African Journal of Science and Technology (AJST)
Science and Engineering Series Vol. 2, No. 2, pp. 44-49

INSECTICIDAL ACTIVITY OF EXTRACTS DERIVED FROM DIFFERENT PARTS OF THE MANGROVE TREE *RHIZOPHORA MUCRONATA* (RHIZOPHORACEAE) LAM. AGAINST THREE ARTHROPODS

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ABSTRACT: *The insecticidal and antifeedant activity of extracts derived from different parts of the mangrove tree Rhizophora mucronata (Rhizophoraceae) Lam. is reported. The 70% ethanol extracts of leaves, bark, stem wood and pith were tested for toxicity against adults of the desert locust Schistocerca gregaria (Forskal), the 2nd instar larvae of Aedes aegypti (L.) and the 1st instar larvae of the brine shrimp Artemia salina (Leach). Antifeedant activity of the extracts was assessed through tests conducted on S. gregaria adults by the paper feeding protection bioassay. In tests carried out on A. aegypti mosquito larvae, bark and pith extracts showed high toxicity with 48 hour LC_{50}'s of 157.4 ppm and 168.3 ppm respectively. Stem wood extracts had low activity with an LC_{50} of 1003.4 ppm while leaf extracts did not exhibit toxic effects at a concentration of 1000 ppm. A similar trend in activity was observed with antifeedant tests conducted on the desert locust S. gregaria and on toxicity tests carried out on A. salina larvae. The bulk of the active compounds are sequestered in the bark, pith and stem wood with the least being found in the leaves. The results indicate that R. mucronata is a potential source of botanical insecticides(s).*

Key words: Botanical insecticides, mangrove, *Rhizophora mucronata*

INTRODUCTION

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, the carbamates and pyrethroids. Out of these the major classes in use today are organophosphates and carbamates (1,2). There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment (3,4,5). The use of organochlorine insecticides has been banned in developed countries and alternative methods of insect pest control are being investigated (4). Botanicals are a promising source of pest control compounds.

The pool of plants possessing insecticidal substances is enormous (6). These have generated extraordinary interest in recent years as potential sources of natural insect control agents. Today over 2000 species of plants are known that possess some insecticidal activity (7). The first insecticides to be used by man were from plants, the biological activities of which were known from the earliest recorded times (8). In the middle of the 17th century, pyrethrum, nicotine, and rotenone were recognised as effective insect-control agents (9).

The most economically important of the natural plant compounds used in commercial insect control are the pyrethrins from the flower heads of pyrethrum *Chrysanthemum cinerariaefolium* Vis. (10). Despite the relative safety of well known botanical insecticides, most of these substances have their drawbacks hindering large scale application. Pyrethrins are unstable in light and are rapidly metabolised thus limiting their potency and application (11). These limitations gave impetus for the synthesis of active analogues, termed pyrethroids (12). Nicotine, isolated from a number of species of *Nicotiana* is insecticidal), but its use in insect control has dropped steadily because of the high cost of production, disagreable odour, extreme mammalian toxicity, instability in the environment and limited insecticidal activity (13). Rotenone is unstable and very toxic to fish (14). Further, several insects have exhibited resistance to pyrethroids (15). For these reasons, the search for new safer and more effective insecticides from plants is justified. Indeed, research in this area has led to the discovery of substances with interesting activities on insects. The substances include insect growth regulators/inhibitors and antifeedants (16,17).

The insect growth regulators/inhibitors specifically affect growth and development of insects. These compounds

include mimics and inhibitors of two groups of insect hormones, namely the juvenile hormones and the moulting hormones (18,19). Antifeedants are substances which when tasted by insects, result either temporarily or permanently, depending on potency, in the cessation of feeding (20).

The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment (17). Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (16). One plant species may possess substances with a wide range of activities, for example extracts from the the neem tree *Azadirachta indica* are antifeedant, antioviposition, repellent and growth-regulating (21). In contrast, the toxicity of conventional synthetic insecticides is mainly restricted to neuro-muscular function (1).

Conventional synthetic insecticides require special safety procedures and equipment during production and application (1). Despite precautions, exposure to humans, the environment (4) and food (5). The synthetic insecticides are expensive and have in many cases only produced moderate results along with major ecological damage (4). In contrast, the low toxicity of botanical insecticides makes processing and application of the product inexpensive. In many cases, the materials are locally available and affordable (22).

Considering the large number of plants that are reputed to possess some form of insecticidal activity, it is a pity that only a few have been scientifically evaluated. A good example of a plant with reputed insect resistant properties is the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. Which grows in the salty muddy shores of the coast of East Africa and various parts of Asia (10). The tree grows to a height of 3-12 metres and has stilt roots. It is often the dominant species on the edge of mangrove swamps (23). From time immemorial poles of the mangrove tree *R. mucronata* have been used to construct dhows and houses and baskets in coastal areas (23,24). The poles made from the tree are known to be highly resistant to rotting and even attack by arthropods e.g. insects (6). The tree is a good source of tannins for the treatment of leather (10). Although this information has been available for hundreds of years and hence the popularity of the mangrove for building purposes, there is no scientific investigation to determine the part of the tree which has toxic activity against insects or the mode of action of the active substances.

Based on the foregoing, we sort to scientifically evaluate the insecticidal activity of the mangrove *R. mucronata* and the distribution of the active substances within the tree with a view to generating data that will lay the foundation for future research work in fractionation, structural determination and application.

MATERIALS AND METHODS

Preparation of mangrove extracts

Rhizophora mucronata (Lam.) was collected from the Kenya coast near Mombasa with the assistance of the Herbarium, Department of Botany, University of Nairobi. The leaves, bark, stem wood and pith were dried at 30^{o}C for 12 hours and pulverized to powder separately in a hammer mill. One hundred grams of powder from each of the plant parts were then extracted three times with 500 ml of aqueous 70% ethanol. After 12 hours, the supernatants were decanted, filtered and dried in a rotary evaporator at 40^{o}C for 30 minutes. The dry extracts were kept desiccated at 4^{o}C.

Experimental organisms

Five-day-old male desert locusts *Schistocerca gregaria* (Forskal) used in this study were reared in the Department of Zoology, University of Nairobi as described by Hunter-Jones, (25). The insects were fed on wheat bran and wheat seedlings. The gregarious colony was reared under crowded conditions in a light:dark regime of 12L:12D at a temperature of 29 ±1 ^{o}C and a relative humidity of 60%. Second instar *Aedes aegypti* (L.) larvae for the larvicidal assays were obtained from a colony maintained in the Department of Zoology. The mosquito larvae were fed on dog biscuit, while the adults were reared on saturated sucrose solution and allowed to take blood meals from the blood vessels of rabbit ears *ad libitum*. The mosquito eggs were hatched in 0.08% NaCl solution (26). Eggs of the brine shrimp *Artemia salina* (Leach) were obtained from Interpet® Ltd. England. First instar larvae for bioassays were obtained by hatching the eggs in a 3.3% solution of natural marine salt (27). The larvae were fed brewer's yeast. Both the *A. aegypti* and *A. salina* larvae were maintained at 25 ±2^{o}C.

Larval toxicity tests

All the extracts were tested for the presence of biologically active substances against the 2nd instar larvae of *A. aegypti* and 1st instar larvae of *A. salina.* The *A. aegypti* tests were performed in 40 ml of 0.08% NaCl solution (26) contained

in 100 ml petri dishes as described by (28). The test material was dissolved in 70% ethanol so that the final volume did not exceed 0.1 ml. Larval food consisted of 0.05 g of brewer's yeast per dish. The number of larvae in each dish was 20. Controls in all cases received 0.1 ml of 70% ethanol. After 48 hours, dead larvae were removed and counted. The extracts were tested at doses of 100, 140, 180, 220, 260 and 300 ppm. In a similar experiment, the toxicity of extracts to the 1st instar larvae of *A. salina* was assessed in 3.3% solution of natural marine salt (27). The extracts were tested at doses of 60, 100, 140, 180 and 220 ppm Each experiment was replicated three times, and the data subjected to probit (29) and regression analysis (30) to determine the LC_{50}'s of the extracts.

Antifeedant tests

All the extracts were assessed for antifeedant activity against the desert locust by the paper feeding protection bioassay. Fifty microlitres of test solution in 70% ethanol were applied to Whatman® number 1 qualitative grade filter paper squares measuring 2x2 cm impregnated with 0.25M sucrose and dried at 40°C for 30 minutes. Control papers were treated with 50µl of 70% ethanol. The papers were then presented to 24-hour starved five-day-old male desert locusts in one cage of dimensions 43 x 43 x 50 cm (choice test). The ratio of the number of paper squares to locusts was 3:1. For tests on bark and pith extracts, there were 21 locusts in the cage. In the case of tests on stem extract there were 24 locusts in the cage. Bark and pith extracts were tested at concentrations of 100, 200, 300, 400, 500 and 600 ppm, while stem extracts were tested at concentrations of 400, 500, 600, 700, 800, 900 and 1000 ppm. After 24 hours, the papers were retrieved and the percentage feeding protection calculated according to the following formula:

$$\text{Percentage feeding protection} = \frac{(C - t)}{T} \times 100$$

Where:

C= Area of one set of control papers consumed (mm^2)
t= Area of one set of treated papers consumed (mm^2)
T= Area of one set of intact papers (mm^2)

The experiment was replicated three times. The antifeedant ED_{50} of each extract was then estimated by subjecting the percentage feeding protection data to probit (29) and regression analysis (30) to determine the ED_{50}'s of the extracts.

Tests for direct toxicity on locusts

All the extracts were assessed for direct toxicity against the desert locust. Solutions of mangrove extracts for injection were prepared in 60% aqueous ethanol. Locusts were injected with 5µl of the solutions in the inter-segmental membrane between the 2nd and 3rd sternites with a microlitre syringe fitted with a gauge 26 hypodermic needle. Control locusts were injected with 5µl of 60% ethanol. Ten locusts were used for each dose tested and the experiment was replicated three times. For tests on bark and pith extracts, the doses used were 50, 100, 150, 200, 250 and 300 ppm. In the case of tests on stem extracts, the doses used were 400, 500, 600, 700, 800, 900 and 1000 ppm. After 48 hours, dead locusts counted and the data subjected to probit (29) and regression analysis (30) to determine the LD_{50}'s of the extracts.

RESULTS

The amounts of dry 70% ethanol soluble material from 100 g of the different parts of *R. Mucronata* were varied (Table 1). The highest weight was obtained from pith, which produced 4.60 ±0.26g. Leaves produced 4.12 ±0.25g while bark and stem wood produced 3.22 ±0.17g and 1.31 ±0.12g respectively. These weights were significantly different (ANOVA F3, 8(1)=141.028, P=0.000).

Table 1. The weight of dry 70% ethanol soluble material from 100 g of different parts of *Rhizophora mucronata*

Part of plant	Weight of extract (g ± S.E.M. n=3)
Pith	4.60 ± 0.26
Leaves	4.12 ± 0.25
Bark	3.22 ± 0.17
Stem wood	1.31 ± 0.12

The results of the toxicity of *R. mucronata* bark, pith and stem wood extracts to the 2nd instar larvae of *A. aegypti* and the 1st instar larvae of *A. salina* are presented in Table 2. The equations of the regression lines from probit mortality versus log dosage plots and the lower and upper confidence limits of the LC_{50}'s of each extract are also shown on Table 2. In this test, bark and pith extracts showed high toxicity with $LC_{50's}$ of 157.4 ppm and 168.3 ppm respectively against *A. aegypti* larvae. Extracts of stem wood had low activity with an LC_{50} of 1003.4 ppm. Toxicity of bark, pith

and stem wood extracts of *R. mucronata* to *A. salina* larvae is presented in Table 2. The equations of the regression lines from probit mortality versus log dosage plots and the lower and upper confidence limits of the LC_{50}'s of each extract are also shown on Table 2. Similarly, bark and pith extracts showed high toxicity with $LC_{50's}$ of 87.3 ppm and 65 ppm respectively against *A. salina* larvae. Extracts of stem wood had low activity with an LC_{50} of 745.4 ppm. The LC_{50} of leaf extracts could not be determined because no mortality was observed even at the highest concentration of 1000 ppm used.

Table 2. Toxicity of *Rhizophora mucronata* bark, pith and stem wood extracts to the larvae of *Aedes aegypti* and *Artemia salina*

Part of plant	Equation of regression line	R-Sq.(%)	48 hour LC50 (ppm)	95% C.L. of LC50 (ppm)		t-Value	d.f.
				Lower	Upper		
***A. aegypti* bioassay**							
Bark	Y=-17.85+10.40x	92.5	157.4	74.8	331.2	7.031*	4
Pith	Y=-23.34+12.73x	93.1	168.3	72.6	390.4	6.360*	3
Stem wood	Y=-14.96+6.65x	98.6	1003.4	637.5	1579	14.912 *	3
***A. salina* bioassay**							
Bark	Y=-9.75+7.60x	91.6	87.3	50.4	111.5	5.70*	3
Pith	Y=-11.19+8.19x	91.0	65	39.3	228.8	5.52*	3
Stem wood	Y=-14.36+6.74x	99.47	45.4	583.6	952.6	26.29*	4

* Significant at P<0.05

Table 3 shows the toxicity of bark, pith and stem wood extracts of *R. mucronata* to *S. gregaria* adults and the equations of the regression lines from probit mortality versus log dosage plots and the lower and upper confidence limits of the LD_{50}'s of each extract. In this test, bark and pith extracts showed high toxicity with $LD_{50's}$ of 337.4 ppm and 287.7 ppm, respectively. As was the case in the *A. salina* and *A. aegypti* larvae bioassays, stem wood extracts had low activity with an LD_{50} of 1013.4 ppm. Leaf extracts were not toxic to *S. gregaria*.

Table 3. Toxicity of bark, pith and stem wood extracts of *Rhizophora mucronata* to adults of *Schistocerca gregaria*.

Part of plant	Equation of regression line	R-Sq.(%)	48 hour LC50 (ppm)	95% C.L. of LC50 (ppm)		t-Value
				Lower	Upper	
Bark	Y=-3.52+3.37x	96.8	337.4	194.6	584.8	9.536*
Pith	Y=-1.59+2.68x	99	287.7	222.1	372.6	19.447*
Stem wood	Y=-10.54+5.17x	99.3	1013.4	800	1284	27.530*

* Significant at P<0.05

The antifeedant activity of bark, pith and stem wood extracts of *R. mucronata* to *S. gregaria* adults are presented in Table 4. The equations of the regression lines from probit feeding protection versus log dosage plots and the lower and upper confidence limits of the ED_{50}'s of each extract are shown. The highest antifeedant activity was observed in bark and pith extracts with $ED_{50's}$ of 191.7 ppm and 188.7 ppm respectively. In this bioassay, the trend of activity in the four extracts was similar to that observed in the toxicity tests. Stem wood extracts had low antifeedant activity with an ED_{50} of 578 ppm. Extracts of the leaves did not show antifeedant action even at the highest concentration of 1000 ppm.

Table 4. Antifeedant activity of bark, pith and stem extracts of *Rhizophora mucronata* to adults of *Schistocerca gregaria*.

Part of plant	Equation of regression line	R-Sq.(%)	48 hour LC50 (ppm)	95% C.L. of LC50 (ppm)		t-Value	d.f.
				Lower	Upper		
Bark	Y=-4.61+4.21x	60.8	191.7	19.2	1913	2.491	4
Pith	Y=-7.13+5.33x	79.9	188.7	44.7	795.3	3.983	4
Stem	Y=-22.15+9.83x	78.6	578	126.3	2645	4.280*	5

* Significant at P<0.05

DISCUSSION

The results of the present study are interesting. The findings indicate the importance of traditional knowledge in science. The bark of *Rhizophora mucronata* has been shown to possess insecticidal and insect repellent components and these protect the wood from insect damage. The wood itself is strong and in addition possesses insecticidal and insect repellent compounds. The soft pith, which would normally be an easy target to pest damage has high insecticidal and insect repellent activity which protects the strong wood from pest damage. Some of the compounds known to be present in mangrove bark are tannins and these are used for treating leather (10). It would be interesting to investigate whether the insecticidal and insect repellent compounds in mangrove are tannins or other classes of compounds. Such studies could also be extended to cover other species of mangrove trees.

A few suggestions can be made from the results of the present study regarding the pest resistant qualities of mangrove. The high yields of active material from the bark is encouraging. During the preparation of mangrove poles, the bark is normally peeled off. Since the yield of active substances is high, it would be possible to produce enough quantities for field application in farms especially at the coast. Another advantage of extracting the material from bark is that this part of the plant is easy to process during extraction due to its softness. The results indicate that the mangrove poles would be resistant to terrestrial insect pests and marine crustaceans. Since mangrove poles are used for constructing boats and houses, it would be interesting to test the extracts against the main pests of wood in the terrestrial environment such as termites and fungi. From the results obtained in this study, it is suggested that future studies concentrate on the activity

of the bark. This part is easily available by peeling, without destroying the poles.

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable. Antifeedant and growth inhibiting activity reduces pest damage to products even without killing the pest. Further, in the long run, populations are reduced through disrupted metamorphosis or reduced fecundity (21). This antifeedant and growth-inhibiting activity can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM). It would be interesting to investigate whether *Rhizophara mucronata* contains substances similar to the antifeedant and growth inhibiting compounds present in the fruits of *Azadirachta indica* (3,21) and *Melia volkensii* (28,31). It would also be important to test for the toxicity of these mangrove extracts on mammals and other non-target organisms. The following conclusions can be made from the results of this study. *Rhizophora mucronata* bark, pith and stem extracts have toxic effects on the larvae of *Aedes aegypti* and *Artemia salina* larvae and adults of *Schistocerca gregaria*. In addition, these extracts have antifeedant effects on the adults of *Schistocerca gregaria*. The bark and pith extracts of mangrove have highest insectidical and antifeedant activity.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance provided by the Departments of Botany and Zoology, University of Nairobi and the German Academic Exchange Service (DAAD) for funding part of the study.

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