

Effect of Plant Flavonoids on Mosquito Larvae

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Abstract: The ethyl acetate soluble fractions of the methanol extracts of the leaves of six mangrove plants and six isolated flavonoid compounds from them, were tested for larvicidal activity against the mosquito, *Aedes aegypti*, 4th instar larvae. The ethyl acetate extracts of *B. gymnorrhiza* species showed highest activity with LD₅₀ value of 616.70 ppm (24 hr) and 393.80 ppm (48 hr). Among the isolated flavonoid compounds, chrysoeriol (2) was found to be effective with LD₅₀ value of 18.90 ppm (48 hr) and 4', 5', 7- trihydroxy — 3', 5- dimethoxy flavone (1) showed highest activity with LD₅₀ value of 19.40 ppm (24 hr) and 100% mortality in 48 hr.

Keywords: Mangrove Plant, flavonoid, *Aedes aegypti*.

Introduction

Plant toxins have been known for centuries [1]. Many of these plant toxins are flavonoids. Rotenone has been used as an insecticide for the control of leaf- chewing beetles and caterpillars [2]. Insect behaviour, development and growth are affected by plant flavonoids. Feeding stimulants of some beetles are known, such as kaempferol-3-O-xylosyl galactoside, quercetin-7-O-glucoside, quercetin-3-O- glucoside [3].

Some flavonoids are also responsible for resistance of plants to insect attack. For example, rhamnosyl (4-ketofucosyl)- 5, 7, 3', 4' — tetrahydroxy flavone present in corn (*Zea mays*) retarded the growth of corn carworm, *Heliothis zea* (Boddi) [4, 5]. Flavone glycosides and aglycones in the cotton plant are larval growth inhibitors for *H. zea* (the cotton bollworm) [6] and *H. virescens* (the tobacco budworm) [7]. Recently, it has found that cyanidin-3-β-glucoside is resistant to tobacco budworm [8,9].

Flavonoids also act as feeding deterrents and in some species as attractants [10]. A number of flavonoid derivatives (e. g. naringin) are among the most bitter substance known which protect plant from mammalia and other vertebrate herbivores [10]. Hence, flavonoids play an important role in plants as insect feeding attractants or excitants, as repellents or deterrents and as oviposition inhibitors [11].

Chemical Studies in mangrove plants showed the presence of large amount of phenolic compounds in these plants [12]. One of the major biological properties of phenolic compounds is their insecticidal and antifeedant activities. For this, mangrove plants are of special interest in the search of biologically active compounds. Since 1950, the chemical constituents of some common genera were reported, but a few studies on biological activity of mangrove plant chemicals have been done [13].

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Therefore, the present study was carried out to determine the toxic activity against mosquito larvae *Aedes aegypti*. The extracts of six mangrove plants and their isolated flavonoid compounds were investigated in this study.

Materials and methods

Leaves of mangrove plants of *Brugiera gymnorrhiza*, *Bruguiera cylindrica*, *Bruguleara paiviflora*, *Avicennia marina*, *Avicennia officinalis* and *Avicennia alba* were collected from the mangrove swamp of Batu Maung, Pulau Pinang, West Malaysia.

The leaves were dried in an oven at 40°C and crushed into powder using a mortar. The powdered leaves were first extracted with petroleum ether and then with methanol. The methanol extracts were evaporated and separated into chloroform and ethyl acetate soluble fractions. The ethyl acetate soluble fraction was evaporated to dryness and subjected to bioassay. Six compounds, chrysoeriol, 4', 5', 7- trihydroxy -3', 5- dimethoxy flavone, (1) isovitexin, vitexin, quercetin and rutin isolated from the ethyl acetate extracts of *B. gymnorrhiza*, *B. cylindrica*, *B. Parviflora* using a standard method [14]. These chemicals were tested for larvicidal activity against mosquito larvae.

Twenty-five fourth instar larvae of *Aedes aegypti* were put in a 250 mL beaker containing 99 mL of distilled water to which 1 mL of extract solution in alcohol was added to give the test concentrations. Four replicates were set up for each concentration and the each extract was tested at three different concentrations (500, 300, 100 ppm). The pure compounds were tested at concentrations of 20, 10 and 5 ppm. A control was set up with 100 ml of distilled water containing 1 ml of alcohol. The mortality was recorded after 24 hr and 48 hr of exposure to the chemicals. The control mortality was zero. The LD₅₀ values were calculated based on the Probit Regression analysis according to the procedure of Daum and Kilcreas by Computer programming [14].

Results and Discussion

Bioassay results of the ethyl acetate fraction of the methanol extract of the leaves of six plant species and of the isolated flavonoid compounds studied are presented in Table 1.

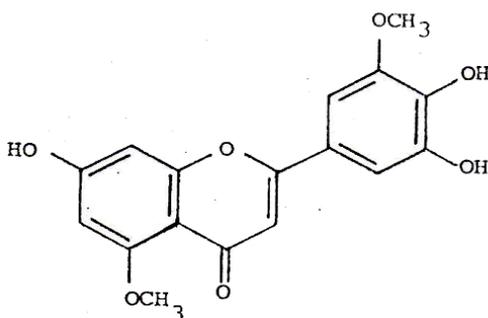
Table 1: Larvicidal activity of the ethyl acetate extract of plants and of isolated compounds.

Treatments	24 hr			48 hr		
	LCL	LD ₅₀	UCL	LCL	LD ₅₀	UCL
Ethyl acetate extracts						
<i>B. gymnorrhiza</i>	570.5	616.7	683.5	379.5	393.8	409.2
<i>B. cylindrical</i>	714.9	816.8	992.0	NSR	NSR	NSR
<i>B. parviflora</i>	—	—	—	552.7	597.1	658.5
<i>A. marina</i>	NSR	NSR	NSR	NSR	NSR	NSR
<i>A. officinalis</i>	NSR	NSR	NSR	319.8	359.1	411.1
<i>A. alba</i>	—	—	—	592.5	704.6	889.8
Isolated compounds						
chrysoeriol (2)	42.1	61.6	115.9	16.4	18.9	22.8
4', 5', 7-tri- hydroxy-3',5-dimethoxy – flavone	18.5	19.4	20.6	NSR	NSR	NSR

(1)		
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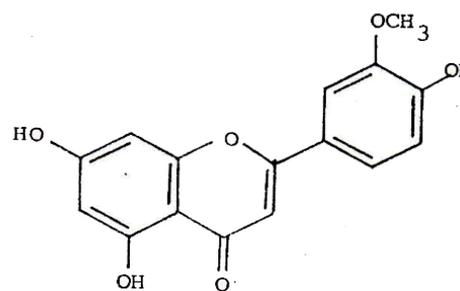
LCL = lower confidence limit; UCL = upper confidence limit; NSR = non — significant regression. — = data statistically not valid.

Of the six ethyl acetate extracts bioassayed, *B. gymnorrhiza* and *A. officialis* leaf extracts, with LD₅₀ values of 393.8 ppm and 359.1 ppm (48 hr) have the highest activity. Flavonoids which were isolated from the ethyl acetate extracts were tested for larvicidal activity. Among the six isolated compounds, only two compounds, chrysoeriol and 4', 5', 7-trihydroxy - 3', 5- dimethoxy flavone, were toxic to the larvae. Chrysoeriol showed LD₅₀ of 18.90 ppm (48 hr) and 4', 5', 7- trihydroxy -3' 5- dimethoxy flavone (1) showed promising activity, effecting 50% mortality at a concentration of 19.40 ppm in 24 hr and 100% mortality in 48 hr.



4',5',7- trihydroxy 3',5-
dimethoxy flavone, isolated
from *B. gymnorrhiza*.

(1)



chrysoeriol, iso-
lated from *A. marina*

(2)

Recently, the effect of South India vetiver oil (*Veteveria zizinioids*) against fourth instar larvae of *Culex quinquefasciatus* was reported and the LC₅₀ of larvae was achieved by 880 ppm (24 hr) [15].

Conclusion

Comparison of this with the present result shows that *B. gymnorrhiza* leaf extract is more effective than vetiver oil and the isolated compound, 4', 5', 7- trihydroxy -3', 5- dimethoxy flavone (1) is about 44 times more effective than vetiver oil. The present work provides an encouraging result and this isolated compound is a potent larvicidal agent.

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