Australian Journal of Basic and Applied Sciences, 4(8): 3342-3346, 2010
ISSN 1991-8178

Antifeedant Activity of Quercetin Isolated from the Stem Bark of *Bobgunnia madagascariensis (Desv.) J.H.Kirkbr & Wiersema. (Caesalpiniaceae)*

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Abstract: The solvent extracted portions of the leaves, stem and root bark of the plant *Bobgunnia madagascariensis* were evaluated for antifeedant activity against the confused flour beetle of maize, *Tribolium casteneum*, Hbst; a storage pest of maize and its products. The compound, 3', 4', 5, 7-tetrahydroxy flavonol (Quercetin), a flavonoid isolated from the chloroform extract of the stem bark of *B. madagascariensis* was also evaluated for antifeedant activity against the confused flour beetle of maize, *T. casteneum* Hbst. using the wafer disc choice bioassay with over 50% feeding deterrent activity observed.A comparative study was also carried out using a known botanical, azadirachtin as a control.

Key words: Antifeedant, Flavonoids, Quercetin, *Bobgunnia madagascariensis*

INTRODUCTION

Plant products are known to be effective means used by small scale farmers for protecting stored grain from insect damage. Tissues from various botanical species have been used to improve storage of grain in the rural areas, for example, the dried fruits of *Capsicum* spp., the powdered roots of *Derris elliptica* and the leaves and seeds of the Neem tree, *Azadirachta indica*.(Paneru, *et al* 1997) The pesticidal properties of many plants have been known for a long time and natural pesticides based on plant extracts were used in pest control during the early half of the last century. The use of plant and plant-derived products to control pests in the developing world is very common. 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 still in use to control a wide variety of pests.(Pavela and Chermenskaya, 2004)

The genus *Bobguinnia* J.H.Kirkbr. & Wiersema belongs to tribe Swartzieae, subfamily Papilionoideae (Faboideae) of the Leguminosae (Fabaceae):Caesalpinioideae and consists of about 140 species. *Bobgunnia madagascariensis* (formerly known as *Swartzia madagascariensis*, Caesalpiniaceae) is a very common tree in many regions of Africa. It is a wild leguminous tree that is widespread throughout the Miombo woodland areas of Tanzania. Various parts of this plant are used by traditional healers in Africa.(Watt and Breyer-Brandwijk, 1962) The tree bears large fruits which have been reported to be toxic to snails and are related to the causative agent of human schistosomiasis. (Mozley, 1939 ; Mozley,1941; Lwambo, and Moyo, 1991) It is an indigenous tree cited as being used for fodder and exhibit termite-resistant properties, the insecticidal activity against termites has also been reported.(Schultes, 1979) The snake bean in its indigenous use has its root bark or/and leaves pounded and mixed with a litre of water, allowed to infuse for twelve to twenty fours hours, strained and sprayed or drenched over termite nests. This formed basis for the interest to ascertain the antifeedant activity of the plant. Traditional healers use the roots of the tree to treat leprosy and syphilis.

Insect antifeedants are substances which on being tasted by insects, result in temporary or permanent cessation of feeding activity.(Kubo and Nakanishi, 1977) Antifeedants that reduce consumption by insects act as behaviour modifiers that deter feeding through a direct action on peripheral sensilla (taste organs) of insects.(Isman,*et al* 1996)

MATERIAL AND METHODS

Samples of *Bobgunnia madagascariensis* comprising the leaves, stem bark and root bark were collected at Sakaru village along Jos road in Saminaka Local Government Area in Kaduna state, Nigeria. The plant

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samples were authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria and voucher specimen were deposited with Herbarium number 430. These plant materials were cut into smaller pieces, separated into leaves, stem and root barks, air-dried and pulverized using a blender.

The air-dried powdered stem barks (400g) were extracted exhaustively with n-hexane by maceration method. The n-hexane extract concentrated in *vacuo* yielded a light brown to greenish brown coloured oily substance 3.89 g (0.97%). The marc (378g) was extracted exhaustively by the Sohxlet extraction method with chloroform to give a reddish brown extract 43.93g (11.62%). Preliminary TLC was carried out on the Chloroform extract of *B. madagascariensis* stem bark and was further fractionated using Column chromatography with solvent mixture Chloroform: Ethyl acetate (1:1) (Silica gel, Particle size 0.13-0.25mm, 60-120 mesh). Preparative TLC using 20x20cm plates with thickness 0.5mm were used for the purification work carried out on the fractions for possible isolation of pure components. In all cases, detection of the various components was carried out using Model UVGL-58 Mineral light lamp with detection at 254 and366 nm.

Chemical characterization of purified active compound was done through IR [SHIDMAZU 8400S FTIR Spectrophotometer], and GC-MS, [MERCURY 200BB NMR Spectrophotometer] 1H- and C13-NMR spectral analyses. The mass spectra NMR were run and compared to reference standard compound from the library database.

The antifeedant wafer disc choice bioassay test method was used to determine the feeding inhibition rate.(Talukder and Howse, 1995) 11 Wheat wafer discs, made of finely ground flour and water and baked at 80ºC were used as the test food or substrate. The discs (2.5 cm diameters within weights of 30-35mg) were saturated by dipping into the solvents and the test solutions in varying concentrations of 5.0 and 10.0mg/ml.

The feeding of the insects were recorded under two conditions: - on the discs treated with the solvent medium, this comprising of two untreated discs CC [Control]; on discs treated with the various extracts, this comprising of two treated (TT) discs (No-Choice Test). Each treatment was replicated five times. After 5-day feeding period, the discs were reweighed. A correction procedure was adopted to take into consideration the disc weight loss in calculating the amount of food consumed (FC) using a formula:

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

Where

FC = Calculated Food Consumed

IW = Initial weight of the wafer disc (control or treated),

FW = Final Weight of the wafer disc (control or treated),

IW_B = Initial weight of the blank wafer disc (treated with solvent only but not offered to insects) and

FW_B is the final weight of the blank wafer disc (treated with only solvents but not offered to insects).

After the feeding deterrency test was completed, to measure the activity of the test compounds, the Antifeedant Index AFI is used. The AFI is a measure of the percentage of the weight of the treated wafer discs consumed in comparison with the untreated wafer discs. That is:

$$AFI = \frac{\%\text{Weight of Treated Disc Consumed}}{\%\text{Weight of Treated Disc Consumed} + \%\text{Weight of Control Disc Consumed}} \times 100$$

The AFI values obtained are then converted to the feeding inhibition rate FI (%).

$$FI\ (\%) = (50 - AFI) \times 2$$

An FI value under 30% when treated at 10mg/ml for a wafer disc is indicated as inactive in the study.(Morimoto,*et al* 2006)

The deterrent test activities were carried out on both the plant extract and the isolated constituent identified by spectroscopic techniques. A comparative deterrent test was also conducted on the methanol extract of the stem bark of the plant, the isolated quercetin and a known compound identified as a natural deterrent in plant material, Azadirachtin, from *Azadirachta indica* was used as a control.

RESULTS AND DISCUSSION

Antifeedant Tests:

In the feeding deterrent tests, (Table I), it was observed that at a concentration of 10mg/ml, the methanol extract of the stem bark of the *B.madagascariensis* plant showed the highest deterrent activity against the red flour beetle, *Tribolium casteneum*. In the study, the *T. casteneum* were highly sensitive to all the test compounds except for the n-hexane extracts of the plant.

Table 1: Results of the Feeding Deterrent tests of the solvent-extracted stem bark of *B. madagascariensis*

	Stem bark in varying solvent of increasing polarities	Concentrations	Feeding Inhibition rate(%)	Status
a.	n-Hexane-BSNX	5.0mg/ml	10.14	Inactive
b.	n-Hexane-BSNX	10.0mg/ml	13.06	Inactive
c.	Chloroform-BSC	5.0mg/ml	51.94	Active
d.	Chloroform-BSC	10.0mg/ml	61.82	Active
e.	Methanol-BSME	5.0mg/ml	44.96	Active
f.	Methanol-BSME	10.0mg/ml	63.04	Active

Characterization of Isolated Compound:

The compound isolated was characterized using IR and NMR Spectrophotometric techniques. From the analyses, the compound was identified as 3', 4', 5, 7-tetrahydroxy flavonol (Quercetin).

IR ν_{max} (Neat) cm^{-1} 3282.40 (w), 1743.70 (w), 1666.75 (w), 1610.96(w), 1517.55(w), 1430.18(s), 1358.18(s), 1210.93 (w), 1094.07 (w), 1001.94 (w), 929.56(w), 882.18(w), 808.85(w), 705.01 (w), 590.36 (w).

Quercetin (70eV) m/z 348 [M^+], 302(98), 257(15), 228(8), 201(8), 154(8), 136(9), 110(9), 70(4) and 23(3). Compound was obtained as a crystalline yellow solid (m.pt. 315 ° C). The ^{1}H-NMR δ (CD_3OD):6.18(1H, d, J=1.7Hz),6.40(1H,d,J=1.7Hz) are due to meta-coupled protons of A-ring (H-6 and H-8) of a flavonoid nucleus. Signals at δ = 6.89 d= 8.3Hz, 7.68d, 2.5Hz and δ = 7.55dd, 2.2Hz, 8.3Hz are assigned to H-5', H-2' and H-6' of the ring. The ^{1}H NMR spectrum showed protons at aromatic regions from 6- 8 ppm, and strong hydrogen bonding at 12.5ppm. These suggest a quercetin nucleus. (Mabry,*et al*1970 ; Markham,*et al* 1978) The ^{13}C-NMR spectra revealed 15 carbon signals typical of flavonoid monoglycoside nucleus. The low field signal at 176.5ppm was due to the carbonyl group at C-4. Complete assignment was aided by DEPT, direct C-H correlation and HMBC. Thus the compound was identified as Quercetin. The NMR Spectral data values as reported in library reference is tabulated along side the observed NMR Spectra of the isolated Quercetin. The spectra compares very well with that reported in literature. (Chien-Chang,*et al*1993)

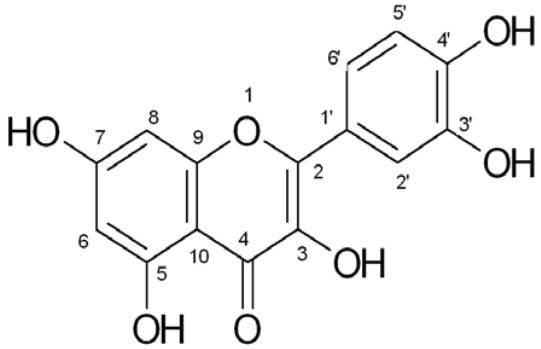

Fig I: Structure of Quercetin

Table 2: ^{1}H and ^{13}C NMR data for Quercetin, 200MHz, DMSO-d_6

Position	^{1}H of Quercetin compound from *B. madagascariensis*	^{1}H from Lit. data	^{13}C of Quercetin compound from *B. madagascariensis*	^{13}C from Lit. data
2			147.5	146.8
3			136.4	135.6
4			176.5	175.7
5			161.4	160.6
6	6.18 (d, J=1.7Hz)	6.18 (d, 2.0Hz)	98.9	98.1
7			164.5	163.8
8	6.40 (d, J=1.7Hz)	6.40 (d, 2.0Hz)	94.0	93. 3
9			156.8	156.1
10			103.7	103.0
1'			122.6	121.9
2'	7.68 (d, J=2.5Hz)	7.67 (d, 2.2Hz)	115.8	115.1
3'			145.7	145.0
4'			148.4	147.6
5'	6.89 (d, J= 8.3Hz)	6.89 (d, 8.3Hz)	116.3	115.5
6'	7.55 (dd, J=12.3 Hz,2.2 Hz)	7.53 (dd, 8.6, 2.2 Hz)	120.7	119.9
5-OH				
Other–OH grp	12.48 s 9.4-11.2	12.42 (s)		

Table 3: Result of the Comparative Antifeedant Tests between the methanol extracts from the stem bark of *B. madagascariensis*, the isolated compound, Quercetin and the control, azadirachtin against the Red Flour Beetles, *Tribolium casteneum*.

S/No	Extracts	Concentration (mg/ml)	Feeding Inhibition Rate (%)	Status
1.	Methanol Extract (Stem bark)	5.0mg/ml	56.28	Active
2.	Quercetin	2.0mg/ml	54.04	Active
3.	Azadirachtin	1.0mg/ml	67.10	Active

Comparative Antifeedant Tests:

From the result obtained, the isolated quercetin had comparable activity at 2.0mg/ml with the crude extract of the plant (5mg/ml) indicating that it has potential to serve as an antifeedant against the stored product pest, the maize weevil, *T. casteneum*. It can also be deduced from this result that increasing concentration of the quercetin compound would lead to higher activity comparable to that of the standard reference (control) Azadirachtin used in the experimental set, though the azadirachtin compound had a higher antifeedant activity with feeding inhibition rate of 67.10% when compared to the isolated quercetin component (54.04%). From the various literature reports cited so far and the results obtained from the antifeedants tests obtained, a confirmation can be made as to the efficacy attributed to the plant by the traditional farmers.

Other reports(Chien-Chang,*et al*1993) have indicated that some partially purified flavonoids including quercetin have shown activity as a post-harvest pesticide against grains and legumes. The effects of partially purified flavonoids obtained from *Calotropis procera* (Ait.) R. Br. and six standard flavonoids on the adults and eggs of *Callosobruchus chinensis* (L.), reared on mung beans (*Vigna radiata* L.), have been studied. (Salunke *et al*. 2005) Quercetin is a well-known allelochemical inhibiting the growth of other plant species and is insecticidal.(Gressel, and Ammann, 2008)

In conclusion, there is an urgent need to build up reliable food production systems in developing countries. The tremendous post-harvest losses sustained in these countries due to physical, nutritional and quality deterioration of stored grains by insects and the detrimental impact of these losses on food security are well known. From the results obtained from the various antifeedant tests, the plant, *Bobgunnia madagascariensis* (Desv.) J.H.Kirkbr & Wiersema has the potential to serve as an antifeedant against the stored product pest of maize and maize flour, *Tribolium casteneum* Hbst. From the phytochemical screening analyses (Hassan, 2009), it was observed that several classes of secondary metabolites are present in all parts of the plant and the flavonoid isolated; quercetin could be attributed to the high level of antifeedant activity observed in the crude extracts.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Dr. M. Bezabih of the Department of Chemistry, University of Botswana, for the NMR analysis. The effort of U.S Gallah of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria in collection of the plant samples and NARICT Technologists W.D Ibrahim and E. Ali in preparation of the extracts is also appreciated.

REFERENCES

Chien-Chang, S., C. Yuan-Shiun and H. Li-Kang, 1993. Nuclear Magnetic Resonance Studies of 5,7-Di-hydroxyflavonoids. Phytochem, 34(3): 843-845.

Gressel, J. and K. Ammann, 2008 Genetic Glass Ceiling-Transgenics for Crop Biodiversity, pp: 268-270. The John Hopkins University Press, Baltimore.

Hassan, M.M., 2009. Evaluation of the Chemical Constituents and Antifeedant Activity of the plant *Bobgunnia madagascariensis* (Desv.) J.H.Kirkbr & Wiersema (Caesalpiniaceae), Ph.D Thesis, Ahmadu Bello University, Zaria, Kaduna state, Nigeria.

Isman, M.B., H. Matsuura, S. MacKinnon, T. Durst, G.H.N. Towers and J.T. Arnason, 1996. Phytochemistry of the Meliaceae: So many Terpenoids, so few Insecticides. Recent Advances in Phytochemistry, 30: 155–178.

Kubo, I. and K. Nakanishi, 1977. Insect Repellants and Antifeedants from African plants. In: Hedin, P.A (Ed), 1977. Host Plant Resistance to Pests. American Chemical Society, Washington, D.C., 157.

Lwambo, N.J. and H.G. Moyo, 1991. The Molluscicidal Activity of Seed pods of *Swartzia madagascariensis* on Marisacornuarietis. East Afr. Med. J., 68(10): 827-830.

Mozley, A., 1939 Freshwater Mollusca of Tanganyika Territory and the Zanzibar Protectorate. Transactions of the Royal Society of Edinburgh, 59: 687-730.

Mozley, A., 1941 Malachite in the control of Bilharzai. Brit. Med. J. October 11, 511.

Morimoto, M., H. Fukumoto, M. Hiratani, W. Chavasir and K. Komai, 2006. Insect Antifeedants, Pterocarpans and Pterocarpol in Heartwood of *Pterocarpus macrocarpus* Kruz. Biosci. Biotechnol. Biochem., 70(8): 1864-1868.

Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids. Springer-Verlag Publication, New York.

Markham, K R., B. Ternai, R. Stanley, H. Geiger and T.J. Mabry, 1978. ^{13}C-NMR Studies of Flavonoids II. Tetrahedron, 34: 1391-1397.

Paneru, R.B., G.N.J. Le-Patourel, and S.H. Kennedy, 1997. Toxicity of *Acorus calamus* rhizome powder from Eastern Nepal to *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Prot., 16(8): 759-763. In: Payne, T.S. (Ed) Harvest and Storage Management of Wheat. www.fao.org/docrep/006/Y4011E/y4011e0t.htm

Pavela, R. and T. Chermenskaya, 2004. Potential Insecticidal Activity of Extracts from 18 Species of Medicinal Plants on Larvae of *Spodoptera littoralis*. Plant Protect. Sci., 40(4): 145–150.

Schultes, R.E., 1979. J. Ethnopharm.., 1, 79. In: - Schaller, F.; Wolfender and K. Hostettmann, 2001. New Antifungal Quinone Methide Diterpenes from *Bobgunnia madagascariensis* and Study of their Interconversion by LC/NMR. Hel. Chim. Acta., 84: 222-229.

Salunke, B.K., H.M. Kotkar, P.S. Mendki, S.M. Upasani and V.L. Maheshwari, 2005. Efficacy of Flavonoids in Controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), A Post-harvest Pest of Grain Legumes. Crop Protectn. October, 24(10): 888-893.

Talukder, F.A. and P.E. Howse, 1995. Evaluation of *Aphanamixis polysatchya* as a Source of Repellents, Antifeedants, Toxicant and Protectants in Storage against *Tribolium casteneum* (Hbst). J. Stored Prod. Res., 31(1): 55-61.

Watt, J.M. and M. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, E & S Livingstone, Edinburgh.