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NOTE

Insect Antifeedant and Growth Regulating Activities of β-Amyrin from *Sarcostemma acidum*

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Sarcostemma acidum (brevistigma) belongs to the family Asclepiadaceae, it is succulent and leafless creeper. The present study involves that the effect of methanolic extract and β-amyrin a pentacyclic triterpene isolated from *S. acidum* (whole plant) was tested for insect antifeedant and growth regulatory activities against the tobacco cutworm, *Spodoptera litura*. β-Amyrin exhibited concentration dependent antifeedant activity against *S. litura*, which increases the larval and pupal duration and mortality.

Key Words: *Sarcostemma acidum*, β-Amyrin, *Spodoptera litura*, Antifeedant, Growth inhibitor.

The cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a polyphagous insect that has about 150 host species[1]. Traditional farmers use the synthetic pesticides to control *S. litura* and hence it has developed resistance against almost all the commonly using pesticides in this area. For this purpose, medicinal plants that have been occasionally attacked by the pests were screened and are being reported to contain bio-pesticidal property[2]. *S. litura* is often used to evaluate antifeedants in plants[3].

Sarcostemma acidum (Roxb) Voigt, a xerophytic plant of the family Asclepiadaceae, has several medicinal uses. The plant has a great phytochemical significance. The literature survey revealed that a variety of secondary metabolites have been reported from *Sarcostemma*. Natural products containing secondary plant compounds contribute to the defense system of plants. Botanical insecticides such as azadirachtin and its analogues are often effective alternatives for insect antifeedants and growth regulators[4]. In present investigation, we tested the antifeedant level and growth inhibitory effects of plant extract and isolated compound such as β-amyrin against the larvae of *S. litura*.

S. acidum collected from wild area near Tambaram, Chennai, India, was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai 600 045. A specimen has been stored at the herbarium unit of Asthagiri Herbal Research Foundation (AHRF), perungudi, Chennai-600 096, India.

FT-IR spectra were recorded in Bruker (ATR, alpha-E). ^{1}H, ^{13}C NMR spectra were measured in $CDCl_3$ using TMS as an internal standard at 500 MHz, respectively and the chemical shifts are expressed in δ ppm. LCMS were recorded in Shimadzu. Silica gel (60-120 mesh) was used for column chromatography. Fractions were monitored by TLC plate (silica gel 60 F_{254}, Merck).

Isolation and identification of β-amyrin: The plant material (500 g) was extracted with methanol (3 × 2 L). The combined extract was concentrated under reduced pressure. The crude methanolic extract was first washed with hexane and then remaining extract was subjected to column chromatography. The elution was carried out with the increasing combinations of ethyl acetate and hexane as a mobile phase. The fraction obtained from column chromatography with 15 % ethyl acetate in hexane, which was further treated with charcoal, offered a pure white powder and the structure was identified by spectroscopic techniques like ^{1}H, ^{13}C NMR, IR and LC-Mass.

Insects: *S. litura* was reared in the laboratory on Ricinus communis (Castor leaves) at 25 ± 2 ºC. Third instar larvae were used for the study.

Dual choice leaf disc method: Methanol soluble fraction and isolated β-amyrin from whole plant of *S. acidum* were tested for anti-feedant activity according to reported procedure[5,6]. Azadirachtin A, a potent insect antifeedant and growth regulatory compound, was kept as active control[7,8] and the negative control the solvent methanol was used. Five larvae per replication with four replications for each concentration were used. Insect antifeedant activity was studied by dual choice leaf disc method[7] and the per cent feed index (PFI) was calculated[9] as:

TABLE-2
DEVELOPMENT DURATION OF INSTAR AND MORTALITY OF *S. Litura* FED ON CRUDE METHANOLIC EXTRACT AND β-AMYRIN TESTED CASTOR LEAVES

Compounds	Total larval duration (days)		Larval mortality (%)	
	5 (μg/cm^2)	10 (μg/cm^2)	5 (μg/cm^2)	10 (μg/cm^2)
Methanolic extract	16.26 ± 0.14^{c}	16.55 ± 0.21^{c,d}	5.0	10.0
β-Amyrin	16.80 ± 0.20^{c,d}	17.16 ± 0.27^{d}	25.0	40.0
Control	11.94 ± 0.14^{a}	11.94 ± 0.14^{a}	5.0	5.0
Azadirachtin A	15.05 ± 0.15^{b}	16.57 ± 0.20^{c,d}	-	65.0

Values are mean ± SD; n = 20; ^{a,b,c,d}Values followed by a common letter are not significantly different at $p < 0.05$ by DMRT.

TABLE-3
PUPAL DURATION AND % MORTALITY OF *S. Litura* FED ON CRUDE METHANOLIC EXTRACT AND β-AMYRIN TESTED CASTOR LEAVES

Compounds	Pupal duration (days)		Pupal mortality (%)	
	5 (μg/cm^2)	10 (μg/cm^2)	5 (μg/cm^2)	10 (μg/cm^2)
Methanolic extract	11.05 ± 0.17^{b,c}	11.12 ± 0.23^{b,c}	–	11.11
β-Amyrin	11.76 ± 0.20^{c}	11.75 ± 0.36^{c}	13.33	33.33
Control	8.05 ± 0.17^{a}	8.05 ± 0.17^{a}	5.26	5.26
Azadirachtin A	10.35 ± 0.22^{b}	10.75 ± 0.47^{b}	30.00	42.86

Values are mean ± SD; n = 20; ^{a,b,c}Values followed by a common letter are not significantly different at $p < 0.05$ by DMRT.

$$PFI = \frac{\%\ \text{area fed in treated}}{\%\ \text{area fed in treated} + \%\ \text{area fed in control}} \times 100$$

duration of larval development, pupal development and mortality were also studied[7].

The isolated compound from the methanolic extract was identified as β-amyrin (Fig. 1) by NMR and the spectral data of this compound stayed in agreement with literature[10]. The methanol extract of the material contained about 0.1 % β-amyrin. A comparison of antifeedant activity against *S. litura* indicated that the methanolic extract and the active compound of *S. acidum*, β-amyrin, are not phago-suppressants compared to azadirachtin A. However, these treatments significantly increased larval and pupal durations and induced mortality which is comparable to Azadirachtin A. Results are reported in Tables 1-3.

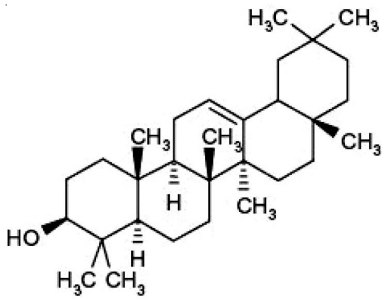

Fig. 1. Structure of β-amyrin

The methanolic extract and β-amyrin, expressed significant growth regulating properties by enhancing the larval and pupal duration thereby effecting changes in the moulting behaviour of the insect. Since β-amyrin is a plant steroid and non-toxic this could be useful in eco-friendly formulations for insect control. The growth inhibitory action displayed by β-amyrin needs to be investigated at physiological and molecular levels.

TABLE-1
PER CENT FEEDING INDEX (PFI) OF *S. Litura* FED ON CRUDE METHANOLIC EXTRACT AND β-AMYRIN TREATED CASTOR LEAVES

Compounds	% Feeding index	
	5 (μg/cm^2)	10 (μg/cm^2)
Methanolic extract	43.53 ± 5.71	42.65 ± 4.96
β-Amyrin	43.25 ± 5.33	40.50 ± 1.84
Azadirachtin A	15.62 ± 3.14	12.59 ± 2.67

Values are mean ± SD; n = 4.

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