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Screening of methanol extract of *Sclerocarya birrea* root bark as antifeedants for *Tribolium casteneum*

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ABSTRACT

The methanol extract of *Sclerocarya birrea* (A. Rich) Hochst was screened for antifeedant activity against the stored product pest in maize, *Tribolium casteneum.* It was found to be a good antifeedant for the insect at various concentrations based on the antifeedant index calculated. Preliminary investigation of the active constituents indicates that the hydrolysate of the extract contains a phenolic compound that might be responsible for the antifeedant activity observed.

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INTRODUCTION

Many tropical plants have developed chemical defenses to deter predation by herbivorous animals or insects. People in many communities around us possess a sophisticated knowledge of these plants and often use them as medicines or poisons. The calabar bean *Physostigma venenosum* was traditionally used as an ordeal poison in West Africa and studies of the active principle of this species led to the development of methyl carbamate insecticides.

Many crude extracts of plants have found use as insecticides, antifeedants, repellents, etc. Insecticides are substances that kill or drive away insects. Antifeedants are substances in plants which when perceived by insects or pests reduce or prevent insect feeding [1] and act as defense mechanism against insect attack in plant systems. Majority of antifeedants do not directly kill the insects, they repel them from eating the plant, upset the insect metabolic system – mould cycle, respiratory system, etc. [2]. Antifeedants, unlike insecticides are often selectively toxic towards some insect species whereas they are not harmful to others and to mammals.

A good natural insecticide or antifeedant should have the following qualities: effectivity - such as in insecticidal activity or insect deterrence; low toxicity in vertebrates and beneficial insects; more than one mode of action to reduce resistance to pest; low persistence in plants, soil and water; extraction and formulation should be simple, cheap and easy; and plant material should be one that can be easily sourced.

Phenolic Compounds as Antifeedants

The phenols are a varied group of compounds found in plants that range from sugar–containing phenolic glycosides to salicyclic acids. Phenolic compounds are known to exhibit anti-inflammatory, antiseptic, anti-viral and anti-oxidant properties. Protection against infection and insect feeding activity (or attack) are the leading theories on why plants produce these compounds [1]. Several phenolic compounds have insecticidal or antifeedant properties and have been correlated with host plant resistance to insects. For example, rotenone (isolated from the roots of three genera: *Derris, Lonchocarpus* and *Tephrosia*) has been used commercially as an insecticide since in the 1930s. From literature [3] and reports of traditional users, *Sclerocarya birrea* (A. Rich). Hochst, has such antifeedant activities, hence this investigation to determine its potency against pests especially *Trobolium casteneum* Hbst. (rust red flour beetle), a stored product pest in maize.

The plant belongs to the *Anacardiaceae* family. It is known as Jelly plum or Maroola plum. It is a tree, about 30ft high and often found in the drier Sahel Savannah from Senegal to Niger in West Africa, South Africa, Ethiopia and Uganda. In Nigeria, the Hausas refer to it as Danya and it is common in Sokoto, Zamfara, Kano, Bornu, Kaduna and Bauchi States [4]. Studies on its nutritional content and value have been reported [3,5,6]. Various parts are reported to be rich in tannins [6]. Physiological studies on its anticrustacean activity to predict for antitumor, antibacterial and antifungal activities using the stem bark and leaf have also been reported [8,9]. The isolation of a compound, (-)–Epicatechin–3–galloylester, from the bark of the plant has been reported [10].

In the Anacardiaceae family, other genus reported to have medicinal and insecticidal activity include *Anacardium occidentale*, *Mangifera indica* L.(Mango), *Rhus tomentosa* L., *Schimus molle*. L. and *Sclerocarya caffra* [6].

EXPERIMENTAL

Plant Sample Collection and Preparation

The plant sample, *Sclerocarya birrea* (A.Rich) Hochst. was collected around the Dakace area in Zaria, Kaduna State and properly identified at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. The voucher specimen number is *S. birrea*: -1079. They were collected after the rainy season, between the months of October and November. The root of the sample was washed in water to remove soil debris, cut into pieces and air-dried under shade. The dried root bark was ground using a mill and was extracted exhaustively using solvents of varying polarities – petroleum spirit, and methanol. The methanol crude extract was screened for antifeedant activity.

Insect Culture

A culture of *Trobolium casteneum* Hbst., rust red flour beetle (a stored product pest in maize), was used for this experiment. The culture was obtained from Biological Sciences Department, Ahmadu Bello Univerisity, Zaria. The insects were raised in wheat powder under laboratory conditions at $30\pm3\ ^{O}C$.

Antifeedant Test

The antifeedant test was conducted using a modified standard method by [11]. Wafer discs of wheat flour were used as the testing food. The discs were prepared and the extracts (E) were applied to the discs using a dropping pipette. A control [C] set of discs received only an equal amount of pure solvent, methanol. After equilibration, the wafers were placed in sets of three tubes with: Tube 1 [CC] containing two control discs; Tube 2 [CE] containing one disc with extract and one control disc; and Tube 3 [EE] containing two extract discs. This was repeated for the varying concentrations (1.0%, 5.0% and 10%w/v in methanol) of the plant extract.

A standard reference; a storage pesticide Pirimiphos methyl-2% dust was also set up under the same experimental condition. Methanol was used as the extruding solvent.

For each set of experiment, fifteen (15) unfed, adult *Tribolium casteneum* were introduced into each tube and the weight loss from each disc in the tubes (representing the amount consumed) after 12 days was taken. The experiment was repeated five times, and observations were made on the conditions and activity or otherwise of the insects towards the wafer. For each set of values, the mean; standard deviation (SD); variance; and standard deviation, were calculated to obtain the antifeedant index, T, a co-efficient to measure the unpalatableness of a substance and this is reported on the antifeedant scale. This scale infers that T values from 200-151 mean Excellent antifeedant; 151–101 mean Good antifeedant; 101–051 mean Medium antifeedant; 050–001 mean Neutral substances; while Negative values mean Attractants.

Purification and Characterization of the Extract

The crude *S. birrea* methanol extract was hydroly- zed with dilute hydrochloric acid and the hydrolysate exhaustively extracted using a sohxlet extractor with chloroform. A reddish brown $CHCl_3$ extract was obtained. The extract was tested for presence of alkaloids, glycosides and phenols. The infrared spectrum of the extract was taken.

RESULTS AND DISCUSSION

Table 1 gives the Antifeedant Test results obtained. The results show that the methanol extract of the root bark of *Sclerocarya birrea* (A. Rich) Hochst has good antifeedant constituents. Inhibition of feeding was observed at all concentrations of the crude methanol extract. An average of two insects was recorded dead in the 1.0% w/v tubes, between three and four in 5.0% w/v tubes and up to 10 or more in some of the 10.0% w/v EE tubes. It was also observed that there was a higher level of inactivity among the insects in the EE tubes compared to those in the CC tubes due to their inability to feed. The insect in CE tubes kept mostly to the C-wafer discs and abstained from feeding on the E – wafer discs. The calculated T values at 1.0% $^{w}/_{v}$ is 80.81; at 5.0% $^{w}/_{v}$ is 102.73 and at 10% $^{w}/_{v}$ is 123.71; which are good antifeedant index indicating that the

Table 1: Antifeedant Activity of methanol extract of the root bark of *S. birrea* on *T. Casteneum*

Concentration		Food Consumed (% wt of wafers) Mean $\pm$ S.D.
1% $^{w}/_{v}$		
Control – Control [CC] tubes	-	64.10 $\pm$ 2.27
Control – Extract [Control] CE tubes	-	68.49 $\pm$ 3.19
Control – Extract [Extract] CE tubes	-	27.87 $\pm$ 2.83
Extract – Extract [EE] tubes	-	23.91 $\pm$ 5.68
T Value	-	80.81
5.0% $^{w}/_{v}$		
Control – Control [CC] tubes	-	65.42 $\pm$ 2.39
Control – Extract [Control] CE tubes	-	64.90 $\pm$ 1.38
Control – Extract [Extract] CE tubes	-	13.28 $\pm$ 0.69
Extract – Extract [EE] tubes	-	14.31 $\pm$ 1.05
T Value	-	102.73
10.0% $^{w}/_{v}$		
Control – Control [CC] tubes	-	66.63 $\pm$ 4.12
Control – Extract [Control] CE tubes	-	67.94 $\pm$ 1.77
Control – Extract [Extract] CE tubes	-	06.33 $\pm$ 1.82
Extract – Extract [EE] tubes	-	04.53 $\pm$ 0.69
T Value	-	123.71

methanol extracts contain constituents responsible for suppressing the feeding habit of *Tribolium casteneum* Hbst. (rust red flour beetle).

For the standard reference, Pirimiphos methyl-2% Dust, inhibition of feeding was observed in all concentrations. The T values at 0.1% w/v is 92.34; at 0.5% w/v is 102.76; and at 1.0% w/v is 114.20. At concentration as low as 0.1% w/v, an average of five insects was recorded dead in the CE tubes, while about eight were recorded in the EE tubes. Most of the insects in the 0.5% and 1.0% w/v EE tubes were dead before the end of the 12 days duration of the experiment. Attempts at using 5.0 and 10.0% w/v lead to the death of all the insects within hours and minutes (respectively) of setting up the experiment. On an average the 10.0%w/v methanol extracts (T=123.7) showed good activity comparable with 1.0% w/v concentrate of the reference standard (T=114.2). At higher concentrations of the crude extract (>10% w/v) or using pure constituent of *S. birrea*, it can be extrapolated that antifeedant activity will increase.

In an attempt to determine the nature of the constituents in the methanol extract, it was hydrolysed with hydrochloric acid, aimed at converting tannin and/or phenolic glycosides to the corresponding phenolic compounds. The decision to hydrolyze the extract was based on literature reports that *S. birrea* is a tannin–producing tree [6,12] and that several tannins, particularly in the condensed form, have antifeedant characteristics [13]. This hydrolysate was positive for phenolic compounds and its IR-spectrum showed absorption frequencies of a strong O-H band at 3446cm^{-1}, C-O$_{str}$ at 1460cm^{-1}, C=O$_{str}$ at 1708cm^{-1} and C-H$_{str}$ at 2853cm^{-1}. Further work on the hydrolysate is being carried out to fully characterize the compound responsible for the activity.

CONCLUSION

The methanol extract of the root bark of *Sclerocarya birrea* (A. Rich) Hochst contains constituents that act as good antifeedant (suppressing the feeding habit) of the rust red flour beetle, *Tribolium casteneum*. The antifeedant index, T, of the extract was calculated to be 80.81, 102.73 and 123.71 at 1.0% $^{w}/_{v}$, 5.0% $^{w}/_{v}$ and 10% $^{w}/_{v}$ concentrations respectively against the insect. The T value at 10% $^{w}/_{v}$ of the crude extract was found to have comparable activity with the 1.0%$^{w}/_{v}$ Pirimiphos methyl-2% Dust, a commercial storage pesticide. From the result of this investigation the methanol extract hydrolysate is a phenolic compound, as a product of the hydrolysis of a condensed tannin which *S. birrea* is known to contain.

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