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Chemical and Biological Evaluation of the Stem Bark of Bobgunnia Madagascariensis (Desv.) J. H. Kirkbr. & Wiersema)

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ABSTRACT

The potential of the Snake bean plant, Bobgunnia madagascariensis (Desv.) J. H. Kirkbr. & Wiersema) as a grain protectant is under study here with the Wheat Wafer Disc Bioassay technique being adopted for the Antifeedant Bioassay tests. The stems bark proving highly active against stored-product insect pest of maize, Tribolium casteneum. The crude chloroform and methanol extracts of the stem bark of the plant Bobgunnia madagascariensis demonstrated strong feeding deterrent activity with antifeedant T-values of 128.94 and 122.53 respectively at 0.10ppm concentrations. Two compounds namely, (1) Methyl paraben and (2) Lupeol (Lup-20(29)-en-3-one) were identified and isolated from the stem bark using spectroscopic techniques.

KEY WORDS: Plant Extracts, Secondary metabolite, Feeding Deterrent, Methyl paraben, Lupeol.

1.0 INTRODUCTION

Alternative strategies in the search for new types of insecticides, and the re-evaluation and use of traditional botanical pest control agents have gained momentum in recent times. From previous experience, farmers have observed that the botanical insecticides/pesticides tend to have a broad-spectrum activity, are safe and relatively specific in their mode of action and easy to process and use in the traditional settings [1]. They also tend to be safe for their animals and environment in general. They can also often be easily produced by farmers and small-scale industries. Prior to the discovery of synthetic pesticides, plant or plant-based products were the only pest-managing agents available to farmers around the world. Even today several plant-based products are used to control a wide variety of pests, for example many oils and formulations from plant extracts are being marketed as pesticides around the world [2, 3]. The potential of the Snake bean plant, *Bobgunnia madagascariensis* as a grain protectant is under study here with the stem bark proving highly active against stored-product insect pest of maize, *Tribolium casteneum.* The investigation has led to the identification of some class of chemical compound(s) with potential to act as deterrents against *Tribolium casteneum* Hbst, a maize weevil of maize grains, maize flour and wheat flour/meals.

1.1. Bobgunnia madagascariensis (Desv.) J.H.Kirkbr & Wiersema

The genus *Bobguinnia* J.H.Kirkbr. & Wiersema belongs to tribe Swartzieae, subfamily Papilionoideae (Faboideae) of the Leguminosae; Fabaceae: Caesalpinioideae. This species occurs as small trees or shrubs in tropical and Southern Africa. *Bobgunnia madagascariensis* is a wild leguminous tree, about 12m tall, the outer bark scales off in small cracks leaving reddish brown stem with dark brown slash and gritty. *Bobgunnia madagascariensis* (formerly known as *Swartzia madagascariensis*) is a very common tree in many regions of Africa. The wood is used for timber, fire woods, for fodder for larger animals, poles, medicines, pods, fish poison and it also exhibit termite-resistant properties. Various parts of this plant are used by traditional healers in Africa for medicinal purposes such as treatment of leprosy and syphilis, among others [4]. Phytochemical investigation of the dried fruits gave triterpenoid saponins, which were shown to be responsible for its high molluscicidal activity against *B. glabrata* [5]. Pterocarpans have also been reported to have been isolated from the heartwood [6, 7, 8]. A LC/UV/MS analysis of the extract demonstrated that a new type of natural product, a 'quinone methide' diterpene with a cassane skeleton and hemiacetals derived from the bark and roots of the *Bobgunnia madagascariensis* tree as being effective against *Candida albicans* and *Aspergillis* infections [9, 10]. A flavonoid, Quercetin has also being reported isolated from the stem bark with strong antifeedant activity [11].

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2.0 MATERIAL AND METHODS

2.1 Preparation of Plant Extracts.

The stem bark of the plant *Bobgunnia madagascariensis* was collected at Sakaru village in Kaduna state, Nigeria. The plant samples were collected in the month of October 2007. The plant sample was authenticated at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria where a voucher specimen was deposited with Herbarium number 430. The stem bark were cut into smaller pieces and air-dried and pulverized using a blender. Extraction was carried out exhaustively using solvents of varying polarity extracts obtained was concentrated using a vacuum rotary evaporator. These were used for the phytochemical screening and antifeedant tests. Laboratory work was conducted in the Department of Chemistry, Ahmadu Bello University, Zaria and the Industrial Chemicals Division of the National Research Institute for Chemical Technology, Zaria.

2.2 Phytochemical Analysis of the plant

The pulverized and solvent-extracted plant samples were screened for its phytochemical constituents using standard procedures [12, 13]. Tests conducted were for carbohydrates, sugars, cardiac and saponin glycosides, steroids tannins, alkaloids and flavonoids.

2.3 Antifeedant Analysis: Feeding Deterrency Tests

The Antifeedant Bioassay tests were determined using the wheat wafer disc bioassay technique [14, 15]. The insects used for the tests were reared under laboratory conditions at $27 \pm 2^{o}C$ temperature and $75 \pm 5\%$ Relative humidity on a diet mixture of wheat meal of 190g of whole meal wheat flour and 10g of brewer's yeast in the ratio 19:1. Wheat wafer discs, made of flour and water and baked at $80^{o}C$ were used as the test substrate. The discs (1cm diameter x 1mm thickness within weights of 0.30-0.35gm) were saturated by coating with prepared concentrations of 0.05ppm and 0.10ppm of each of the extracts, BSX, BSC and BSMe and a control was set-up using only the solvents. The wafers were allowed to dry by evaporating off the solvent medium (30mins of air-drying). These wafers were weighed and introduced into Petri dishes into which sets of ten (10) flour beetles/Petri dish (previously starved for about 24hrs) had been placed and each set-up was monitored over a period of five (5) days. Some blank discs (treated with solvents but not offered to the insects were also prepared). The wafer discs were weighed before the experiments and 5days after the test insects had been feeding on them.

Each treatment was replicated five times. After the 5-day feeding period, the discs were reweighed. The disc weight loss which was the estimate of the amount of food consumed (FC), was calculated using an adopted formula [16].

$$FC = IW_S - [(FW_S \times IW_B)/FW_B)]$$

Where $\mathbf{IW_S}$ is Initial weight of the disc after treatment with extract or solvent;
$\mathbf{FW_S}$ is the Final Weight of the treated wafer disc;
$\mathbf{IW_B}$ is the Initial weight of the Blank disc (treated with solvent only but no insects present) and $\mathbf{FW_B}$ is the final weight of the blank disc though no insects were present.

The weight of food consumed was the basis for calculation of three co-efficient f deterrence: Absolute (A), Relative (R) and Total (T=A+R). The three feeding deterrent co-efficient was calculated by using the following formulae:

Absolute Coefficient of Deterrence (Control and No-Choice Test)

$$A= \frac{CC - TT}{CC + TT} \times 100$$

Relative Coefficient of Deterrence (Choice Test)

$$R= \frac{C - T}{C + T} \times 100$$

Total Coefficient of Deterrence

$$T= A + R$$

The values of the total co-efficient served as an index of antifeedant activity expressed on a scale between 0 and 200, the index zero (0) signified an inactive compound and 200, compounds with maximum activity. Antifeedants having an index of 150-200 were designated ++++; 100-150 +++; 50-100 ++ and 0-50 +. All experimental data were analyzed by analysis of variance (ANOVA).

2.4 Chromatographic Analyses

The Chloroform extract from the stem bark was further partitioned in Diethyl ether and TLC carried out. A total of four (6) components were observed and the R_F values noted (0.12; 0.38; 0.48; 0.52, 0.64 and 1.00). Detection was carried

out at 254 and 366 nm with a UV-Visible Lamp. Column Chromatograph (CC) further separated the components, (Silica gel; Chloroform: Ethyl acetate 1:1) various fractions collected and pooled into four components were collected. Further purification of the various combined fractions was carried out using Prep TLC (Silica gel, Ethyl acetate) and two of the fractions obtained as relatively pure compounds were further confirmed Spectrophotometric techniques.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical Test Results

The phytochemical analysis indicated the presence of carbohydrates, glycosides, flavonoids, sterols, tannins and alkaloids.

3.1.2 Antifeedant Test Results

From the feeding deterrent test results as tabulated in Table I, the crude chloroform and methanol extracts were also observed to have the highest T-values, most especially at the 0.10ppm concentrations with T-values of 128.94 and 122.53 respectively indicating strong feeding deterrent activity in the extracts. From these antifeedant results, chromatographic partitioning of the components of the chloroform extracts from the stem bark of the plant was carried out.

Table I: Feeding Deterrent Co-efficients of *Bobgunnia madagascariensis* Extracts against Red Flour Beetles, *Tribolium casteneum.*

Name/Type of Extract	Coefficient of Deterrency			Efficacy of Extract
	Absolute	Relative	Total	
Stem Bark Extracts/Conc. [ppm]				
0.05 -BSX	07.19	06.90	14.09	+
0.10 -BSX	08.44	09.52	17.96	+
0.05 -BSC	52.16	46.26	98.42	++
0.10 -BSC	65.66	63.28	128.94	+++
0.05 -BSMe	58.58	56.32	114.90	+++
0.10 -BSMe	**72.53**	**50.00**	**122.53**	+++

Key:-BSX-n-Hexane; BSC-Chloroform and BSMe-Methanol Extracts of Bobgunnia madagascariensis.

3.3. Chromatographic Analysis

After evaporation under vacuum the stem bark extract of *B. madagascariensis* was subjected to a series of chromatographic purifications, using Thin-Layer, Column and Prep Thin-Layer Chromatography to afford four components (Chloroform: Ethyl acetate 1:1;R_f values 0.38, 0.52, 0.64 and 0.78). GC/MS analysis resulted in the identification of triterpenoid and a methyl ester compounds. Further spectroscopic analyses using the IR [SHIDMAZU 8400S FTIR Spectrophotometer], and GC-MS, 1H- and C13-NMR spectral analyses, two compounds namely: (1) Methyl paraben and (2) Lupeol (Lup-20(29)-en-3β-ol) were identified.

Compound I was obtained as a white crystalline powder (132° C m.pt) and its GC-MS spectrum showed an ion peak at *m/z* 152, consistent with the molecular formula $C_8H_8O_3$. The IR spectrum of Compound **I** showed an absorption bands at 3282.40cm^{-1}, 1743.70cm^{-1}, 1430.18cm^{-1} and 1001.94cm^{-1} which were consistent with the presence of hydroxyl, carbonyl, aromatic ring and ether groups respectively. The GCMS values: Methyl paraben (70eV) m/z 152 [M$^+$], 121 (100), 93 (25), 65(25), 53(10), and 39(20).

Compound II was obtained as colourless crystalline substance (257°C m.pt.). Spectroscopic data is as detailed below:

IR (CHCl3) *max*: 3311, 2944, 2872, 2860, 2342, 1731, 1640, 1465, 1379, 1317, 1246, 1216, 1149, 1105, 1027, 979, 944, 883, 757, 667 cm−1.

GCMS with %abundance: m/z 424 [M$^+$], 424 (70), 409(25), 313(30), 245(25), 218(40), 189(45), 175(25), 149(50), 121(65), 109(75), 81(50), 69(45), 55(45), and 40(25).

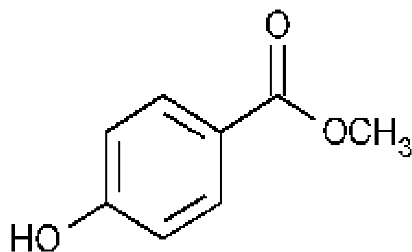
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.74 (1H, br. s, Ha-29), 4.61 (1Hb, br. s, H-29), 3.19 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 1.69 (3H, s , Me-30), 0.98 (3H, s, Me -26), 0.97 (3H, s, Me -23), 0.94 (3H, s, Me -27), 0.82 (3H, s, Me -25), 0.76 (3H, s, Me -24).

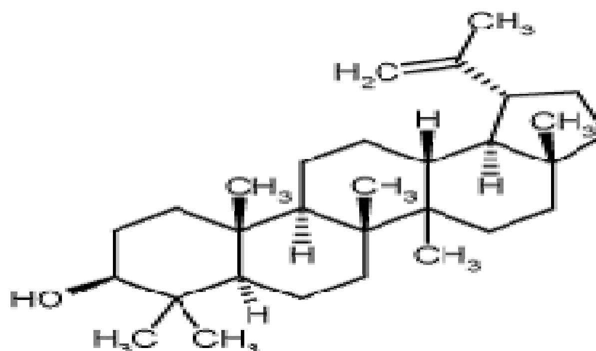
The 1H NMR spectrum (400 MHz, $CDCl_3$) of compound **II** showed one double doublet of one proton intensity at δ 3.19 (*J*=11.2, 4.8 Hz) typical for H-3 of a triterpene type carbon skeleton. The spectrum displayed two singlets at δ 4.74 and δ 4.61 (1H each) assignable to protons at C-29. A multiplet of one proton intensity at δ 2.36 was assigned to H-19.

The spectrum also displayed six singlets at δ 0.76, 0.82, 0.94, 0.97, 0.98, and 1.69 (3H each) assignable to protons of methyl groups at C-4 (H3-23, H3-24), C-10 (H3-25), C-8 (H3-26), C-14 (H3 -27), and C-20 (H3-30), respectively.

The 13C-NMR CDCl3) showed signals of 30 carbon atoms, which were identified with the assistance of its DEPT spectrum as seven methyls, ten methylenes, seven methines, and six quaternary carbons. Some of these are supported by the values of the 13C-NMR data below:

^{13}C NMR ($CDCl_3$ δ values: 150.82, 110.10, 78.60, 55.10, 48.60, 42.28, 41.28, 36.40, 33.40, 31.10, 27.90, 25.10, 22.80, 19.10 and 14.80. These values have been verified with that of previously published values and are in good agreement for the structure of Lupeol (II) [17, 18, 19]. Below are the structures of compound I and II.





Compound I- Methyl paraben

Compound II- Lup-20(29)-en-3β-ol or Lupeol

3.2 DISCUSSION

Methyl paraben with chemical formula $C_8H_8O_3$ and molecular weight 152.15 is a methyl ester of p-hydroxybenzoic acid. It is a stable, non-volatileantifungal compound used as an antimicrobial preservative in foods, drugs and cosmetics for over 50 years in a variety of health and beauty products to preserve and rejuvinate the skin. Methylparaben is a water-soluble anti-microbial and anti-irritant agent. Methylparaben is classified as both an ester and a phenol. The compound is often found in carpules of local anaesthetic, acting as a bacteriostatic agent and preservative. Methylparaben (also called Nipigin, Tegosept, and Mycocten) is commonly used as a fungicide in *Drosophila* food media. Usage of methylparaben is known to slow *Drosophila* growth rate in the larval and pupal stages [20].

Lup-20(29)-en-3β-ol with chemical formula $C_{30}H_{50}O$, molecular weight 426.7 is also generally known as lupeol, clerodol, fagarsterol and lupenol. It is mainly identified by its ^{1}H and ^{13}C NMR spectral data which reveal typical signals of a pentacyclic lupine-type triterpene with olefinic protons/carbons. This triterpene also has reports of antifungal activity [21] and is known to have vast occurrence in diverse plant families. Lupeol has been shown to exhibit strong anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial activity in *in vitro* and *in vivo* systems. [22]

From literature studies conducted, these compounds, though already known are being reported in the plant *Bobgunnia madagascariensis* for the first time. Previous work done on the antifeedant activity of this plant, had led to the isolation of a flavonoid compound, quercetin from the stem bark of the plant *B. madagascariensis* which had proved to have strong deterrent activity against the maize weevil, *Tribolium casteneum* comparable to azadirachtin from the Neem plant [11]. Insecticidal activity against termites and anti-malarial activity have also been reported [22, 23]. An anti fungal diterpenoid compound 'quinone methide' believed to be useful in treating *Candida albicans*, which is responsible for many fungal skin conditions and for the mycosis that affects the mouths, eyes and other parts of AIDS patients have also been identified in the bark of this plant. The Swiss team said it can also kill *Aspergillis*, which can cause a fatal lung condition and several other species of fungi that infect people [24].

Possibility of these isolated compounds working in synergy with other identified components of this plant cannot be ruled out. The antimicrobial and anti fungal properties reported from these two compounds isolated could also have had synergistic effect in the antifeedant activity reported in the crude chloroform and methanol extracts earlier screened. It also gives credence to some of the claims made by the traditional healers and farmers as regard the efficacy of the plant. There is a need to re-visit some of these known and identified compounds from plants (most of them already have synthetic analogues) for possible use in formulation new post harvest storage pesticides.

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