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Antifeedant Constituents from *Leucaena leucocephala*

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ABSTRACT

Hexane and methanolic extracts of *Leuceana leucocephala* Lam. leaves were tested against *Spodoptera litura* L. a polyphagus pest of cotton rice, tomato, ground nut, castor and legume and found to have antifeedant potential in the concentration of 2.5 µg/cm^2. The bioassay-guided fractionation yielded the three compounds quercetin 3-*O*-rhamnoside (**1**), quercetin (**2**) and D-onanitol, (**3**) with antifeedant activity.

INTRODUCTION

Botanical insecticides have long been used by men since ancient times in insect pest management and crop protection and are attractive alternatives to synthetic chemical insecticides for pest management because of little threat to the environment and human health. The mechanism of action of botanical pesticides may differ greatly and are often not yet well understood. They have an advantage that they combine a wide range of toxic potencies hence reducing the chance of pest to develop resistance (Nelson and William, 2004). In addition to that, residues are hardly expected on the products or in the environment since botanical pesticides are generally considered to be non persistent under field condition as that are readily degraded by light, oxygen and microorganism to less toxic products (Isman and Akhtar, 2007). The deleterious effects of crude plant extracts on insects are manifest in several ways, including toxicity, (Hiremath, 1997) feeding inhibition (Wheeler and Isman, 2001). The search for plant derived chemicals that have potential use as crop-protectant (insecticide, antifeedant, and growth inhibitor) often begins with screening of plant extracts (Peta and Pathipati, 2008). Insecticides of plant origin have been in use for long time. By applying plant extracts to other susceptible plant species the defense of susceptible plant is improved and use of natural products in agro ecosystem is emerging as one of the prime means to protect crops (Rattan, 2010). The cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a polyphagous pest that has near about 150 host species. The cutworm, *Spodoptera litura* is an economically important polyphagous pest found in major part of the world. In India, the larval stages causes severe damage to a number of crops including tobacco, caster, groundnut, tomato, cabbage, cauliflower, cotton and other various crucsiferous crops (Rao *et al.*, 2001) Crop loss due to insect pests varies between 10% and 30% for major crops (Ferry *et al.*, 2004; Isman *et al.*, 2007). Traditionally synthetic pesticides are used to control *S. litura* and hence the pest developed resistance against the commonly using pesticides. For this purpose, medicinal plants were screened and are being reported to contain bio-pesticidal property (Selvaraj *et al.*, 2005).

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S. litura is often used to evaluate antifeedants in plants. (Pung and Srimongkolchai, 2011). Number of plants exhibited significant antifeedant activity against this pest. Several Himalayan plants are documented as insecticidal agents against *spodoptera littura* and other forest pests (Negi *et al.*, 2016; Negi *et al.*, 2006) Extracts of *Swertia corymbosa, Phyllanthus deblis, Syzygium lineare, Curculigo orchioides, Evolvulus alsinoides,* and *Zanthoxylum limonella* showed significant antifeedant activity against *spodoptera litura* (Jeyasankar *et al*., 2010; Arivoli et. al., 2012). *Leucaena leucocephala* (Mimosaceae) Lam. (*Shu babol*) is an unarmed small deciduous tree to 5 m height, generally used for soil conservation, fuel and feed purposes in India.(Gaur, 1999) It was known to be a rich source of tannin, proteinous and non-proteinous amino acid and other phenolics (Azeemoddin *et al.*, 1988; Hossain *et al.*, 1998).The plant commonly used as foliage, as a source of *β*-carotene, vitamin K, *β*-carotene, green manure, fuel wood or as drought resistance **(**Lalitha *et al.*, 1993). It is also known to have great medicinal importance (Salem *et al.*, 2011). The roots of *Leucaena leucocephala* contains tannins which are known to exhibited nitrification inhibition effect (Erickson *et al.*, 2000).The seed oil could be used as a potential bio inhibitor for corrosion of mild steel and copper.(Meena *et al.*, 2013). The phytochemical investigation of *Leucaena leucocephala* revealed the presence of coumarins, terpenes, sterols and flavonoids. The seed extract of *Leucaena leucocephala* have been reported as antidiabetic, anthelmintic and has a broad spectrum antibacterial activity (Irene *et al.*, 1997; Ademola *et al.*, 2005; Syamsudin *et al.*, 2010). The significant antioxidant activity and antimicrobial activity was recorded from the extract and the compounds isolated from *Leucaena leucocephala* (Reda *et al.*, 2015).

In present investigation, we tested the antifeedant level of plant extract of *Leucaena leucocephala* and isolated compounds *of Leucaena leucophela* against third instar larvae of *S. litura*. (Lapidoptera), a polyphagous pest of groundnut, tomato, cotton, rice, tobacco, castor and legumes.

MATERIAL AND METHODS

General Experimental Procedure

Melting points is uncorrected and was taken in open capillary. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on Bruker AVANCE 400 spectrometer in DMSO-d_6, $CDCl_3$ with TMS as internal standard. Proton detected heteronuclear correlation were measured using HMQC (optimized for $^{1}J_{HC}$=145 Hz) and HMBC (optimized for J_{HCCC} =7 Hz). The IR spectra were recorded on a Perkin Elmer Infrared 15 spectrometer using KBr pellets. MS data were obtained on a JEOL SX-102 spectrometer. Silica gel (60-120 mesh Merck) for column chromatography and silica gel G (Merck) for TLC were used.

Plant Material

Leaves *of Leucaena leucocephala* were collected at Chauras campus of HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India. A voucher specimen (GUH 16280) is deposited in the Herbarium of Chemistry Department HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India.

Pest

Field collected *Spodoptera litura* L. larvae were cultivated in the laboratory at 25±2°C and third instar larvae from laboratory culture were used for antifeedant assay.

Extraction and Isolation

Shed dried powder leaves part (2.4kg) was exhaustively extracted at 60^0 C with 90 % EtOH. The extract was evaporated *in vacuo* to give the crude residue A-1. It was partitioned with hexane and methanol, and gave hexane-soluble fraction, A-2 and methanol-soluble fraction, A-3. A-3 was charged to gross chromatography on silica gel using $CHCl_3$-MeOH as eluent with increasing proportion of methanol, which afforded Fr 01 and Fr 02. Fr 01 was re-chromatographed on CC using $CHCl_3$-MeOH (9:1) afforded **1** (45mg) and **2** (18mg), while Fr 02 gave **3** (108mg) further purified by MeOH.

Quercetin 3-O-rhamnoside (1) was isolated as yellow solid from $CHCl_3$-MeOH (9:1), 45 mg, mp 208°C, IR (KBr) ν_{max} cm^{-1}: 3465(OH), 1664(lactone C=O), 1596(aromatic), 824; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRMS: 448.4741 calcd for 448.3769.

Quercetin (2) was obtained as yellow solid from $CHCl_3$-MeOH (9:1),18 mg, mp 313-314° C, IR (KBr) ν_{max} 3470(OH), 1645(lactone C=O), 840 cm^{-1}; HRMS: 302.3214 calcd for 302.2357.

D-Onanitol (3) was isolated as white solid from $CHCl_3$-MeOH (9:1), 108mg, IR (KBr) ν_{max} : 3378, 1070, 711 cm^{-1}; HRMS : 194.2145 calcd for 194.1825.

Antieedant Activity

Crude extract and isolated compounds were tested against third instar larvae of *Spodoptera litura* L. (Lepidoptera). The dual choice leaf disc method was performed (Kannan, *et al.*, 2013). Field collected *Ricinius communis* leaves were cut in to circular discs (180 cm^2) with the medium vain as marker between two equal halves. Hexane and methanolic extracts and isolated compounds were dissolved in solution, which was sprayed on half of circular leaf disc with 2.5-µg/cm^2 concentrations. Other half of the leaf treated with solvent.

Azadirachtin A, a potent insect antifeedant and growth regulatory compound, was kept as active control (Govindachari, *et al.*, 1995; Govindachari, *et al.*, 1996). After drying, each leaf disc was placed in a Petri dish (15 cm dia). Five freshly moulted insect third instar larvae of *S. litura* were placed in the center of leaf and left to feed for 36 hr. For each extracts and compounds, five replicate were maintained. After 24 hrs the leaves were removed

and unfed area in the treated and control halves were measured using ΔT area measurement meter.

Percent feeding index (PFI) was calculated as:

$$PFI = \frac{\%\ \text{area fed in treated}}{\%\ \text{area fed in treated} + \%\ \text{area fed in control}} X100$$

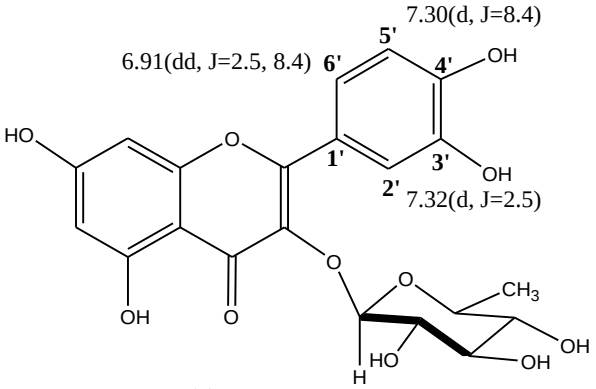
RESULTS AND DISCUSSION

Compound **1** was isolated as yellow solid. The high-resolution mass spectroscopy gave molecular mass 448.4741 suggested for $C_{21}H_{20}O_{11}$. A positive Shinoda test and $FeCl_3$ color reaction suggested it as phenolic flavonoid. The ^{1}H-NMR spectrum showed signal for 20 protons including two doublets at δ 6.19 and 6 .36 (*J*=2.0 Hz) as H-6 and H-8, two downfield doublets 7.30 and 7.32 for H-5' and H-2' one double doublet 6.91 assigned for H-6' respectively. Anomeric proton H-1'', detected at δ 5.34 with coupling constant of 1.0 Hz and a doublet methyl signal at δ 0.84 (*J*=6.0 Hz) suggested sugar is rhamnose. Exact proton and carbon positions were confirmed by COSY, DQF HSQC and HMBC experiments. Since in the HMBC spectrum, an anomeric H-1'' showed long-range coupling with C-3 (δ 136.23) and C-2''(δ 72.10), the exact position of rhamnose was confirmed to be at C-3. Further acid hydrolysis of **1** gave aglycone and sugar identified as rhamnose by comparison of co-TLC with that of authentic sample. On the basis of above data and its comparison with data reported in literature compound **1** was identified as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-methyl-oxan-2-yl]oxy-chromen-4-one(Lowry *et al.*, 1984) (Table 1).

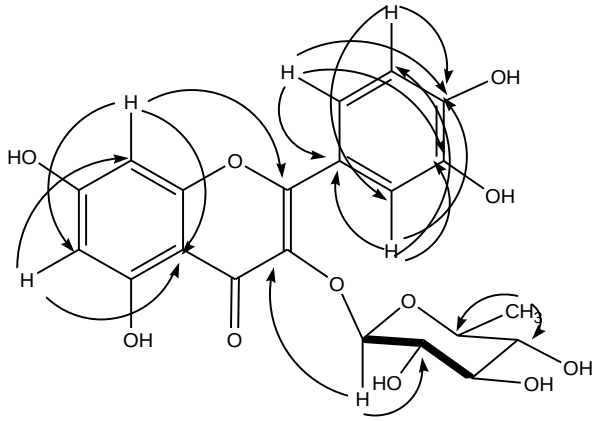
Table 1: ^{1}H and ^{13}C data and HMBC correlation of **1**

H/C atom	^{13}C δ *J* in Hz	^{1}H δ *J* in Hz	HMBC ^2J	HMBC ^3J
2	158.52	-	-	-
3	136.23	-	-	-
4	179.64	-	-	-
5	163.23	-	-	-
6	99.76	6.19(d, *J*=2.0)	C-5,C-7	C-8,C-4a
7	165.86	-	-	-
8	94.69	6.36(d, *J*=2.0)	C-7	C-6,C-4a,C-2
4a	105.89	-	-	-
8a	159.31	-	-	-
1'	122.85	-	-	-
2'	116.91	7.32(d, *J*=2.5)	C-1'	C-5',C-4'
3'	116.35	-	-	-
4'	149.7	-	-	-
5'	146.41	7.30(d, *J*=8.4)	C-2',C-4'	C-6'
6'	122.96	6.91(dd, *J*=2.5, 8.4)	C-1',C-5'	C-3',C-4',C-8a
1''	103.54	5.34(d, *J*=1.0)	C-2''	C-3
2''	72.10	3.43(dd, *J*=6.0, 8.5)	-	C-6'',C-4''
3''	72.03	3.32(t, *J*=9.0)	-	C-5''
4''	73.24	3.74(t ,*J*=9.0)	C-3''	-
5''	71.90	4.21(q, *J*=1.6, 3.2)	C-4''	C-3''
6''	17.65	0.87(d,*J*=6.0)	C-5''	C-4''

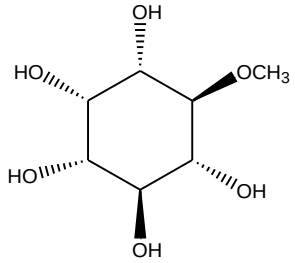
Compounds **2** and **3** were identified as quercetin and D-onanitol by comparison of co-TLC with authentic samples and NMR data with those of data reported in literature (Lowry *et al.*, 1984; Dorman *et al.*, 1970) (Fig 1). For antifeedant activity the crude extracts were tested on dual choice leaf disc method to know the Percentage Feeding Index (PFI).



(1)



Selected HMBC correlation of compound (1)



(3)

Fig 1: Isolated Compounds : Quercitin 3-*O*-rhamnoside (1) HMBC Correlation of (1) and D –Onanitol (3)

Table 2: Antifeedant activity of extracts and isolated compounds.

Particular	Percent Feeding Index (PFI)
Hexane extract **A02**	68.24 ± 4.62
Methanol extract **A03**	32.68 ± 2.49
Quercitin 3-*O*-rhamnoside **(1)**	35.41 ± 1.64
Quercitin **(2)**	40.10 ± 6.24
D-onanitol (3)	54.18 ± 8.34
Azadirachtin A	16.81± 4.24

Hexane extract A-2 showed 68.24 ± 4.62 and methanol extract A-3 showed 32.68 ± 2.49 PFI. Fraction Fr 01 and Fr 02

from A-3 exhibited 46.22 ± 1.4 and 70.12 ± 6.12 PFI. The Fr 01 fraction led to isolation of two active compound **1-2** while Fr 02 afforded **3** in which **1** showed higher antifeedant activity as compared to **2** and **3** (Table 2).

CONCLUSION

Flavonol glycoside Quercitin 3-*O*-rhamnoside along with Quercitin and D-onanitol were isolated from the dried leaves *Leucaena leucocephala* leaves. Hexane and methanolic extracts and isolated compounds were test for antifeedant activity against *spodoptera litura* by duel choice leaf disc method and result was evaluated in terms of Percentage feeding index. A comparison of antifeedant activity against *S. Litura* indicated that the methanolic extract and the active compound Quercitin 3-*O*-rhamnoside expressed significant antifeedant potential. These results could be useful in eco-friendly formulations for insect control.

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Conflict of Interests: There are no conflicts of interest.

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