Pest Manag Sci 59:1159–1161 (online: 2003)
DOI: 10.1002/ps.740

Effect of rotenoids from the seeds of *Millettia dura* on larvae of *Aedes aegypti*

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Abstract: A crude chloroform extract of seeds of *Millettia dura* Dunn (Leguminosae) showed high activity (LC_{50} = 3.5 μg ml^{-1} at 24 h) against second-instar larvae of the mosquito, *Aedes aegypti* L (Diptera: Culicidae). The rotenoids, deguelin and tephrosin, isolated from the seeds of this plant also showed potent activities, with LC_{50} values of 1.6 and 1.4 μg ml^{-1} at 24 h, respectively. The related rotenoids millettone and millettosin were inactive at 20 μg ml^{-1}. Saturation at the B/C ring junction and the presence of methoxy groups at C-2 and/or C-3 in deguelin and tephrosin appear to be important for the observed larvicidal activity.

Keywords: *Millettia dura*; Leguminosae; rotenoids; tephrosin; deguelin; *Aedes aegypti*; larvicides

1 INTRODUCTION

The appearance of insect populations resistant to conventional insecticides, together with the growing environmental concern over the use of many synthetic insecticides, in particular halogenated compounds such as DDT, has resulted in high prevalence of mosquito-transmitted diseases in Africa. With the intention of discovering cost-effective alternatives for the control of disease vector insects, plant extracts and pure compounds have been tested for larvicidal activity.[1–3]

Rotenone, one of the most extensively used natural insecticides, has been reported to be highly active against fourth-instar larvae of *Aedes aegypti* L.[4] The insecticidal activities of rotenone and some other rotenoids (including deguelin and tephrosin) against a variety of insect species are well known.[5] Commercially, rotenone is mainly extracted from the roots of *Derris* species in Asia and *Lonchocarpus* species in South America.[5] Rotenone and other rotenoids are also known to occur in the related genus, *Millettia* Wight et Arn.[6,7] These three genera (*Derris*, *Lonchocarpus* and *Millettia*) are taxonomically related and their generic delimitation is not yet satisfactorily resolved.[8]

Whereas *Lonchocarpus* and *Derris* are used commercially as a source of rotenoids, the genus *Millettia* has not been exploited commercially in this way, even if the seeds of some of these plants are known in traditional practice for their insecticidal and piscicidal properties.[9] Some 100 *Millettia* species are known worldwide, and in Kenya this genus is represented by six species, namely, *M dura* Dunn, *M lasiantha* Dunn, *M leucantha* Vatke, *M oblata* Dunn, *M tanaensis* Gillett and *M usaramensis* Taub.[10] Of these *M dura* is cultivated as a timber tree and also as a shade and ornamental tree.[10,11] Rotenoids and isoflavones have been isolated from the seeds, seed pods, stem bark and root bark of this plant.[9,12–14] In our effort to identify botanical agents for the control of disease vector insects, we report here the activities of the crude chloroform extract of the seeds of *M dura* and the rotenoids deguelin and tephrosin, isolated from this extract, against the larvae of *Ae aegypti*.

2 EXPERIMENTAL

2.1 Plant material

The seeds, seed pods, leaves, stem bark and root bark of *M dura* were collected in March 1995 in Nairobi. The plant was identified by Mr SG Mathenge of the University Herbarium, Botany Department, University of Nairobi, Kenya, where a voucher specimen is deposited.

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Contract/grant sponsor: Deutsche Forschungsgemeinschaft; contract/grant number: Pe 264/14-2
Contract/grant sponsor: Bundesministerium für Zusammenarbeit; contract/grant number: Pe264/14-3
Contract/grant sponsor: Natural Products Research Network for Eastern and Central Africa (NAPRECA)
Contract/grant sponsor: German Academic Exchange Service (DAAD)
(*Received 12 September 2002; revised version received 17 February 2003; accepted 24 March 2003*)
Published online 12 June 2003

2.2 Extraction and isolation

The dry and ground seeds (1 kg) of *M dura* were extracted with chloroform (3 × 1.5 litre) by cold percolation. The solvent was removed to give an oily residue, labelled as seed-extract-1 (100 g). The extract was partitioned between hexane and methanol to give seed-extract-2 (hexane layer, 35 g) and seed-extract-3 (methanol layer, 65 g). The rotenoids millettone (Fig 1,**1**), millettosin (**2**), deguelin (**3**) and tephrosin (**4**) were isolated from seed-extract-3 for larvicidal testing. The details of the isolation and identification of these compounds and other compounds from the seeds of *M dura* have been described previously.[12]

The crude chloroform extract from the seed pods and stem bark of *M dura* and the isoflavones durallone, durmillone and calopogonium isoflavone A, were obtained as described by Yenesew *et al*[13] Dehydrodeguelin (Fig 1, **3a**) was prepared by dehydration of tephrosin (**4**) under acidic condition as described by Ollis *et al*[12] The leaves and root bark of *M dura* were also extracted by percolation of the plant material (100 g) in chloroform. Removal of the solvent gave crude leaves extract (6 g) and root bark extract (9 g).

2.3 Larvicidal activity assay

The eggs of *Ae aegypti* (Diptera:Culicidae) were obtained from the Department of Zoology, University of Nairobi. The eggs were flooded with sodium chloride solution (0.8 g litre^{-1}) and left to hatch at 28 °C. Twenty second- or fourth-instar larvae were transferred into Petri dish containing sodium chloride solution (0.8 g litre^{-1}; 40 ml) solution. Different concentrations (40, 20, 10, 5, 2.5, 1.25 mg ml^{-1}) of test samples in dimethyl sulfoxide (DMSO) were prepared by serial dilution. From each test solution 20 µl was transferred into Petri dishes containing larvae in sodium chloride solution, giving final concentrations of 20, 10, 5, 2.5, 1.25 and 0.75 µg ml^{-1}, respectively.[2] Control larvae in all cases received 20 µl of DMSO. Mortality was checked after 24 h, and, in the case of fourth-instar larvae, up to the fourth day. LC_{50} values were calculated from the mean of three observations for each concentration using Finney's probit analysis for quantal data.[15]

3 RESULTS AND DISCUSSION

The chloroform extract of the seeds of *M dura* were tested for larvicidal activity against the second-instar larvae of *Ae aegypti*, and showed high and dose-dependent larvicidal activity (LC_{50} = 3.5 µg ml^{-1} at 24 h; confidence interval 3.1–3.9; χ^2_4 = 82.99). This activity improved (Fig 2) when the oil was removed by partitioning between hexane and methanol. The LC_{50} for the methanol layer (seed-extract-3) was found to be 0.9 µg ml^{-1}, (confidence interval 0.7–1.0; χ^2_4 = 33.16), while the hexane layer (seed-extract-2) was inactive at 20 µg ml^{-1}. Seed-extract-3 was also

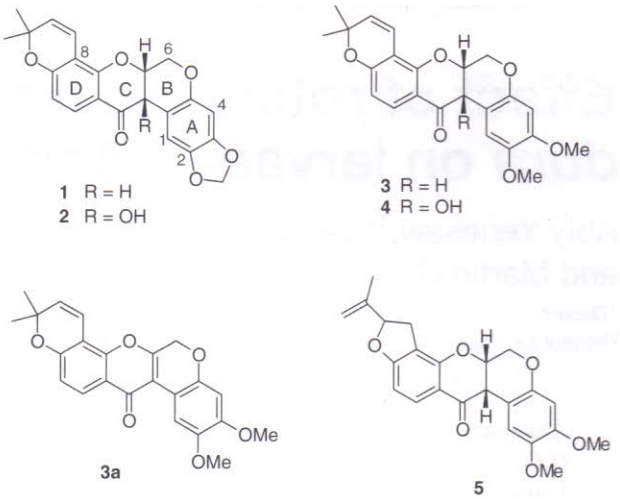

Figure 1. Structure of rotenoids from *Millettia dura*.

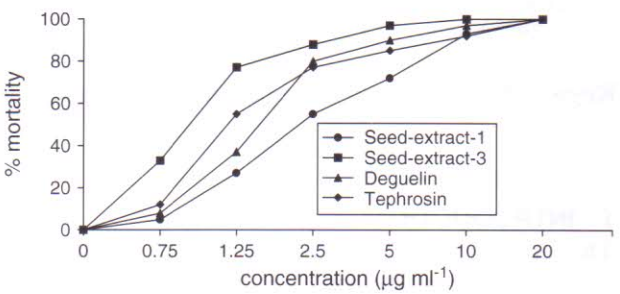

Figure 2. Effect of different concentrations of crude extracts and rotenoids obtained from the seeds of *Millettia dura* on the second instar larvae of *Aedes aegypti*.

tested for activity against fourth-instar larvae and caused 100% mortality at 5 µg ml^{-1} within 4 days.

Five rotenoids [millettone (**1**), millettosin (**2**), deguelin (**3**), tephrosin (**4**) and rotenone (**5**)] have been isolated earlier from *M dura*.[12] We have re-isolated compounds **1** to **4** from the seed of this plant and these were tested for larvicidal activities on second-instar larvae of *Ae aegypti*. Among these rotenoids, deguelin (**3**) and tephrosin (**4**) showed potent and dose dependent activities (Fig 2) with LC_{50} values of 1.6 (confidence interval 1.5–1.8; χ^2_4 = 150.99) and 1.4 µg ml^{-1} (confidence interval 1.3–1.6; χ^2_4 = 19.16) at 24 h, respectively. It is interesting that 6a,12a-dehydrodeguelin (**3a**) was inactive even at 20 µg ml^{-1}, indicating the importance of saturation at the B/C ring junction for larvicidal activity of rotenoids. This observation is in line with an earlier report that 6a,12a-dehydrorotenone is entirely inactive against insects.[5] It is worth noting here that larvicidal activity of rotenone (**5**) against fourth-instar larvae of *Ae aegypti* has been reported, and it caused 100% mortality at 10 µg ml^{-1} within 3 days.[4] We have tested compounds **3** and **4** against the fourth-instar larvae at 10 µg ml^{-1} and, as with rotenone, both compounds caused 100% mortality within 3 days. Even at 5 µg ml^{-1} compounds **3** and **4** were toxic to the fourth-instar larvae, causing 100% and 95% mortality within 4 days, respectively.