



**Phytochemical screening and insecticidal activity of leaf extracts
of *Bryophyllum pinnatum* and *Eucalyptus globules* against rice
weevil (*Sitophilus oryzae*)**

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ABSTRACT

Ethanollic extracts of the leaves of *Bryophyllum pinnatum* and *Eucalyptus globules* were screened for secondary metabolite constituents and insecticidal activity against rice weevil (*Sitophilus oryzae*). Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins and terpenoids in the plants investigated. Phlobatannins and terpenoids were found to be absent in ethanol extract of *Eucalyptus globules* (leaf) while steroidal compounds were absent in *Bryophyllum pinnatum* (leaf). The extracts of *B. pinnatum* and *E. globules* of different concentrations were also investigated for their insecticidal activity against *S. oryzae*. Average mortality percentage indicated that the extracts caused significant mortality on the targeted insects. The bioassay indicated that the toxic effect of the extracts was proportional to the concentration, and higher concentrations had stronger effects. The observed mortality percentage also increased with increase in time intervals after treatment. The mortality percentage at 0.25, 0.50, 0.70, 1.00 and 1.50 h after treatment (HAT) indicated that 4% solution of the extracts of *E. globules* and *B. pinnatum* showed the highest mortality of 37.00% and 35.00% respectively in *S. oryzae* at 1.50 h after treatment. It could be inferred from the study that extract from *E. globules* leaf could cause significant mortality compared to extract of *B. pinnatum*.

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INTRODUCTION

The protection of stored grains from insect damage is currently dependent on synthetic pesticides (Rahman et al., 2007). But the repeated use of synthetic insecticides for insect pests and vectors control has disrupted

natural biological control systems. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures (Brown, 1986;

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Hayes and Laws, 1991; Macêdo et al., 1997; Rahman et al., 2007).

Plants are considered as rich sources of bioactive chemicals and they may provide an alternative source of insect control agents (Wink, 1993). Pest control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence, crude plant extracts have played an important role in this aspect (Mahadevan, 1982). Mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties (Baladrin, 1985; Rawls, 1986; Sukumar et al., 1991).

Natural insecticides such as pyrethrum, nicotine and rotenone, among others, have been extensively used until recently for insect control (Baladrin, 1985). It has been reported that many compounds with insecticidal potential have been isolated from the genus *Piper*, such as Piperidine, isolated from *Piper nigrum* (black piper) has been found to be just as active against adjuki bean weevils as the pyrethroids (Mwangi and Mukiyama, 1988). It has also been reported that essential oils of leaf and bark of some plants demonstrated high larvicidal and insecticidal activity against insect pests (Cheng et al., 2003). Limonoids such as azadirachtin and gedurin present in species from the Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world (Dua et al., 1995; Nagpal et al., 1996; Harve and Kamath, 2004).

Bisht and Kamal (1994) observed that there is a strong need to investigate the chemical composition of many plants to determine their ability to be used as fungicides or insecticides. Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents, which accounted for various uses by man. The most important of these constituents are alkaloids, terpenoids, steroids, phenolics, saponins and tannins (Abayomi,

1993). Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers (Babu and Murugan, 1998; Venketachalam and Jebasan, 2001). However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance (Das et al., 2007).

In the present study, the ethanolic extracts of two botanicals namely: *Bryophyllum pinnatum* and *Eucalyptus globules* were screened for their secondary metabolite constituents and insecticidal activities against rice weevil (*Sitophilus oryzae*).

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Bryophyllum pinnatum* and *Eucalyptus globules* were collected from the premises of Crown Estate, Okada in Ovia North-East Local Government Area of Edo State, Nigeria on the 10th March, 2009. The plant materials (leaves) were identified and authenticated by a Taxonomist at the Biological Sciences Department, Igbinedion University, Okada, Nigeria.

The leaves were air-dried at room temperature (26 °C) for 8 weeks, after which it was milled into a uniform powder with the aid of an electrical grinder. The ethanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 300 ml of 80% ethanol at room temperature for 48 h. The extracts were filtered after 48 h through a Whatmann filter paper No. 42 (125 mm). The extracts were concentrated using a rotary evaporator at a maximum temperature of 45 °C. The weights of dried crude extracts were found to be 10.8% for *B. pinnatum* and 11.66% for *E. globules*. The extracts were then dissolved in distilled water to prepare solutions of different concentrations (1.0, 2.0, 3.0 and 4.0%). The bioassay of the extracts was done for direct toxicity (mortality) test. The solvent used was an analytical grade and purchased from E. Merck (Germany).

Collection of rice weevil

The rice weevils were collected at the Zoology Laboratory of Igbinedion University, Okada, Nigeria.

Phytochemical screening

Chemical tests were carried out on the ethanolic extracts for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Toxicity test

Direct toxicity test with rice weevils was carried out following the method described by Talukdar and Howse (1993), and Rahman et al. (2007). Insects were chilled at 2 °C for a period of 10 min. The immobilized insects were individually picked up and 1ml solutions of different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0% w/v) were applied to the dorsal surface of the thorax of each insect by using a micro capillary tube. Ten insects per replicate were treated. The insects were then transferred into a 9 cm diameter Petri dish containing food. Insect mortality rate was recorded 0.25, 0.50, 0.75, 1.0, 1.5 h after treatment. All the experiments were conducted following a completely randomized design with three replications and turned to statistical analysis. Finally, the mean values were compared using Duncan Multiple Range Test (DMRT), (Duncan, 1955).

RESULTS AND DISCUSSION

The result of the phytochemical screening (Table 1) reveals that tannins, alkaloids, flavonoids and saponins were present in both ethanolic extracts of *Bryophyllum pinnatum* and *Eucalyptus globules*. Phlobatannins and terpenoids were detected only in ethanolic extract of *Bryophyllum pinnatum* while steroids were detected only in the ethanolic extract of *Eucalyptus globules*. These phytochemicals may be responsible for their insecticidal properties (Kabaru and Gichia, 2001).

Tables 2 & 3 summarize the results of the average mortality percentage with ethanol

extracts of *B. pinnatum* and *E. globules* respectively, using Duncan significance test (Duncan, 1955). With the crude extract of *B. pinnatum* (leaf), the average mortality percentage indicated that 4.00% concentration resulted in the higher toxicity of 35.00% in *S. oryzae*. It is also observed that 3.00% showed a toxicity of 31.80%, 2.00% showed a toxicity of 23.30% whereas 1.00% showed the average toxicity of 22.60% in *S. oryzae*. However, with the crude extract of *E. globules* (leaf), the average mortality percentage indicated that 4.00% concentration resulted in the higher toxicity of 37.00% in *S. oryzae*. The 3.00% concentration showed a toxicity of 33.40%, 2.00% showed a toxicity of 24.40% and 1.00% concentration showed the average toxicity of 23.40% in *S. oryzae*.

Results from the present investigation showed that *B. pinnatum* and *E. globules* are very rich in phytochemicals, even though the phytochemical screening of the two plants revealed some differences in their constituents. The present work also reveals that the extracts from the leaves of *B. pinnatum* and *E. globules* possess good insecticidal potential because of their phytochemical constituents (Nadi, 2001; Kabaru and Gichia, 2001). The order of toxicity of four different concentrations were 4.00 > 3.00 > 2.00 > 1.00 percentage. Similar direct toxicity effect of the leaf extracts of *Shiyalmutra* on rice weevils has been carried out by Roy et al. (2005) and reported the order of toxicity to be 3 > 2 > 1%.

The observed mortality percentage also increased with increase in time intervals after treatment. The comparison of mortality on four different concentrations of the extract solution of *B. pinnatum* is shown in Table 2, while Table 3 shows the comparison of mortality of four different concentrations of the extract solution of *E. globules*. Mortality percentage at 0.25, 0.50, 0.75, 1.00 and 1.50 h after treatment indicated that 4.00% solution showed the highest mortality of 35.00% and 37.00% in *S. oryzae* at 1.50 h after treatment with *B. pinnatum* and *E. globules* respectively.

Table 1: Phytochemical screening of ethanol extracts of leaves of *B. pinnatum* and *E. globules*.

Phytochemicals	<i>Bryophyllum pinnatum</i>	<i>Eucalyptus globules</i>
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	-	+
Tannins	+	+
Phlobatannins	+	-
Terpenoids	+	-

Key: + = Present ; - = Absent

Table 2: Corrected mortality with ethanol extract of leaves of *B. pinnatum* on *S. oryzae*.

Concentration	Corrected Mortality (HAT)					Mean Mortality
	0.25	0.50	0.75	1.00	1.5	
0.00% (Control)	0.00	0.00	2.50	7.50	10.00	4.00
1.00	8.00a	15.50a	27.50a	25.50a	36.50a	22.60
2.00	8.00a	15.50a	25.50a	27.50a	40.00a	23.30
3.00	10.00b	28.00b	34.00b	42.00b	45.00b	31.80
4.00	13.00b	32.00c	37.00c	44.00c	49.00c	35.00

HAT = Hours after treatment; Values with different letters in a column are significantly different using Duncan's Multiple Range test at 5% level.

Table 3: Corrected mortality with ethanol extract of leaves of *E. globules* leaf on *S. oryzae*.

Concentration	Corrected Mortality (HAT)					Mean Mortality
	0.25	0.50	0.75	1.00	1.5	
0.00% (Control)	0.00	0.00	2.50	8.50	10.00	4.20
1.00	8.50a	16.50a	28.50a	26.50a	37.00a	23.40
2.00	8.50a	16.50a	26.00a	28.50a	42.50a	24.40
3.00	10.50b	29.00b	36.50b	44.00b	47.00b	33.40
4.00	14.50b	34.50c	39.00c	47.00c	50.00c	37.00

HAT = Hours after treatment; Values with different letters in a column are significantly different using Duncan's Multiple Range test at 5% level.

As a result, mortality percentages of both plants showed parallel responses to the level of concentration at different time intervals after treatment. Moreover, the ethanol extract of the leaf of *E. globules* was found to be more active against *S. oryzae* than

the ethanol extract of the leaf of *B. pinnatum*. This study suggested that *B. pinnatum* and *E. globules* possess insecticidal properties and they can be used to control variety of insect pests and vectors. However, further work is necessary to elucidate the structures of the

biologically active components that are responsible for the insecticidal activity of these plants.

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