Antifeedant activity of twenty six plant extracts and pure compounds from the root bark of Toddalia asiatica (L) (Rutaceae) against the anomalous emperor moth Nudaurelia belina (Saturnidae)

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Abstract: *The antifeedant activity of petroleum ether (PE) (40-60%) extract of the root bark of T. asiatica and twenty five other plant extracts was investigated against the anomalous emperor moth N. belina. The petroleum ether extract of stem bark of Albizia anthelmintica, leaves of Cassia occidentalis, Balanites aegyptica, seeds of Cucumis sativaus, root bark of T. asiatica, root bark of Celtis durandii, B. aegyptica and whole plant of Cisus quadrangularis were found to be active against N. belina at a concentration of 1% w/v in a modified leaf disc method. Also, methanol extracts of the root bark of A. muricata and Eucalyptus spp were found to be active against N. belina at a concentration of 1% w/v. Three cumarius; dihydrotoddanol, toddanol and toddalactone and the alkaloid dihydrochelerynthrine isolated from the PE extract of the root bark of T. asiatica exhibited antifeedant activity at as low as 0.05% w/v. Toddaculine and toddanone which were also isolated from the P.E. extract had no activity at 0.05% w/v.*

Keywords: Antifeedants, Toddalia asiatica, Rutaceae, Nudaurelia belina, Saturnidae

INTRODUCTION

Insect pests can cause significant crop losses and they are a major threat to agriculture in Tanzania. Synthetic pesticides have and are still being used as a major control method of pests.

The use of synthetic pesticides poses a substantial risk to the environment in the form of bio-magnification and toxicity to non-target organism, and the use of alternative structural analogues is often rendered ineffective by cross or multiple resistance (Stoll, 2000). The

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other major drawback associated with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment (Omena et al., 2007). Also, in developing countries, inadequate product knowledge, supply uncertainties, and high prices cause inefficient pesticide use leading to the creation of additional socio-economic problems (Kaoneka, B. et al., 2011).

The toxicity problem, together with the growing incidence of insect resistance, underscores the need for development of effective insecticides, which are environmentally safe, target specific and biodegradable. Plant natural products may contribute new avenues of pest control materials or prototypes for such materials and, to that end, there has been a worldwide effort aimed at screening plant species for bioactivity against pest species (Roy et al., 2005).

The damage caused by the larvae of the moth, *Nudaurelia belina* to Mangifera indica (mango) and *Anarcadium occidentale* (cashew nut) family Anarcadiaceae, has not been assessed but could be extensive. The caterpillars locally known as “washawasha” around the coastal regions of Tanzania are sometimes capable of foraging almost all the leaves of a *Mangifera. Indica* tree.

Economically feasible plant secondary metabolites are considered to be a potential alternative approach against the ravages of various pests of both agricultural and public health importance due to their excellent properties like cheap availability, environmental safety nature and the presence of rich source of bioactive compounds, such as larvicidal, repellent, insect growth regulators, antifeedants, ovicidal, oviposition deterrence and reduction of fecundity and fertility (Rajkumar and Jebanesan, 2005; Elango et al., 2009; Kostic et al., 2008; Pavela et al., 2005).

Further studies on biological activities of some plants against various pests have exhibited potency on the targeted pests. For instance, some plant natural products have been shown to exhibit antifeedant activity against some lepidopterans. For example, Plagiochilin isolated from Plagiochilia sps. (Fam. Graminaceae) was found to be active against Africana armyworm Spodoptera exempta (Asakawa et al., 1980). Nerolidol isolated from Melaleuca leucadendron (fam. Mytaceae) was active against Gypsy moth *Lymantria dispar* (Dozkotch et al., 1980).

Taking into account of the abundance of plants with pesticidal activities available in Tanzania, twenty six plants were screened against the caterpillars of *N. belina*.

This paper presents the results of studies on extracts from twenty six plants and pure compounds isolated from Toddalia asiatica (L.) (fam. Rutaceae) against the larvae of Nudaorelia belina.

MATERIALS AND METHODS

Plant material

The root bark of *Toddalia asiatica* (L.) was collected from Uluguru Mountains and was identified by the late Mr. Samwel Kibuwa, a botanist who was working with the National Herbarium of Tanzania. The voucher specimen number 43 was deposited at the herbarium. The other plants were collected from different parts of Tanzania.

Nudaurelina belina

Wild caterpillars in their third instars collected from *M. indica* were introduced in wood cages covered with wire mesh and reared in the insectary of the Tropical Pesticides Research Institute under laboratory conditions. The caterpillars were allowed to grow until moth emerged. Eggs laid by the adult moth were allowed to pass through all the stages of development until caterpillars emerged. The caterpillars were fed on fresh leaves of *M. indica* twigs dipped in water before being used in the antifeedant tests. Fresh twigs collected from the same plant were introduced into the cages after every twelve hours.

Bioassay

The choice method as previously described by Yano (1986) was used to test for antifeedant activity against third instar caterpillars of N. belina. The extracts and pure compounds used were obtained from twenty six Tanzanian plants. Fresh M. indica leaves of 2.5cm diameter were dipped into a 1% acetone solution of the crude extracts. The control disc was dipped into acetone. The discs were left to dry under a drift of air. The treated discs (TD) and the control disc (CD) were then introduced into the cage (10 X 10 X 10 cm) made of wood and wire mesh. The larvae were placed between the TD and CD. The experiment was carried out at ambient temperature. After 3hours, the discs were removed and an estimation of the eaten areas was determined. Each test was carried out in triplicate. The relative antifeedant percentage (RAP) was then determined as per equation given below:

$$RAP = \frac{[1- \text{Average \% of consumed TD}] \times 100}{\text{Average \% of consumed TD} + \text{Average \% of CD}}$$

Where TD =Treated disc
CD= Control disc

The RAP values were coded as follows:

0- 49	(-) no activity
50-59	(+) poor activity
60-79	(++) moderate activity
80-89	(+++) good activity and
90-100	(++++) very good activity

Source; Yano, K. 1986. Insect Biochemistry

Plant extracts which exhibited 3+ or 4+ activity were diluted to 0.5% and 0.25% for further screening tests. Pure compounds isolated from T. asiatica were also tested against the larvae of *N. belina*.

Isolation and purification of compounds from T. asiatica

About 3g of the crude extracts were eluted over silica gel (400 mesh) in diethyl ether: hexane (1:3) v/v) to afford four fractions. About 0.6g of fraction two was chromatographed over silica gel (70-230 mesh) using diethyl ether: hexane (1:4 v/v). Two compounds dihydrocheleryxthrine (19 mg) mp 157 - 159° and toddaculine (8 mg) mp 95°C were afforded. About 250 mg of fraction three was crystallized in hexane to afford about 120 mg of toddanone mp 116°C. Preparative thin layer chromatography of fraction four (100 mg) eluted with hexane : ethyl acetate (4:1) afforded 20 mg of toddanol mp 125°C, 30 mg dihydrotoddanol mp 85°C and 40 mg toddalactone mp 125°C.

RESULTS AND DISCUSSION

The results of the antifeedant activities of various plant crude extracts are shown in Table 1. From these results it is observed that various plants belonging to different families deter feeding of the *Nudaurelia belina*. Plants which exhibited high potency included the petroleum ether extracts of stem bark of *Albizia anthelmintica*, leaves of *Cassia occidentalis*, stem bark *Balanites aegyptica*, seeds of *Cucumis sativaus*, root bark of *T. asiatica*, root bark of *Celtis durandii*, *Balanites aegyptica* and whole plant of Cisus quadrangularis at a concentration of 1% w/v. Methanol extracts of the root bark of *Annona muricata* and stem bark Eucalyptus spp were also found to be active against *N. belina* at a concentration of 1% w/v.

Plant extracts which exhibited the highest potency against *N. belina* larvae were *Cucumis sativus*, *Eucalyptus* sps, *Azadirachta indica*, *Celtis durandii* , *Toddalia asiatica* and *Cissus quadrangularis* (Table1). It was also shown that petroleum ether extracts were the most active while methanol extracts were the least active (Table1).

Table 1: Antifeedant activity of extracts from some Tanzanian plants against Nudaurelia belina

Plant species	Family	Part of plant	Activity PE	Activity CH_2Cl_2	Activity CH_3OH
1. *Annona reticulate*					
2. *Annona muricata*	Annonaceae	Stem bark	+ +	+ +	+ + + +
3. *Andansonia digitata*	Bombaceae	Seeds	+	+	+
4. *Cassia didymobtrya*	Caesalpinaceae	Leaves	+ +	+ + +	-
5. *Albizia anthelmintica*	Caesalpinaceae	Stem bark	+ + + +	-	+ +
6. *Cassia occidentalis*	Caesalpinaceae	Leaves	+ + + +	+ +	+
7. *Dichrostachys cinerea*	Caesalpinaceae	Stem bark	+ +	+ +	-
8. *Kigelia africana*	Bignoniaceae	Stem bark	+ + +	-	+ +
9. *Balanites aegyptica*	Balanitaceae	Stem bark	+ + + +	+ +	+
10. *Cussonia arborea*	Araliaceae	Stem bark	+	-	-
11. *Sesbania bispinosa*	Fabraceae	Whole plant	+ +	+	+ +
12. *Vismia orientalis*	Guttifereae	Stem bark	+ +	+ + +	+ +
13. *Cucumis sativus*	Cucurbitaceae	Seeds	+ + + +	+ + + +	+ + +
14. *Mirabilis jalapa*	Nyctaginaceae	Whole plant	-	+	+
15. *Boerhavia diffusa*	Nyctaginaceae	Roots	-	+	+
16. *Eucalyptus sp.*	Myrtaceae	Stem bark	-	+ + + +	+ + + +
17. *Azadirachta indica*	Meliaceae	Stem bark	+ + +	+ + +	+ + + +
18. *Plantago major*	Plantaginaceae	Whole plant	+ +	+ + +	+
19. *Gardenia jovis tonantis*	Rubiaceae	Stem bark	-	-	+ +
20. *Cinchona sp.*	Rubiaceae	Stem bark	+	+	+ +
21. *Vangueria infausta*	Rubiaceae	Stem bark	-	+ +	+
22. *Toddalia asiatica*	Rutaceae	Root bark	+ + + +	+ + +	+
23. *Rauwolfia mombassiana*	Verbanaceae	Stem bark	+	+ +	+
24. *Celtis durandii*	Ulmaceae	Stem bark	+ + + +	+ + + +	+ + +
25. *Cissus quandrangularis*	Vitaceae	Plant	+ + + +	+ + +	+ +
26. *Parinari exelsa*	Chrysobalanceae	Stem Bark	-	+ +	+

Key:
PE = Petroleum ether 40-60°C

Table 2 shows that the petroleum ether and methanol extracts of Cassia occidentalis, Albizia anthelmintica and Annona muricata exhibited more potency at concentrations 1% and 0.5% as compared to the other extracts.

Table 2: Anti – feedant activity of extracts from Tanzanian plants at varying concentrations

Plant species	**Part of Plant**	**Extracting Solvent**	**Activity (W/V)** Conc. 1%	0.5%	0.25%
1. *T. asiatica*	root bark	PE	+ + + +	+ +	+ +
2. *C. occidentalis*	leaves	PE	+ + + +	+ + + +	+ +
3. *A. anthelmintica*	stem bark	PE	+ + + +	+ + + +	+ +
4. *A. muricata*	root bark	Methanol	+ + + +	+ + + +	+ +
5. *C. durandii*	root bark	PE	+ + + +	+ +	+
6. *C. quadrangularis*	plant	PE	+ + + +	+ +	+
7. *C. sativus*	seeds	PE	+ + + +	ND	ND
8. *B. aegyptica Balanitaceae*	Stem bark		+ + + +	+ +	+
9. *Eucalyptus sp.*	Stem bark	Methanol	+ + + +	ND	ND

Key:

ND	=	Not determined
PE	=	Petroleum ether (40-60° C)
a	=	Activity determined as RAP

Due to high potency exhibited by extracts of some of the screened plants, isolations were carried out on the PE extract of T. asiatica. Three cumarin namely, dihydrotoddanol, toddanol and toddalactone and an alkaloid dihydrocheleryn thrine were isolated from the PE extract of the root bark of *T. asiatica*. The presence of coumarin derivatives, such as toddaculin, coumurrayin, toddalenol, toddalenone, toddalosin, toddasin and toddanone, have also been described (Madhavan. et al; 2010)

The activities of the pure compounds isolated from the petroleum ether extract of the root bark of *T. asiatica* are shown on Table 3. Dihydrochererythrine, toddanol dyhydrotoddanol and toddalactone showed the highest activities at as low as 0.05% (w/v) while toddaculine and toddanone were the least active at this concentration.

Table 3: Antifeedant activity of pure compounds isolated from T. asiatica

Compound	Concentration(w/v)		
	0.1%	0.05%	0.025%
1. Dihydrochelerynthrine (1)	+ + + +	+ + + +	+ +
2. Toddaculine (2)	+	ND	ND
3. Toddanone (3)	+	ND	ND
4. Toddanol (4)	+ + + +	+ + + +	+ +
5. Dihydrotoddanol (5)	+ + + +	+ + + +	+ +
6. Toddalactone (6)	+ + + +	+ + + +	+ +

Key:

ND	=	Not determined
a	=	Activity determined as RAP

In view of this increasing interest in the biological activities of T. asiatica, an attempt was made in another study to assess the larvicidal efficacy of this plant against two mosquito vectors namely, A.aegypti and *C. quinquefasciatus*. This plant exhibited high potency against the larvae of the two species of mosquitoes (Sarma, 2003).

Alkaloids have been shown to have antifeedant properties. For instance, the alkaloid, dihydrochelerynthrine (T) exhibited high antifeedant activity against N. bellina and an alkaloid isolated from Lycoria radiata showed antifeedant activity against the larvae of yellow butterfly, *Eurema hecabe mandarina* (Numata, 1983).

It is clear from tables 1 and 2 that it is not possible to correlate the level of activity with the family, plant or the part of plant used. This observation is not unexpected since even the mostly widely studied African armyworm *Spodoptera exempta* antifeedants have been isolated from a number of plant species (Asakawa, et al. 1985; Kubo, et al. 1977; Simmonds, et al. 1985). From this study, it has been observed that most of the antifeedant activities were due to the presence of non polar and moderately polar components.

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