LC₅₀ of the extract be determined later, since it became difficult to evaluate at the time of the present study. However, the current result could serve as additional baseline data valuable for use in the development of ethnopharmacologically active substance(s) with the potential for use as insecticides and in modern medicine.

Keyword: Lethality, *Acacia nilotica*, *Guiera senegalensis*, *Kigelia africana*, *Securidaca longepedunculata*, *Culex* mosquito.

1. Introduction

Presently, ethnopharmacognosy in conjunction with phytochemistry provide one of the greatest areas of biotechnology that paves the way for drugs discovery from natural products (WHO/TDR, 2004). It is along this line that investigations have been on the increase in order to discover more beneficial plants, the exact active chemical constituents present in them and their possible roles as therapeutics in modern medicine.

Guiera senegalensis ("Sabara" in Hausa) has a special reputation as one of the preventive agents of leprosy in Sokoto state in Northern Nigeria (Irvine, 1961). Mukhtar and Okafor (2002) reported that the ethanolic extract of *G. senegalensis* (leaf and stem bark) was active against *Staphylococcus aureus*, and *Salmonella typhi* but less active against *Pseudomonas aeruginosa* and *Escherichia coli*. *Kigelia africana* ("Sanya" in Hausa) was used as an abortive and for diarrhoea (purgative) by

traditional doctors (Anokbongo *et al.*, 1990). The aqueous extract was used to treat syphilitic sore, abdominal pains, wound abscess and ulcer (Shahney *et al.*, 1978 and Sharma *et al.*, 1993). The aqueous extract of *K. africana* was also found to have antimicrobial activity. It was found to be active against *Plasmodium falciparum*. *Securidaca longepedunculata* ethanolic extract was found to be active against *S. aureus*, *E. coli*, *Ps. Aeruginosa*, and *K. Pneumoniae* (Desta, 1993). It was also used to treat epilepsy in East Africa and has been reported to be used in the treatment of wounds and sore (Detomassi *et al.*, 1993).

The ethnomedicinal promises and claims regarding these selected plant species could perhaps be harnessed if more studies pertaining to their cytotoxic activities are promoted. Investigations on the chemotherapeutic index of any pharmacological

+ corresponding author (email: mukhtardaouda@yahoo.com)

candidate should include toxicity tests either by employing Brine Shrimps, Mosquito larvae or even tadpole or all. This is before going on to test its toxicity effects at pre-clinical as well as clinical levels (Emos and Wambebe, 1998).

Therefore, the objectives of the present study were therefore to investigate the lethal effects of aqueous extracts of *Acacia nilotica*, *Guiera senegalensis*, *Securidaca longepedunculata*, and *Kigelia africana* by contact method using *Culex* mosquito larvae. This was with the aim of developing an additional base line data that will be valuable for the assessment, and the pharmacological potentials of the extracts of the selected plant specie for chemotherapy and as mosquitocides.

2. Materials and Methods

The plant species used for the test were fruits of *Acacia nilotica adansonii*, leaves of *Guiera senegalensis*, the stem bark of *Kigelia Africana* and the root of *Securidaca longepedunculata* which were collected from different locations in Kano state. These were identified locally by Mallam Ali Garko of the Department of Biological Sciences and also with the help of the keys provided by Dalziel (1946).

(a) Preparation of aqueous extract of the plant materials.

The plant materials collected were air dried and ground into powder separately using pestle and mortar. Two hundred grams of each plant were percolated in 4 litres of ethanol for 2 weeks. The percolates were then filtered and the solvent evaporated using the rotary evaporator at 40 °C (Fatope *et al.*, 1993). The residues were dissolved in 200 ml of water and Trichloromethane mixture ($H_2O:CHCl_3$) and then shaken for about 15 minutes and left to stand overnight. The mixture was separated into a distinct water-soluble layer which was drained and labeled ($F0_1$) and a chloroform-soluble layer, labeled ($F0_2$). These were all evaporated and the $F0_1$ aqueous extract was used in the bioassay.

(b) Collection and rearing of culex mosquito

The eggs of *Culex* mosquito were identified by their appearance as always fastened together vertically in batches of about 100-300 forming a raft like structure which can float (Mukhtar *et al.*, 2004). These were scooped from gutters around the old campus Bayero University, Kano. The eggs were placed in a jar of sterile water to which 0.3 g/l of ascorbic acid had previously been added in order to create low oxygen tension required to facilitate egg hatching. The larvae were harvested and transferred to several fresh beakers of sterile water to which a few grains of bakers yeast was added daily. Every 2-3 days a

Pasteur pipette was used to suck fecal matter and decomposing dissolved yeast (Mukhtar *et al.*, 2004).

As the larvae turned to pupa, they were removed and placed in fresh beakers of sterile tap water and transferred into "Mosquitories" which was the laboratory fume chamber covered with a net to prevent flying adults from escaping or stray mosquitoes entering. The mosquitories were sterilized by subjection to perpetual ultraviolet radiation for 48 hours in addition to thorough cleaning with disinfectants (Arias and Muller, 1975) prior to pupal introduction.

After two days, the pupae hatched out into imagoes that were fed with glucose solution. As *Culex* mosquito is unautogenous, a mouse (for blood meal) was placed in the mosquitories and left to stand overnight. After successful mating some female mosquitoes proceeded to lay eggs in the containers of sterile water. The containers were daily examined and any batch of eggs laid was immediately transferred into fresh beakers of sterile water containing little amounts of ascorbic acid to stimulate egg-hatching. Emergent larvae were harvested for the bioassay (Gerberg, 1970).

(c) Preparation of sample concentrations and biolethality test procedure

Each of the four different species of the test plant extracts were prepared into 400 µg/ml, 200 µg/ml and 40 µg/ml and 0.0 µg/ml (distilled alone as control) solution (Mukhtar *et al.*, 2004). Ten larvae were then added in each case. The solution with 0 µg/ml served as control. At each dose, test was carried out in triplicate at room temperature. After a period of 24 hours, the survivors were counted and the average percentage mortality at each dose was determined.

3. Results

It was observed that at physical point of view, all the aqueous extracts of the four different plant species namely fruits of *Acacia nilotica*, leaves of *Guiera senegalensis*, stem bark of *Kigelia africana* and the roots of *Securidaca longepedunculata* are water-soluble. Texturally however, and before dissolution in water the extract of *A. nilotica*, *K. Africana* and *S. longepedunculata* were sticky; whereas that of *G. senegalensis* was powdery and fine to touch (Table 1).

In terms of lethality (larvicidal) effects which was monitored as percentage mortality, *S. longepedunculata* induced the greatest killing effect, and this was directly proportional to the concentration of the extract. Thus the mortality was

Table 1: Some physical parameters of the aqueous extract of *A. nilotica*, *G. senegalensis*, *K. Africana*, and *S. longepedunculata*.

S/N	Plant extract s	Solubility in water	Texture
1	<i>A. nilotica</i>	Soluble	Sticky
2	<i>G. senegalensis</i>	Soluble	Powdery
3	<i>K. Africana</i>	Soluble	Sticky
4	<i>S. longepedunculata</i>	Soluble	Sticky

Table 2: Percentage mortality in *Culex* mosquito larvae on exposure to different concentrations of aqueous extracts of *Securidaca longepedunculata*, *Kigelia Africana*, *Guiera senegalensis* and *Acacia nilotica*.

Plant	Conc. of extract ($\mu\text{g/ml}$)	Initial No. of larvae	Total death in each compartment.			Average mortality (%)
			i	ii	iii	
<i>Securidaca longepedunculata</i>	400	10	9	8	7	80
	200	10	6	4	5	50
	40	10	2	1	1	13.3
	0	10	0	0	0	0
<i>Kigelia Africana</i>	400	10	8	7	6	70
	200	10	4	4	4	40
	40	10	1	1	1	10
	0	10	0	0	0	0
<i>Acacia nilotica</i>	400	10	5	4	6	50
	200	10	2	2	2	20
	40	10	1	1	0	7.0
	0	10	0	0	0	0
<i>Guiera senegalensis</i>	400	10	6	5	5	53.30
	200	10	3	4	2	30.0
	40	10	0	0	0	0.00
	0	10	0	0	0	0.00

80% at concentration of 400 $\mu\text{g/ml}$, 50% at 200 $\mu\text{g/ml}$ and 13.3% at 40 $\mu\text{g/ml}$ (Table 2).

K. Africana appeared next to *S. longepedunculata* in terms of larvicidal effect on *Culex* mosquito larva (Table 2). Though the activity was also concentration dependent, the severity was in the order 400 $\mu\text{g/ml}$ (70% mortality) > 200 $\mu\text{g/ml}$ (40%) > 40 $\mu\text{g/ml}$ (10% mortality).

Acacia nilotica showed lower mortality effect on *Culex* mosquito larvae as compared with those of the two aforementioned plant species. This was because the orders of mortalities shown in Table 2 were 50%, 20% and 7.0% at concentration of 400 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$ respectively.

Comparatively, *Guiera senegalensis* extract induced a mean mortality rate of 53.30%, to 3.3% high up a little above that of *Acacia nilotica* at the highest test concentration (400 $\mu\text{g/ml}$). It was however, the least lethal plant among all the four tested species in the present study. This was demonstrated by the fact that there was only 30% mortality at 200 $\mu\text{g/ml}$ concentration while at 40 $\mu\text{g/ml}$ none of the test larva was killed (Table 2).

Throughout the experimental period none of the test larvae in the control groups with only distilled water (0.0 $\mu\text{g/ml}$ extract) was shown to have died.

4. Discussion

The current study demonstrated the larvicidal activities of aqueous extracts of fruits of *Acacia nilotica*, leaves of *Guiera senegalensis*, stem bark of *Kigelia Africana* and roots of *Securidaca longepedunculata*. There were cytotoxic effects which culminated in killing of the test *Culex* mosquito. The lethality index was however dose-dependent although it differs in intensity among the plants tested.

It is therefore important to bear this aspect of cytotoxicity in mind where extracts of these plants are considered for assay towards any useful pharmacognostic agent. For example literature revealed that aqueous extracts of the fruits of *A. nilotica* is being used variously as an antimicrobial agent ethnomedicinally, locally in both man and domestic animals (Fatope *et al.*, 1993).

With aqueous extracts of *G. senegalensis* cytotoxic activity against living cells (*Culex* mosquito larva) at 400 $\mu\text{g/ml}$ was 53.3% and at 200 $\mu\text{g/ml}$ it was 30%, while it was 0% at 40 $\mu\text{g/ml}$. This may mean that the plant extracts can be safer to use at moderate concentration than the other three plant types. Perhaps this may account for the reason why it has been used widely for treating diseases traditionally.

Kigelia africana was found to have a pronounced larvicidal activity and this justifies an old tradition in Africa where the leaf of this plant was reportedly used as an abortive agent by traditional healers (Anokbongo *et al.*, 1990). The consumption of its root decoction was valued in the eradication of intestinal worms, cancer of the uterus and alimentary canal (Msonthi and Magambo, 1983). Thus, if the various fractions can be tapped, purified, and tested further the plant may give additional source of anti-cancer, family planning and anti-helminthic agents. This is in addition to its anti-*Staphylococcal* activity (Taniguchi *et al.*, 1978).

The aqueous extract of *Securidaca longepedunculata* was found to be more active than all the other three aqueous extracts of the plants tested. The lethality of its roots was put to use for a long period of time by some women in South Africa who used to insert its root concoction into the vagina as a way of committing suicide. It was claimed that when inserted it causes necrosis of the liver in addition to local caustic effects; although this later use has to be discouraged. Nevertheless this plant plays a vital role in the war against insects, pests and vectors and perhaps pathogenic microorganisms, according to Irvine (1961).

However, due to situational unavailability of the required computer software for the evaluation of LC_{50} , the lethal concentration of the aqueous extracts of the four plants on the larvae of *Culex* mosquito have not been determined. This will necessitate further research along this dimension.

6. Conclusion

The present study shows that aqueous extracts of fruits of *Acacia nilotica*, leaves of *Guiera senegalensis*, and stem bark of *Kigelia africana* and roots of *Securidaca longepedunculata* possessed cytotoxic effect which culminated in lethality action on *Culex* mosquito. The larvicidal activities were directly proportional to the concentration of the extracts and the intensities differ with the plant varieties. It was highest for *S. longepedunculata* followed by *K. africana* then *Acacia nilotica* and the least being *G. senegalensis* which demonstrated 50% larvicidal action at the maximum test concentration of 400 µg/ml. However, the LC_{50} indices for these plants have not been determined due to unavailability of appropriate computer software at the time of the investigation.

It is therefore, recommended that further and detailed pharmacognostic studies be intensified on these candidates so that their potential chemotherapeutic, insecticidal and other economic values could be harnessed.

Acknowledgement

The authors are grateful to Aminu Mohammed and Nura Hassan of the Department of Biological Sciences who rendered great assistance during the laboratory work.

REFERENCES

- Adoum, O.A., Dabo, N.T. and Fatope, M.O., 1997. *Pistia stratiotes* and its antimicrobial activity. *International Journal of pharmacognosy*. *F*, 118, 199.
- Anokbongo, W.W., Odoi-Adome, R. and Oliy, P.M., 1990. Traditional methods in the management of diarrheal diseases in Uganda. *Bulleting of WHO* 63(3), 359-363.
- Arias, J. and Mulla, M.S., 1975. Post emergence of two insect growth regulators on the mosquito *Culex tarsalis* (*Diphtheria culicidae*). *Journal of Medicinal Research* 12, 317-322.
- Dalziel, J.M., 1996. The Useful Plants of West Tropical Africa. Crown Agents for the Colonies, London. pp. 1-80.
- Desta, B., 1993. Antimicrobial activity of 63 medicinal plants. *J. Ethnopharmacol* 213, 253-257.
- Detomassi, N., Piacenter, S., Deamone, F. and Pizza, C., 1993. New sucrose derivatives from the bark of *Securidaca longepedunculata*. *J. Nat. Prod.* 561, 134-137.
- Emos, S. and Wambebe, C., 1998. Lethality bioassay using Brine Shrimp and mosquito larvae. In: *Proceedings of the Workshop on Biomedical and African Society for Pharmacology (WASP)*. Ahmadu Bello University, Zaria. Oct., 1998.
- Fatope, M.O., Ibrahim, H. and Takeda, Y., 1993. Screening of higher plants reputed as pesticides using the brine shrimp lethality bioassay. *International Journal of Pharmacognosy* 31, 250-256.
- Gerberg, E.J., 1970. Manual for mosquito rearing and experimental techniques. *Bulletin of American Mosquito Control Association* No. 109pp.
- Irvine, F.R., 1961. Woody Plants of Ghana with Special References to their Uses. Oxford University Press, London. pp. 124-128.
- Msonthi, J.D. and Magambo, D., 1983. Medicinal herbs in Malawi and their uses. *Hamdard* 262, 94-100.
- Mukhtar, M.D. and Okafor, A.C., 2002. Bioactive evaluation of ethanolic extract of leaf and stem bark of *Guiera senegalensis*. *The Nigerian Journal of Research and Production* 1(1), 114-121.
- Mukhtar, M.D., Sani, A. and Yakasai A.A., 2004. Cytotoxicity of fractions of *Pistia stratiotes* L. on *Culex* mosquito and *Artemia salina*. *Journal of Animal Research International* 4(2).
- Shahney, A.N., Khan, M.P., Ndaalio, G. and Nkunya, M., 1978. Studies on the rational of African traditional medicine. Part II, 295pp.
- Sharma, P.K. and Kaul, M.K., 1993. Specific ethnomedicinal significance of *Kigelia Africana* in India. *Fitoterapia* 64(5), 467-468.
- Taniguchi, M., Chagya, A., Kubo, I. and Nakanishi, K., 1978. Screening of east Africa plant for antimicrobial activity. *Chem. Pharm. Bill.* 26, 2910.
- WHO/TDR, 2004. Tropical disease research. Drug Discovery from Natural Products. TDR News No. 72 UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.