

Full Length Research Paper

Protectant, insecticidal and antimicrobial potentials of *Dalbergia saxatilis* Hook f. (fabaceae)

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The protectant, insecticidal and antimicrobial potentials of the Nigerian plant, *Dalbergia saxatilis* (Fabaceae) were investigated. The dried powdered leaves of *D. saxatilis* were established as a control agent for the cowpea pest, *Callosobruchus maculatus*. Damage was drastically reduced when cowpea seeds were treated with dry powdered leaves of *D. saxatilis*. Doses of 0.75 g/150 seeds and above significantly reduced crop damage, oviposition and larval development without affecting the viability of the seeds. Similarly, the crude 95% ethanol extract and the aqueous methanol and hexane fractions of the bark extract showed insecticidal activity against adult mosquitoes at 0.2% concentration in ethyl acetate. The crude 95% ethanol extracts of the leaves and bark were screened against six pathogenic microorganisms (ATCC). While the leaves extract was active against only *Staphylococcus aureus* at minimum inhibition concentration (MIC) 1000 µg/ml, the bark extract displayed broad and interesting antimicrobial spectra. The bark extracts gave activity at MICs 250, 125, 1000 and 1000 µg/ml against *S. aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. The above properties demonstrate the great potentials of *D. saxatilis* for use in agriculture and medicine. The relevance of bioassay-guided fractionation in ensuring consistency and enhancement of efficacy and quality of phyto-medicines and bio-pesticides is discussed.

Key words: *Dalbergia saxatilis*, protectant, *Callosobruchus maculatus*, insecticidal, adult mosquitoes, antimicrobial, pathogens, bioassay-guided fractionation.

INTRODUCTION

For more than three decades now, much attention has been drawn to pollution of the environment by chemical pesticides and the need to develop safer methods of pest control. In this regard plant-based pesticides in various forms have been generally accepted as good alternatives because they are biodegradable and therefore less persistent. The folkloric use of higher plants as pesticidal and antimicrobial agents by the natives of various parts of the world is well known (Dalziel, 1937; Ayensu, 1978). A number of Nigerian higher plants are traditionally noted for their medicinal and pesticidal properties (Ayensu, 1978; Irvin, 1961; Okwute, 1992), but regrettably very few have been screened (Osisiogu and Agbakwuru, 1978;

Ivbijaro and Agbaje, 1986; Huffort et al., 1989; Takeda and Fatope, 1988; Okwute, 1989).

Several species belonging to the genus *Dalbergia* have been shown to possess biological properties (Irvin, 1961; Oliver, 1978; Dutta, 1979). When *D. saxatilis* leaves were powdered and burnt the odour affected the tracheal tubes of insects and suffocated them (Dutta, 1979). Recently, the aqueous root extract was found to exhibit sedative and anticonvulsant effects (Yemitan and Adeyemi, 2003). This paper assesses the protectant properties of the leaves against the cowpea pest, *Callosobruchus maculatus*, the insecticidal activity of the stem bark against adult mosquitoes and the activity spectra of both the bark and leaves extracts against some pathogenic microorganisms. The relevance of bioassay-guided fractionation in ensuring consistency in efficacy and quality of plant-based medicines and bio-pesticides is also discussed.

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Table 1. Evaluation of protectant potentials of *D. saxatilis*.

Treatment (g)	No. of eggs laid on seeds	No. of damaged seeds	Insect mortality	% Germination
2.00	3.2 ± 0.84	0 ± 0.00	10.0 ± 0.00	83.3
1s.75	3.4 ± 0.55	0 ± 0.00	10.0 ± 0.00	91.7
1.50	3.6 ± 0.95	0 ± 0.00	10.0 ± 0.00	83.3
1.25	3.8 ± 1.00	0 ± 0.00	10.0 ± 0.00	75.0
1.00	4.8 ± 1.64	0 ± 0.00	10.6 ± 0.89	100
0.75	5.8 ± 1.48	0 ± 0.00	12.0 ± 0.71	100
0.50	10.6 ± 2.70	9.8 ± 1.30	14.0 ± 1.92	100
0.25	16.4 ± 3.11	10.8 ± 1.95	0.0 ± 0.00	91.7
0.0 g (Control)	17.0 ± 3.16	32.0 ± 3.49	0.0 ± 0.00	91.7

Values are means of 5 replicates.

MATERIALS AND METHODS

Collection and preparation of materials

The bark and leaves of *D. saxatilis* were collected from Apofin forest, Idu, Abuja, Nigeria, authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja and air-dried. The leaves were further dried to constant weight at 80 g for 24 - 36 h. They were then separately pounded in a mortar into powder.

The powdered bark (570 g) was extracted with 95% ethanol (2.5 L) using a Soxhlet extractor. The extract was filtered and evaporated to dryness using a Rotavapor to give a dark brown gum (56 g). The leaves (96 g) on extraction with 95% ethanol (2.0 L) and evaporation gave a dark green residue (29 g).

A *C. maculatus* culture was maintained in the laboratory on pre-fumigated cowpea seeds in Kilner jars (500 ml capacity), covered with fine muslin mesh. Adult insects emerging over a 48-h period were used in screening for protectant properties. Cages with perforations and adult mosquitoes were used for the insecticidal screening of the bark extract. 0.2% solutions of the crude extract and the two fractions in ethyl acetate in triplicates were prepared for the screening.

The test organisms for antimicrobial screening were from ATCC except *Bacillus subtilis*, which was local. They were prepared by incubating them in freshly prepared broth at 37°C for 8 h. Serial dilutions of the organisms and concentrations of the extracts at 1000 µg/ml and below were prepared according to a standard procedure (Mitscher et al., 1972).

Pesticidal screening

150 clean pre-fumigated cowpea seeds were taken in each of appropriately marked 40 Kilner jars (500 ml capacity). The jars were arranged in eight groups of five jars each. Each group was treated with the powdered *D. saxatilis* leaves at doses of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 g/150 seeds. An untreated group was used as control. The dosage was not based on any particular recommendation. The treated jars were shaken thoroughly and five male and five female *C. maculatus* adults were introduced into each jar.

To determine the mode of action of the leaves 2.0 g was introduced into the Kilner jars and were properly covered with filter paper. 150 cowpea seeds were introduced into the jars. The insects were then introduced into the jars and tied up to determine the fumigation efficacy of the leaves. This was replicated 5 times. The jars were left for 30 days after which they were observed for ovi-

position, seed damage, insect mortality, viability of undamaged treated seeds and fumigant potentials of the leaves.

For seed viability test 10 seeds were randomly selected from each level of treatment after 30 days and placed in Petri dishes with moist papers. The percentage germination was determined after 5 days.

Insecticidal screening

The insecticidal activity of the crude 95% ethanol extract and the hexane and aqueous methanol fractions of the bark of *D. saxatilis* was done according to a standard procedure (Ogebegwu, 1973) using three different cages each. A cage with solvent only and a cage without extracts or solvent were used as controls. Ten adult mosquitoes were counted into each of the cages. The cages were observed at intervals of 10 min for 60 min.

Antimicrobial screening of the crude extracts

The standardized test organisms (100 ml each) were inoculated onto the nutrient agar plates at the various concentrations of the extracts in triplicates and incubated at 37°C for 24 - 48 h. Absence of growth indicated activity against a particular microorganism. Activity at concentrations higher than 1000 µg/ml was considered not significant in the screening.

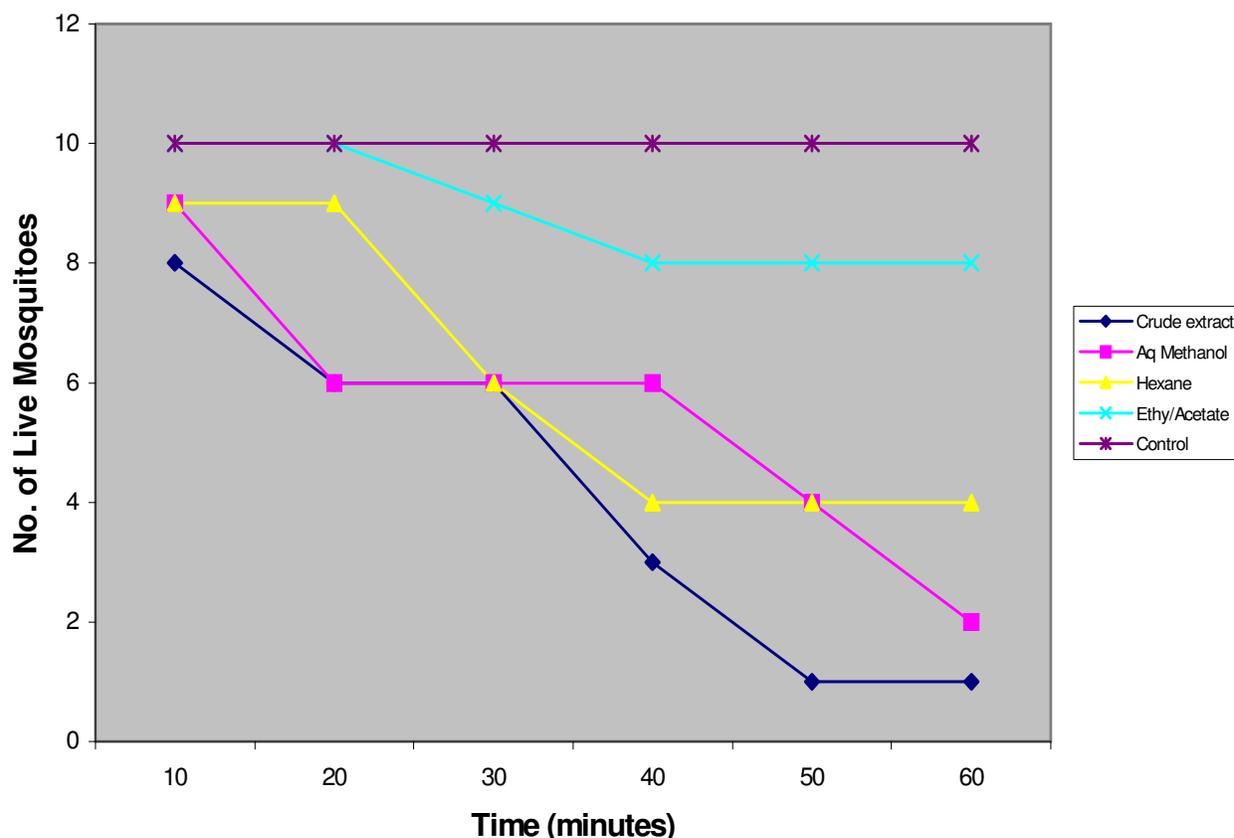
RESULTS AND DISCUSSION

Table 1 shows the data on the number of eggs laid on cowpea seeds, number of damaged seeds, insect mortality and viability of seeds (% germination) after 30 days. From the table, more eggs were laid at lower concentrations and at concentrations of 1.0 g and above significantly low numbers of eggs were laid. Thus, *D. saxatilis* treatment affected the oviposition of *C. maculatus*. There was no damage to the seeds at treatment levels of 0.75 g and above and damage at 0.25 and 0.5 g was much lower than that of the control. The plant therefore showed very promising effects in controlling damage to cowpea seeds. After 30 days, no live insect was recorded at different levels of treatment. However, a greater number of dead insects were recorded at lower levels indicating that there were reproductive activities leading to the development of F₁

Table 2. Determination of mode of protectant activity of *D. saxatilis* leaves.

Effect of leaves (2.0 g)	Contact poison	Respiratory poison
Insect mortality	10.0 ± 0.00	14.2 ± 1.30

Values are means of 5 replicates.

**Figure 1.** Mortality rate of mosquitoes exposed to 0.2% solutions of the crude extract and fractions of *D. saxatilis*.

progenies. Total mortality observed in this work indicates the effectiveness of *D. saxatilis* as a protectant for cowpea seeds against *C. maculatus*. The % germination at all levels of treatment was above 70% but seeds at lower levels of treatment were generally more viable. Thus, the treatment did not adversely affect germination of the seeds.

Table 2 shows that mortality was much more with *D. saxatilis* as a contact poison than as a respiratory poison. The mortality of 14 ± 1.30 suggested the development of F₁ progenies with *D. saxatilis* as a respiratory poison and therefore it is more suitable as a contact bio-pesticide. The results of insecticidal screening are presented in Figure 1.

After one hour of exposure in the cages 9 dead mosquitoes were recorded for the crude extract, 8 for the aqueous methanol and 6 for the hexane fractions and 2 for the solvent. Thus, compared to the controls, 0.2% solutions of the total crude 95% ethanol extract and the

aqueous methanol and hexane fractions of the crude extract displayed appreciable and almost equal insecticidal activity against adult mosquitoes in the first 30 min of application. Preliminary chemical studies in this work have suggested the presence of fatty acid esters, sterols and phenols in both the bark and leaves of *D. saxatilis*. These classes of compounds have been associated with biological activity, particularly insecticidal activity (Hill, 1975). Studies by other groups have also established that *Dalbergia* species contain cinnamylated phenols, which are known to possess sterilizing effects on flies (Gregson et al., 1978).

The antimicrobial screening results (Table 3) showed the crude 95% ethanol extract of the leaves of *D. saxatilis* to be active only against *S. aureus* (1000 µg/ml) among the six pathogenic organisms used in the screening. Though the spectrum of activity of the leaves is narrow the level of activity is quite adequate because minimum inhibitory concentrations at 1000 µg/ml and below are

Table 3. Antimicrobial activity of crude 95% ethanol extracts of the bark and leaves of *D. saxatilis*.

Extract	Microorganism/MIC ($\mu\text{g/ml}$)					
	1	2	3	4	5	6
Bark	250	125	1000	-	1000	-
Leaves	1000	-	-	-	-	-
Streptomycin SO ₄	5.0	-	5.0	-	-	2.5

(-) = No activity; microorganisms: 1 = *S. aureus* ATCC 13709, 2 = *Bacillus subtilis*, clinical isolate, 3 = *E. coli* ATCC 9637, 4 = *C. albicans* ATCC 10231, 5 = *P. aeruginosa* ATCC27853, 6 = *K. pneumoniae* ATCC 10031.

considered promising (Mitscher et al., 1972). The bark on the other hand displayed broad antimicrobial spectra. It was active against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* at concentrations of 250, 125, 1000 and 1000 $\mu\text{g/ml}$, respectively, belonging to both Gram-positive and Gram-negative classes of bacteria. Thus relative to the leaves the bark has a greater potential as a source of new anti-infective agents.

Given the above biological activities of *D. saxatilis* there is no doubt that it ranks high among the native Nigerian higher plants that need to be harnessed for use in agriculture and medicine. However, there is need to examine the modalities for their utilisation, particularly with respect to consistency of constituents as well as efficacy and quality of the products, vis-à-vis the production of bioactive plant-based products using western models or utilize the plants according to traditional procedures that eliminate purification. For example, the anti-sickle cell anaemia drug, *NICOSAN* has been found to be less potent and more toxic on separation into individual components (XeCHEM, 2006). Thus, there are some advantages in the traditional procedures of preparing herbal products in a manner that preserves the constituents of the plants. However, while appreciating the low cost of production of phyto-medicines and bio-pesticides by eliminating sophisticated purification and formulation procedures, a middle of the road approach that ensures consistency of active constituents and enhances efficacy and safe delivery is necessary. This may be using bioassay-guided fractionation which has been shown by some workers to ensure that bioactive compounds of the same chemical class in a crude plant extract are consistently pooled together. The procedure has been shown to improve activity dramatically and has been used to obtain active compounds from plants that were previously considered to be inactive (Mitscher et al., 1987).

Thus, cheap plant-based bioactive products may be prepared with improved efficacy if processed using bioassay-guided fractionation of the crude extract. The content of the identified components can be used to standardize the crude drug or bio-pesticide as gedunin has been proposed for crude Neem-based antimalarial drugs (MacKinnon et al., 1997).

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