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Laboratory evaluation of *Dalbergia oliveri* (Fabaceae: Fabales) extracts and isolated isoflavonoids on *Aedes aegypti* (Diptera: Culicidae) mosquitoes

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ABSTRACT

We explored the potential of Burma rosewood, *Dalbergia oliveri* in dengue fever vector control by evaluating various extracts as larvicides and growth disruptors of *Aedes aegypti* Linnaeus (Diptera: Culicidae) under laboratory conditions. Third instars and pupae of the mosquito were tested using WHO guidelines. Highest larvicidal effect was obtained with dichloromethane and hexane extracts (LC_{50} = 289.1 and 325.3 ppm, respectively) within 24 h post-treatment. However, toxicity was about 1.78-fold more in dichloromethane extracts than hexane extract, if treatment was given for longer duration of 48 h (LC_{90} = 5048.5 ppm for hexane and 2848.1 ppm for dichloromethane extracts, respectively). In these treatments moderate inhibition of pupation was also seen with some larvae staying as 4th stage larvae for 8 days before dying. There were no significant differences in the sex ratio of emerged adults compared with the controls. Dichloromethane extract, being most active, was subjected to sequential chemical separation to obtain series of isoflavonoids. Three compounds (+)-medicarpin, formononetin and (±)-violanone were identified as active larvicides of *Ae. aegypti*. Significant pupal mortality was observed with dichloromethane extract treatment of 1–3 h old pupae after 48 h post-treatment (LC_{50} = 1004.5 ppm). Isoflavonoid medicarpin (LC_{50} = 296.72 ppm) was most active compound against pupae. Eclosion inhibition (EI) was also observed in pupae treated with dichloromethane extract (EI_{50} of 668.7 ppm). Thus, naturally occurring larvicides can be particularly effective where larval habitats are readily identifiable. Results obtained should be of value in the search for new natural larvicidal compounds from other extracts as well and represent the first report of this plant species being used to control a mosquito vector.

1. Introduction

Over 40% of the world's populations in some 100 countries are exposed to varying degrees of vector borne diseases like malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and visceral leishmaniasis that result in thousands of deaths annually (El Hag et al., 1999; Dhiman et al., 2010). *Aedes aegypti* (Diptera: Culicidae) is the principal vector which transmits dengue fever and dengue hemorrhagic fever. It is also reported to infect more than one hundred million people every year in more than 110 countries in the tropics (Halstead, 2000). Factors that may cause outbreaks include an increase in vector breeding sites, migration of infected people into a vector-rich area, populated with susceptible individuals, arrival of new efficient vectors, breakdown of vector control measures and resistance of the vectors to insecticides. Pesticides are known to cause environmental pollution and they are biomagnified through food chain, and have been demonstrated as being toxic to non-target organisms, with residual effects and resistance by their indiscriminate use. Hence, an effort is required to find alternative method to replace or reduce the use of chemical pesticides. The search for new strategies or natural products to control destructive insects and 144 vectors of diseases is desirable, due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic nonbiodegradable residues contaminating the environment and undesirable effects on nontarget organisms (Jantan et al., 2005). Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to

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Table 1
Amount of *D. oliveri* heartwood crude extract (% w/w) after Soxhlet extraction.

Solvent used for extraction	Characteristics of the extract	Amount (%) of extract (w/w)
Hexane	Orange brown oil	1.08
Dichloromethane	Black brown sticky gum	46.2
Ethyl acetate	Black brown sticky gum	37.65
Methanol	Black brown gum	74.25

their excellent larvicidal, pupicidal, and adulticidal properties. The extracts or essential oils from plants may be alternative sources of mosquito control agents, because they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for the use of controlling mosquitoes (Mehlhorn et al., 2005; Amer and Mehlhorn, 2006a,b; Rahuman et al., 2009a,b). Use of naturally occurring compounds from plant sources have shown promise for commercial insecticides, such as azadiractin, pyrethrins, rotenone, nicotine and toosendanin. (Dev and Koul, 1997; Koul and Walia, 2009). For these various reasons, interest in the screening of medicinal plants for their mosquito control remains of great scientific interest.

Dalbergia species, isoflavonoids there in and especially the family Fabaceae plants are known to have potential to control pests (Barron and Ibrahim, 1996; Dixon and Steele, 1999; Ding and Wang, 2005). Pesticidal activity of *Dalbergia oliveri* against termites and fungi are known (Sekine et al., 2009) and other species of this genus also can control leaf minors, defoliators, stem borers, and termites (Intari et al., 1995; Nair, 2000). Although some studies demonstrated their antifeedant (Simmonds, 2003) and insecticidal activities (Herath et al., 1998); no insecticidal reports are currently available to show effects on *Ae. aegypti*. Hence, the present study aims at analyzing the larvicidal effect of crude and some isolated compounds from *D. oliveri* on this dengue vector. The results of the present study would be useful in promoting research, aiming at the development of new agent for vector and pest control, based on bioactive chemical compounds from indigenous plant source.

2. Materials and methods

2.1. Extraction and isolation

Heartwood of *D. oliveri* (Gamble) was obtained from Singburi Province (100 km from Bangkok), Thailand. Air-dried *D. oliveri* (4 kg) heartwood was powdered and subsequently extracted with hexane, dichloromethane, ethyl acetate and methanol, in this order by Soxhlet extractor. The powder was first extracted with *n*-hexane by soaking in this solvent at room temperature for seven days. The hexane extract was filtered and then concentrated under vacuum. This process was repeated three times to obtain crude extract as orange brown oil (4.3 g). The solid residue was further extracted successively with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH). CH_2Cl_2 extract was black-brown sticky gum (184.8 g), EtOAc extract was a black-brown sticky gum (150.5 g) and MeOH extract was black-brown gum (296.9 g) (Table 1). As CH_2Cl_2 extract was most active it was further subjected to fractionation. CH_2Cl_2 extract (140 g) was fractionated by column chromatography (Merck Kieselgel 60 G) with a gradient elution system (hexane, 50% EtOAc in hexane, EtOAc and 50% MeOH in EtOAc). Nine fractions (Fr. I–IX) were obtained. All the fractions were further evaluated and fraction III, V and VI were observed to contain the activity. Fr. III (11.7 g) was subjected to similar fractionation with 10% and 60% EtOAc in hexane to yield (+)-medicarpin (2.8 g). Fr. V (5.5 g) yielded formononetin (329.2 mg) after eluting with 100% CH_2Cl_2 and 5% EtOAc in CH_2Cl_2. Fr. VI (14.1 g) was re-fractionated and eluted with 40% EtOAc in hexane and 70% EtOAc in hexane to give formononetin (405.8 mg) and (±)-violanone (380.5 mg), respectively. Both ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury plus 400 (Varian, Palo Alto, CA, USA) with tetramethylsilane as an internal reference which operated at 399.84 MHz for ^1H and 100.54 MHz for ^{13}C nuclei. The FT-IR spectra were recorded on Nicolet Impact 410 (Nicolet Analytical Instruments, Madison, WI). Specific rotations were measured on a Perkin-Elmer 3410 (Perkin-Elmer, Waltham, MA). Melting points were determined with a Fisher-Johns melting point apparatus (Fisher Scientific, Pittsburgh, PA). The analytical data of active compounds is given as under:

(+)-Medicarpin: white needle crystal, m.p. 132.0–133.5 °C (Herath et al., 1998); $[\alpha]_D^{20}$: +223.1 (c 0.16, acetone); IR (KBr, cm^{-1}): ν = 3249, 1626, 1494, 1443, 1369, 1256, 1210, 1167, 1112, 1081, 1042, 949; ^1H NMR ($CDCl_3$): δ 7.40 (1H, d, J = 8.5 Hz), 7.16 (1H, d, J = 8.8 Hz), 6.58 (1H, dd, J = 8.5, 2.4 Hz), 6.50 (2H, br), 6.45 (1H, d, J = 2.4 Hz), 5.53 (1H, d, J = 6.7 Hz), 4.26 (1H, dd, J = 10.9, 4.8 Hz), 3.80 (3H, s), 3.65 (1H, dd, J = 10.9, 10.9 Hz,), 3.55 (1H, m); ^{13}C NMR ($CDCl_3$): δ 161.0, 160.5, 157.3, 156.5, 132.3, 124.9, 119.2, 112.4, 110.0, 106.6, 103.7, 96.9, 79.7, 66.5, 55.6, 39.5.

Formononetin: pale brown solid, m.p. 260.8–261.5 °C (Goda et al., 1992); IR (KBr, cm^{-1}): ν = 3311, 1606, 1517, 1455, 1276, 1256, 1182, 1108, 1022; ^1H NMR (DMSO-d_6): δ 8.36 (1H, s), 7.99 (1H, d, J = 8.8 Hz), 7.52 (2H, d, J = 8.8 Hz), 7.01 (2H, d, J = 8.8 Hz), 6.96 (1H, dd, J = 8.8, 2.2 Hz), 6.89 (1H, d, J = 2.2 Hz), 3.80 (3H, s); ^{13}C NMR (DMSO-d_6): δ 175.1, 163.0, 159.4, 157.9, 153.7, 130.6 (2C), 127.8, 124.7, 123.6, 117.1, 115.7, 114.1 (2C), 102.6, 55.6.

(±)-Violanone: white needle crystal, m.p. 204.3–206.2 °C (Farkas et al., 1974); IR (KBr, cm^{-1}): ν = 3292, 1669, 1591, 1494, 1245, 1100; ^1H NMR (acetone-d_6): δ 7.81 (1H, d, J = 8.6 Hz), 6.71 (1H, d, J = 8.9 Hz), 6.65 (1H, d, J = 8.9 Hz), 6.61 (1H, m), 6.45 (1H, br), 4.62 (1H, dd, J = 11.0, 11.0 Hz), 4.48 (1H, dd, J = 11.0, 5.4 Hz), 4.15 (1H, dd, J = 11.0, 5.4 Hz), 3.85 (3H, s), 3.81 (3H, s); ^{13}C NMR (acetone-d_6): δ 190.5, 164.1, 163.8, 148.3, 146.0, 139.5, 129.2, 122.2, 119.5, 114.9, 110.4, 106.5, 102.6, 71.2, 59.2, 55.6, 48.1.

2.2. Mosquito culture

Eggs of *Ae. aegypti* (Thailand laboratory strain) were received from Ministry of Public Health, Thailand. Larvae were reared in 500-ml glass beakers containing water and fed a diet of brewer's yeast. Pupae were also maintained in similar beakers and were maintained in our culture room at 28 °C, 70% RH and 14:10 DL photoperiod where adults emerged. Adults were maintained in glass cages and were continuously provided with 10% sucrose solution in a jar with a cotton wick.

2.3. Bioassays

Larvicidal bioassay against 3rd instars of *Ae. aegypti* was done following WHO (1981) method in the laboratory. Third-instars were placed in a small cup (n = 10 per cup) filled with 50 ml of distilled water to which 0.5 ml of each concentration of the extract was added. The final concentration range in each treatment was 62.5–8000 ppm. In all 8 concentrations were used in 10 replicates per concentration and experiments were conducted under laboratory conditions at 28 °C, 70% RH and 14:10 DL photoperiod. In controls 0.5 ml of acetone was used in each case. During aqueous dispersion test, mosquito larvae were never provided with food. After 24 h, mortality of the larvae in each treatment was recorded and LC_{50} values determined. The experiments were continued and after 48 h the toxicity was recorded again and now both the LC_{50} and LC_{90} with 95% confidence limit were calculated by Probit analysis using StatPlus version 2008. The behavior of treated larvae comparing control group were also observed. Three isoflavonoids isolated were also evaluated in similar fashion, however, at lower