CHAPTER FIVE

RESULTS AND DISCUSSION - BIOASSAY RESULTS

5.1 Introduction

The number of dengue fever victims in Malaysia has increased tremendously over the last few years. This fever is caused by a virus, transmitted through the *Aedes aegypti* mosquito bites. Controlling the population of *Aedes aegypti* by killing them, especially at the larval stage, will definitely reduce dengue fever cases. Some of the control methods currently practiced are adulticidal fogging and aerosol sprays, mosquito repelling vapourisers and destruction of breeding grounds. However, adulticides do not affect mosquito eggs or larvae. The search for potential larvicides from natural resources such as plants, let it be crude extracts which contain mixtures of bioactive compounds, or pure compounds which are safe and environmentally-friendly is the theme of this work.

A series of plant extracts from the genera *Goniothalamus*, *Disepalum* and *Mezzetia* were screened for biological activity and the active extracts selected to be further studied in detail. Bioassay directed fractionations were carried out and the bioactive compounds identified.

5.2 Bioassay of crude plant extracts

Bioassay for the toxicity of the crude extracts was performed against the larvae of *Aedes aegypti* using the WHO (1981a) standard procedures with slight modifications. A stock solution was prepared by dissolving a weighed amount of the crude extract in absolute ethanol. Serial dilutions of the stock were prepared in 250 ml drinking glasses containing 25 ml of chlorine-free tap water. Ten late 3rd-instar mosquito larvae were introduced into each glass and the volume made up to 50 ml with chlorine-free tap water. The range of concentrations tested were pre-determined in earlier trials using the similar technique. All bioassays were carried out in duplicates or triplicates. A small amount of larval food was also added and the larval mortality was observed 24 hours after continuous exposure. The mortality data were analysed using probit analysis programme for personal computer in order to obtain the LC_{50} and LC_{90} values (Raymond, 1985).

5.3 Larvicidal activity for crude plant extracts of various plant species of the genera *Goniothalamus*, *Disepalum* and *Mezzetia*

Natural products of plant origin such as rotenone, pyrethrins and nicotine have long been used to control destructive insects and vectors of disease (Matsumura, 1975). One of the earliest report on the toxicity of plant extracts on mosquito larvae was by Campbell and Sullivan which reported that the plant alkaloids, nicotine, anabasine, methylanabasine and lupinine killed larvae of *Culex pipiens*, *Culex territans* and *Culex quinquefasciatus* (Campbell and Sullivan, 1933). Subsequently many researchers have reported about the effectiveness of plant extracts against mosquito larvae (Haller, 1940; Amonkar and Reeves, 1970). Zebitz (1986) reported that the neem seed kernel extract (*Azadirachta indica*) was active against fourth instar larvae of *Aedes aegypti* with an LC_{50} value ranging from 1.19 to 18.10 μg/ml. No report has been made on the larvicidal activity of plant extracts from the genera *Goniothalamus*, *Disepalum* and *Mezzetia*.

5.3.1 Crude hexane extracts of the stem bark from the plants of the genera *Goniothalamus*, *Disepalum* and *Mezzetia*

The table that follows summarises the results obtained from bioassay testings carried out on the hexane extracts of the various plants.

Table 5. 1: Larvicidal (*Aedes aegypti*) activity of crude hexane extract

Plant	LC_{50} (μg/ml)[a] (95% C.L.)[b]	LC_{90} (μg/ml)[a] (95% C.L.)[b]	Slope ± S.E.[c]
Goniothalamus andersonii	42.3 (36.2-47.8)	87.9 (74.2-116.8)	4.03±0.63
Goniothalamus dolichocarpus	166.7 (148.6-184.8)	313.6 (265.9-420.6)	4.67±0.75
Goniothalamus velutinus	200-250	-	-
Goniothalamus malayanus	187.6 (163.6-213.9)	402.4 (325.4-591.8)	3.87±0.64
Goniothalamus ridleyi	81.2 (71.5-90.9)	164.1 (137.2-223.6)	4.19±0.65
Goniothalamus macrophyllus	60.5 (53.4-66.5)	106.9 (94.2-131.5)	5.19±0.79
Goniothalamus gigantifolius	>400		
Goniothalamus sinclairinius	119.6 (107.5-134.1)	253.3 (206.7-361.9)	3.93±0.60
Goniothalamus montanus	242.6 (228.3-255.8)	334.6 (303.3-417.1)	9.17±1.91
Goniothalamus macranii	103.9 (90.7-118.9)	205.1 (170.0-276.3)	4.34±0.60
Goniothalamus uvarioides	47.7 (28.2-80.0)	171.8 (55.4-590.7)	2.30±0.75
Disepalum anomalum	202.8 (186.9-219.7)	320.0 (282.3-398.0)	6.47±1.01
Mezzetia umbellata	248.4 (231.9-270.4)	389.6 (336.9-522.0)	6.56±1.19
*Azadirachta indica**	1.2-18.0	N.A.	N.A.
Asimina triloba (EtOH)**	20.0	N.A.	N.A.
*Dipterocarpus kerrii****	146.1 (139.7-152.3)	N.A.	N.A.
*Litsea elliptica****	16.0 (14.3-18.1)	N.A.	N.A.
*Piper aduncum****	23.4 (21.1-25.8)	N.A.	N.A.
*Polygonum minus****	47.9 (43.2-52.8)	N.A.	N.A.
*Cinnamomum iners****	62.9 (59.4-66.8)	N.A.	N.A.
*Cinnamonum zeylanicum****	87.5 (77.9-97.8)	N.A.	N.A.
Stemona tuberosa Lour[+]	197.5 (highly variable)	1408.2 (highly variable)	N.A.
Termephos (synthetic)	0.005 (0.004-0.006)	0.009 (0.008-0.01)	5.89±0.81
DDT***	0.07 (0.05-0.11)	N.A.	N.A.

* Zebitz (1986), **Mikolajczak *et al.* (1988), ***Jantan *et al.* (1995), [+]Lee and Chiang (1994), [a] LC_{50} = lethal concentration, [b] 95% CL = confidence interval at 95% confidence limit, [c] SE = standard error, N.A. = not available.

5.3.1.1 *Goniothalamus andersonii*

The larvae of *Aedes aegypti* were susceptible to the hexane extract of the stem bark of *Goniothalamus andersonii*. Even at 87.9 μg/ml there was a 90% mortality and a 50% mortality at 42.3 μg/ml (LC_{50} = 42.3 μg/ml and LC_{90} = 87.9 μg/ml). For details of probit analysis, see Appendix 1.

5.3.1.2 *Goniothalamus dolichocarpus*

The larvae of *Aedes aegypti* were less susceptible to the hexane extract of the stem bark of *Goniothalamus dolichocarpus* compared to *G. andersonii*. There was a 90% mortality at 300 μg/ml and at 166.7 μg/ml 50% of the population had died (LC_{50} = 166.7 μg/ml). This LC_{50} value is four-fold that obtained for *Goniothalamus andersonii*. The LC_{90} for *Goniothalamus dolichocarpus* (313.6 μg/ml) is also about four-fold that of *G. andersonii.*

5.3.1.3 *Goniothalamus velutinus*

The mosquito larvae were not very susceptible to the hexane extract of *Goniothalamus velutinus* since even at a dosage of 300 μg/ml only 70% mortality was observed. The LC_{50} value was high with an approximate value of 200-250 μg/ml.

5.3.1.4 *Goniothalamus malayanus*

A slightly lower dosage of extract was required to obtain a 90% mortality compared to *Goniothalamus dolichocarpus*. For a 90% mortality, a dosage of 402.4 μg/ml of extract was required. This is about five-fold the amount that is required for *Goniothalamus andersonii*. (LC_{90} = 87.9 μg/ml). The LC_{50} value (LC_{50} = 187.6 μ g/ml) is close to that for *Goniothalamus dolichocarpus* (LC_{50} = 166.7 μg/ml) but is four-fold that for *Goniothalamus andersonii* (LC_{50} = 42.3μg/ml).

5.3.1.5 *Goniothalamus macrophyllus*

A very low dosage of 106.9 μg/ml was required for a 90% mortality. This is low compared to a 313.6 μg/ml dosage for *Goniothalamus dolichocarpus* and an LC_{90} value of 402.4 μg/ml for *G. malayanus*. The LC_{50} was only 67.7 μg/ml which is of a slightly higher value than that for *G. andersonii* (LC_{50} = 42.3 μg/ml) but one-third the LC_{50} value for *G. malayanus* (LC_{50} = 187.6 μg/ml). The LC_{90} (90% mortality) for *G. macrophyllus* was about one-quarter that for *G. malayanus* (LC_{90} = 402.4 μg/ml) and one-third that for *G. dolichocarpus* (LC_{90} = 313.6 μg/ml). However, the LC_{90} for *G. andersonii* was slightly lower than that for *G. macrophyllus*. Overall, the mosquito larvae of the *Aedes aegypti* were most susceptible to the bark extract of *G. andersonii* compared to the other four species mentioned so far.

5.3.1.6 *Goniothalamus ridleyi*

The larvae of *Aedes aegypti* were also susceptible to the hexane extract of *Goniothalamus ridleyi*. A dosage of 164.1 μg/ml was required for a 90% mortality. This is about two-fold the dosage required for *G. andersonii* (LC_{90} = 87.9 μg/ml) and two and a half-fold that for *G. malayanus* (LC_{90} = 402.4 μg/ml). However, the LC_{90} value for *G. dolichocarpus* was two-fold that for *G. ridleyi*. The LC_{50} value for *G. ridleyi* (LC_{50} = 81.2 μg/ml) is two-fold the LC_{50} value for *G. andersonii* (LC_{50} = 42.3

μg/ml). It is, however, half the LC_{50} value for *G. dolichocarpus* which required a 166.7 μg/ml dosage to effect a 50% mortality.

5.3.1.7 *Goniothalamus gigantifolius*

The mosquito larvae of *Aedes aegypti* were not very susceptible to the hexane extract of the *Goniothalamus gigantifolius*. The LC_{50} value was more than 400 μg/ml.

5.3.1.8 *Goniothalamus sinclairinius*

The LC_{90} value for *Goniothalamus sinclairinius* (LC_{90} = 253.3 μg/ml was about three-fold that for *G. andersonii* (LC_{90} = 87.9 μg/ml) and two and a half times that for *G. macrophyllus* (LC_{90} = 106.9 μg/ml). *G. sinclairinius* gave an LC_{50} value of 119.6 μg/ml which is about three-fold that for *G. andersonii* (LC_{50} = 42.3 μg/ml) and two-fold that for *G. macrophyllus* (LC_{50} = 60.5 μg/ml). Hence, compared to the other tested and mentioned *Goniothalamus* species extracts, *G. sinclairinius* is less cytotoxic than *G. andersonii*, *G. macrophyllus* and *G. ridleyi* but more cytotoxic than *G. dolichocarpus*, *G. velutinus*, *G. malayanus* and *G. gigantifolius*.

5.3.1.9 *Goniothalamus montanus*

Again the larvae of *Aedes aegypti* were not very susceptible to the hexane extract of *Goniothalamus montanus*. A dosage of more than 300 μg/ml was required for a 90% mortality. The LC_{50} value of an extremely high figure of 242.6 μg/ml was four-fold that *for G. macrophyllus*, six-fold that for *G. andersonii* but only three-fold the LC_{50} value for *G. ridleyi*. Overall, the hexane extract of *G. montanus* can still be considered cytotoxic compared to the extract of *G. gigantifolius* which gave an LC_{50} value of more than 400 μg/ml.

5.3.1.10 *Goniothalamus macranii*

By applying a dosage of more than 200 μg/ml it was possible to obtain a one hundred percent mortality of the mosquito larvae. The extract gave an LC_{90} value of 205.1 μg/ml which is approximately one and a half times less than the value obtained for *G. montanus*. However, this value is close to that for *G. sincalirinius* and half the LC_{90} value for *G. malayanus* (LC_{90} for *G. malayanus* = 402.0 μg/ml). The LC_{50} value for *G. macranii* (LC_{50} = 103.9 μg/ml) was half the value for *G. montanus* (LC_{50} = 242.6 μg/ml) but almost similar to that for *G. sinclairinius*.

5.3.1.11 *Goniothalamus uvarioides*

The larvae of the *Aedes aegypti* were very susceptible to the stem bark extract of *Goniothalamus uvarioides*. A dosage of only 171.8 μg/ml was required to give a 90% mortality of the larvae. This value is comparable to that obtained for *G. ridleyi* (LC_{90} = 164.1 μg/ml) but slightly higher than that for *G. macrophyllus* (LC_{90} = 106.9 μg/ml). However, the LC_{90} value for *G. uvarioides* is about two-fold that for *G. andersonii* (LC_{90} = 87.9 μg/ml). A very low LC_{50} value of 47.7 μg/ml was obtained for *G. uvarioides* which is almost equal to that for *G. andersonii* but one-quarter that for *G. malayanus* and half the value for *G. ridleyi*.

5.3.1.12 *Disepalum anomalum*

The mosquito larvae were not very susceptible to the crude hexane extract of *Disepalum annomalum*. An extremely high dosage of 320.0 μg/ml was required to get a 90% mortality. This value is almost equivalent to that for *G. dolichocarpus* and *G. montanus*. The extremely high LC_{50} value of 202.8 μg/ml indicated the extract to be not very cytotoxic compared to the *G. andersonii*, *G. macrophyllus*, *G. ridleyi* and *G. uvarioides* extracts which gave LC_{50} values of 42.3 μg/ml, 60.5 μg/ml, 81.2 μg/ml and 47.7 μg/ml respectively.

5.3.1.13 *Mezzetia umbellata*

The hexane extract of *Mezzetia umbellata* gave a very high LC_{50} value of 248.4 μg/ml and an extremely high LC_{90} value of 389.6 μg/ml, indicating that this was not very cytotoxic compared to some of the other extracts obtained from the various *Goniothalamus* species mentioned earlier. However, the LC_{50} value for *Mezzetia umbellata* was lower than that for the extract of *Goniothalamus gigantifolius* so it is still relatively more cytotoxic than the hexane extract from *G. gigantifolius.*

5.3.2 Conclusions

The susceptibility or resistance of mosquito larvae to insecticide test on crude hexane extracts of various *Goniothalamus*, *Mezzetia* and *Disepalum* species revealed that some samples were cytotoxic to the mosquito larvae, with LC_{50} values mostly <200 μg/ml. The hexane extracts of *Goniothalamus andersonii*, *G. uvarioides*, *G. macrophyllus* and *G. ridleyi* were very cytotoxic to the *Aedes aegypti* with LC_{50} values of 42.3, 47.7, 60.5 and 81.2 μg/ml, respectively. The other extracts also show cytotoxicity with LC_{50} values ranging from 103.9 to 187.6 μg/ml. The best larvicide would be the hexane extract of *Goniothalamus andersonii* with an LC_{50} value of 42.3 μg/ml, followed by that from *G. uvarioides* (LC_{50} 47.7 μg/ml), *G. macrophyllus* (LC_{50} 60.5 μg/ml) and *G. ridleyi* (LC_{50} 81.2 μg/ml). Hence, these extracts have potential as natural larvicides since they possess potent or significant levels of bioactive principles. Although the crude extracts were very much less toxic than that of *Azadirachta indica* (Zebitz, 1986) and *Asimina triloba* (Mikolajczak, 1988), they were comparable to other larvicidal plant extracts (Jantan *et al.*, 1995; Lee and Chiang, 1994). Preliminary gas chromatography screenings of the crude hexane extracts of the *Goniothalamus* species indicated a complex mixture of essential oils with *G. andersonii*, *G. dolichocarpus*, *G. velutinus*, *G. macrophyllus*, *G. ridleyi*, *G. montanus* and *G. macranii* containing sesquiterpenes. Probably this is the contributing factor to the high cytotoxicity in *G. andersonii* which contains a high percentage of goniothalamin as well. Goniothalamin could well be the contributing factor as well. On the other hand, *G. uvariodes* showed strong cytotoxicity (LC_{50} 47.7 µg/ml) but did not contain any essential oils. However, goniothalamin was found to be present in the gas chromatographic screening. The other two good larvicides from *G. ridleyi* and *G. macrophyllus* were found to contain both essential oils and goniothalamin.

The log-probit regression lines (LPRL) of these extracts all have slopes of more than 1.0. It is interesting that the LPRL of the four most toxic extracts, *Goniothalamus andersonii*, *G. macrophyllus*, *G. ridleyi* and *G. uvarioides* have different slopes. *G. macrophyllus* has the largest slope while *G. uvarioides* has the smallest slope. This shows that the *Aedes aegypti* is very much more sensitive to changes in concentrations of *G. macrophyllus* than of *G. uvarioides*.

Preliminary *in vitro* cytotoxicity screening against P388 cell lines of the crude hexane extracts of the above plants indicated some bioactivity.

5.3.3 Crude ethanol extracts of the stem bark from the plants of the genera *Goniothalamus*, *Disepalum* and *Mezzetia*

The following table summarises the results obtained from the bioassay testings carried out on the ethanol extracts of the various plants.

Table 5. 2: Larvicidal (*Aedes aegypti*) activity of crude ethanol extracts

Plant	LC_{50} (µg/ml)[a] (95% C.L.)[b]	LC_{90} (µg/ml)[a] (95% C.L.)[b]	Slope ± S.E.[c]
Goniothalamus andersonii	58.1 (45.3 - 73.6)	171.8 (55.4 - 590.7)	7.24±2.42
Goniothalamus dolichocarpus	146.3 (119.5 -176.4)	548.9 (396.2 - 962.3)	2.23±0.33
Goniothalamus velutinus	12.1 (10.8 - 13.5)	22.5 (19.0 - 30.5)	4.77±3.99
Goniothalamus malayanus	33.9 (27.9 - 40.4)	110.9 (84.3 - 171.9)	2.49±0.34
Goniothalamus macrophyllus	100.3 (88.4 - 122.3)	209.7 (157.4 - 406.7)	4.00±0.84
Goniothalamus ridleyi	132.2 (117.0 - 145.0)	219.2 (192.1 - 279.6)	5.83±1.04
Goniothalamus gigantifolius	208.7 (194.0 - 224.5)	351.7 (311.3 - 425.5)	5.65±0.71
Goniothalamus sinclairinius	inactive	-	-
Goniothalamus montanus	inactive	-	-
Goniothalamus macranii	150 - 180	-	-
Goniothalamus uvarioides	123.4 (110.0 - 137.5)	246.2 (206.3 - 333.1)	4.27±0.64
Mezzetia umbellata	5.5 (4.9 - 6.2)	10.7 (8.9 - 15.2)	4.41±0.73
Disepalum anomalum	19.6 (15.4 - 24.7)	89.7 (60.4 - 176.6)	1.94±0.29
*Azadirachta indica**	1.2 - 18.0	N.A.	N.A.
*Asimina triloba***	20.0	N.A.	N.A.
*Dipterocarpus kerrii****	146.1 (139.7 - 152.3)	N.A.	N.A
*Litsea elliptica****	16.0 (14.3 - 18.0)	N.A.	N.A.
*Piper aduncum****	23.4 (21.1 - 25.8)	N.A.	N.A
*Polygonum minus****	47.9 (43.2 - 52.8)	N.A.	N.A.
*Cinnamomum iners****	62.9 (59.4 - 66.8)	N.A.	N.A.
*Cinnamomum zeylanicum****	87.5 (77.9 - 97.8)	N.A.	N.A.
Stemona tuberosa Lour.[+]	197.51 (highly variable)	1408.16 (high variable)	N.A.
Termephos	0.005 (0.004 - 0.006)	0.009 (0.008 - 0.01)	5.89±0.81
DDT***	0.07 (0.05 - 0.11)	N.A.	N.A.

* Zebitz (1986), **Mikolajczak *et al.* (1988), ***Jantan *et al.* (1995), [+]Lee and Chiang (1994), [a] LC = lethal concentration, [b] 95% CL = confidence interval at 95% confidence level, [c] SE = standard error, N.A. = not available.

5.3.3.1 *Goniothalamus andersonii*

The larvae of the *Aedes aegypti* were very susceptible to the crude ethanol extract of *Goniothalamus andersonii*. A reasonably low LC_{50} value of 58.1 μg/ml was obtained. A dosage of 171.8 μg/ml was required to give a ninety percent mortality of the larvae.

5.3.3.2 *Goniothalamus dolichocarpus*

The mosquito larvae were less susceptible to the ethanol extract of *Goniothalamus dolichocarpus*. The LC_{50} value was two and a half times that *for G. andersonii* (LC_{50} = 58.1 μg/ml).

5.3.3.3 *Goniothalamus velutinus*

The crude ethanol extract of *Goniothalamus velutinus* was very bioactive to the larvae of the *Aedes aegypti*. It gave an LC_{50} value of 12.1 μg/ml and only a dosage of 22.5 μg/ml was required to obtain a 90% mortality. This extract is five times more bioactive than that of *G. andersonii* (LC_{50} for *G. andersonii* is 58.1 μg/ml).

5.3.3.4 *Goniothalamus malayanus*

The larvae of the *Aedes aegypti* were susceptible to the ethanol bark extract of *Goniothalamus malayanus*. An LC_{50} value of 33.9 μg/ml which is almost three-fold that for the bark extract of *G. velutinus* was obtained. However, this extract showed a better activity than the extract of *G. andersonii* which gave an LC_{50} value of 58.1 μg/ml. The LC_{90} value of 110.9 μg/ml was also lower than that for *G. andersonii* which was 171.8 μg/ml. However, it was about five-fold that for *G. velutinus*.

5.3.3.5 *Goniothalamus macrophyllus*

The crude ethanol extract of *Goniothalamus macrophyllus* was about half as bioactive as that of *G. andersonii*. The LC_{50} values for *G. macrophyllus and G. andersonii* were 58.1 and 100.3 μg/ml, respectively. Also, this extract gave an LC_{50} value which was about three-fold that for *Goniothalamus malayanus* (LC_{50} 33.9mg/ml) and eight-fold that for *Goniothalamus velutinus*. A high dosage of 209.7 μg/ml was required to obtain a 90% mortality.

5.3.3.6 *Goniothalamus ridleyi*

The crude ethanol extract of *Goniothalamus ridleyi* gave almost the same LC_{50} value as *G. macrophyllus* except that it was thirty-two units higher (LC_{50} 132.2 μg/ml). This value was almost four-fold that for *G. malayanus* (LC_{50} 33.9 μg/ml) and eleven-fold that for *G. velutinus*. However, this LC_{50} value was only two-fold that for *G. andersonii*.

5.3.3.7 *Goniothalamus gigantifolius*

The larvae of the *Aedes aegypti* were not very susceptible to the crude ethanol extract of *Goniothalamus gigantifolius*. An extremely high LC_{50} value of 208.7 μg/ml was obtained. A very high LC_{90} value of 351.7 μg/ml was also obtained. This extract is about three and a half times less bioactive than that of *G. andersonii* and six times less bioactive than that of *G. malayanus*. Also, the LC_{50} for *G. gigantifolius* was two fold that for *G. macrophyllus* and *G. ridleyi*.

5.3.3.8 *Goniothalamus sinclairinius*

The crude ethanol extract of *Goniothalamus sinclairinius* was not cytotoxic to the mosquito larvae of the *Aedes aegypti*.

5.3.3.9 *Goniothalamus montanus*

The crude ethanol extract *of Goniothalamus montanus* was also not cytotoxic to the *Aedes aegypti* larvae.

5.3.3.10 *Goniothalamus macranii*

The larvae of the *Aedes aegypti* appeared not to be very susceptible to the crude ethanol extract of *Goniothalamus macranii*. The bioassay indicated an LC_{50} value of approximately 150-180 μg/ml. This is almost equivalent to the LC_{50} value obtained for *G. dolichocarpus* (LC_{50} for *G. dolichocarpus* is 146.3 μg/ml). However, this extract appeared to be more cytotoxic than that for *G. gigantifolius*.

5.3.3.11 *Goniothalamus uvarioides*

The crude ethanol extract of *Goniothalamus uvarioides* indicated almost the same LC_{50} value as that for *G. ridleyi* (LC_{50} 132.2 μg/ml). This LC_{50} value is also close to that for *G. dolichocarpus* (LC_{50} 146.3 μg/ml) and *G. macrophyllus* (LC_{50} 100.3 μg/ml). The LC_{50} value for the extract of *G. uvarioides* is about two-fold the LC_{50} value for *G. andersonii* and four-fold the value of *G. malayanus*.

5.3.3.12 *Mezzetia umbellata*

The crude ethanol extract of *Mezzetia umbellata* was extremely cytotoxic to the mosquito larvae. An extremely low LC_{50} value of 5.5 μg/ml was obtained. This is about ten times less than the LC_{50} value for *Goniothalamus andersonii* but half that for *G. velutinus*. The LC_{90} value was equally as low (10.7 μg/ml). This value is half that for *G. velutinus* (22.5 μg/ml). Hence, the extract of *M. umbellata* is more bioactive than that of *G. velutinus*. For details of probit analysis, see Appendix 2.

5.3.3.13 *Disepalum anomalum*

The mosquito larvae of the *Aedes aegypti* were extremely susceptible to the crude ethanol extract of *Disepalum anomalum*. A very low LC_{50} value of 19.6 μg/ml was obtained. This value is about one-third that for *Goniothalamus andersonii* (58.1 μg/ml) and slightly less than half that for *G. malayanus* (33.9 μg/ml). However, it is slightly more than one and half fold the LC_{50} value for *G. velutinus* (12.1 μg/ml). A dosage of 89.7 μg/ml was required to give a ninety percent mortality. This is half the amount required for *G. andersonii* (LC_{90} 171.8 μg/ml) but four fold that for *G. velutinus*.

5.3.4 Conclusions

The susceptibility or resistance of mosquito larvae to insecticide test on crude ethanol extracts of various *Goniothalamus*, *Mezzetia* and *Disepalum* species revealed that most of the species were cytotoxic to the mosquito larvae and had LC_{50} values less than 200 μg/ml. Among the species tested, the ethanol extracts of *Goniothalamus andersonii*, *G. velutinus*, *G. malayanus*, *Mezzetia umbellata* and *Disepalum anomalum* were very cytotoxic to the *Aedes aegypti* with LC_{50} values of 58.1 μg/ml, 12.1 μg/ml, 33.9 μg/ml, 5.5 μg/ml and 19.6 μg/ml, respectively. The best among these five plants were *M. umbellata*, *G. velutinus* and *D. anomalum* with LC_{50} values below 20 μg/ml. These values are comparable to those of the crude extracts of *Azodirachta indica* (Zebitz, 1986), the crude extracts of *Asimina triloba* (Mikolajczak *et al.*, 1988) and *Litsea elliptica* (Jantan *et al.*, 1995). The ethanol extracts of *G. andersonii* and *G. malayanus* were also cytotoxic and comparable to the extracts of *Polygonum minus* and *Cinnamomum iners* (Jantan, 1995). The other extracts also show cytotoxicity with LC_{50} values ranging from 100.3 μg/ml to 146.3 μg/ml. These include the plant extracts of *G. macrophyllus*, *G. ridleyi*, *G. uvarioides* and *G. dolichocarpus*. Not too cytotoxic were the ethanol extracts of *G. gigantifolius* and *G. macranii* with LC_{50} values of 208.7 μg/ml and 150-180 μg/ml, respectively. The extracts of *G. sinclairinius* and *G. montanus* were not cytotoxic to the larvae of the *Aedes aegypti*.

The LPRL of all the ethanol extracts have slopes of more than 1.0. The five most toxic extracts all have different slopes ranging from 1.94 to 7.24. *Goniothalamus andersonii*, the least toxic among the group has the highest slope while *Disepalum anomalum* has the lowest slope. This means that the *Aedes aegypti* is very sensitive to changes in concentration of *G. andersonii* than of *Disepalum anomalum*.

Preliminary *in vitro* cytotoxicity screening against P388 cell lines of the crude ethanol extracts of the above plants indicated some bioactivity with the extract of *Goniothalamus andersonii* being the most bioactive and that of *G. malayanus* the least bioactive. It is quite surprising that the bark extract of *Disepalum anomalum* which was strongly cytotoxic against the larvae of *Aedes aegypti* is not very bioactive against P388 cell lines. *G. dolichocarpus*, *G. velutinus* and *G. macrophyllus* gave the same IC_{50} value (9.5 μg/ml) even though they indicated completely different cytotoxicity with *Aedes aegypti*. With *Aedes aegypti* *G. velutinus* was the most cytotoxic followed by *G. macrophyllus* and *G. dolichocarpus*. The extract of *G. uvarioides* which was not too cytotoxic to *Aedes aegypti* is very bioactive to P388 cell lines with an IC_{50} value comparable to that for *G. andersonii* (IC_{50} = 7.0 mg/ml). The results are shown in Table 5.3 below.

Table 5. 3: *In vitro* cytotoxicity activity of crude ethanol extracts

Plant	IC_{50} (μg/ml)[d]
Goniothalamus dolichocarpus	9.5
Goniothalamus velutinus	9.5
Goniothalamus malayanus	80.0
Goniothalamus ridleyi	8.0
Goniothalamus gigantifolius	10.0
Goniothalamus macrophyllus	9.5
Goniothalamus uvariodes	7.0
Goniothalamus andersonii	5.5
Goniothalamus sinclairinius	10.0
Goniothalamus montanus	8.0
Goniothalamus macranii	8.5
Mezzetia umbellata	9.0
Disepalum anomalum	15.0

[d] IC = inhibition concentration

5.3.5 Crude methanol soluble fractions of the ethanol bark extracts of the genera *Goniothalamus*, *Mezzetia* and *Disepalum*

The following table summarises the results of the bioassay testings on the methanol soluble fractions of the crude ethanol extracts.

Table 5. 4: Larvicidal (*Aedes aegypti*) activity of methanol soluble fractions of crude ethanol extracts

Plant	LC_{50} (μg/ml)[a] (95% C.L.)[b]	LC_{90} (μg/ml)[a] (95% C.L.)[b]	Slope ± S.E.[c]
Goniothalamus andersonii	56.1 (52.4 - 60.0)	111.9 (99.5 - 131.6)	4.27 ±0.39
Goniothalamus dolichocarpus	81.0 (73.8 - 91.9)	198.6 (151.9 - 326.9)	3.28±0.52
Goniothalamus velutinus	3.8 (3.3 - 4.2)	9.5 (8.2 - 11.6)	3.19±0.31
Goniothalamus malayanus	7.1 (5.3 - 8.8)	25.4 (18.6 - 43.3)	2.30±0.37
Goniothalamus macrophyllus	78.2 (70.3 - 87.5)	163.7 (136.2 - 219.6)	3.99±0.52
Goniothalamus ridleyi	54.4 (43.5 - 68.3)	256.1 (169.1 - 539.4)	1.91±0.30
Goniothalamus gigantifolius	200.6 (158.5 - 375.0)	646.3 (355.3 - 4472.3)	2.52±0.69
Goniothalamus sinclairinius	160.1 (137.9 - 180.4)	297.2 (247.7 - 433.3)	4.77±0.95
Goniothalamus montanus	inactive	-	-
Mezzetia umbellata	9.2 (8.3 - 10.7)	18.0 (14.2 - 28.3)	4.43±0.77
Disepalum anomalum	5.3 (3.8 - 7.3)	33.7 (16.4 - 76.2)	1.59±0.29
*Azadirachta indica**	1.2 - 18.0	NA	NA
*Asimina triloba***	20.0	NA	NA
*Dipterocarpus kerrii****	146.1 (139.7 - 152.3)	NA	NA
*Litsea elliptica****	16.0 (14.3 - 18.0)	NA	NA
*Piper aduncum****	23.4 (21.1 - 25.8)	NA	NA
*Polygonum minus****	47.9 (43.2 - 52.8)	NA	NA
*Cinnamomum iners****	62.9 (59.4 - 66.8)	NA	NA
*Cinnamomum zeylanicum****	87.5 (77.9 - 97.8)	NA	NA
Stemona tuberosa Lour.[+]	197.51 (highly variable)	1408.16 (highly variable)	NA

* Zebitz (1986), **Mikolajczak *et al.* (1988), ***Jantan *et al.* (1995), [+]Lee and Chiang (1994), [a] LC = lethal concentration, [b] 95% CL = confidence interval at 95% confidence level, [c] SE = standard error, N.A. = not available.

5.3.5.1 *Goniothalamus andersonii*

The larvae of the *Aedes aegypti* were susceptible to the methanol soluble fraction of the ethanol bark extract of *Goniothalamus andersonii*. The testing gave an LC_{50} value of 56.1 μg/ml and an LC_{90} value of 111.9 μg/ml.

5.3.5.2 *Goniothalamus dolichocarpus*

The methanol soluble fraction of the bark extract of *Goniothalamus dolichocarpus* was cytotoxic to the mosquito larvae. An LC_{50} value of 81.0 μg/ml which is about half that obtained for the ethanol extract indicated the methanol fraction to be more cyctotoxic. Moreover, the dosage required for a 90% mortality had dropped from 548.9 μg/ml to 198.6 μg/ml in moving from ethanol extract to methanol soluble extract. Also the methanol fraction of *G. dolichocarpus* is 1.5 times less cytotoxic than the methanol fraction of *G. andersonii* compared to a two and half times for the ethanol extracts.

5.3.5.3 *Goniothalamus velutinus*

The methanol soluble fraction of the bark extract of *Goniothalamus velutinus* showed very strong cytotoxicity to the mosquito larvae with an extremely low LC_{50} value of 3.8 μg/ml. This value is about one third that for the ethanol extract of the same plant implying that the methanol fraction is very much more cytotoxic. This fraction is now about fifteen times more cytotoxic than that for *G. andersonii* compared to a five fold cytotoxicity in the ethanol extracts. The LC_{90} value is also very low with a value of 9.5 μg/ml compared to 22.5 μg/ml for the ethanol extract. Overall, the methanol fraction for *G. velutinus* is about twenty one times more cytotoxic than the extract of *G. dolichocarpus*.

5.3.5.4 *Goniothalamus malayanus*

The *Aedes aegypti* mosquito larvae were again very susceptible to the methanol fraction of the *Goniothalamus malayanus*. The LC_{50} value (7.1 μg/ml) was very much lower than that for the ethanol extract (LC_{50} 33.9 μg/ml). This is about a five times drop in LC_{50} value. The LC_{90} value had also dropped from 110.9 μg/ml (EtOH extract) to 25.4 μg/ml which is about a four-fold drop. This methanol fraction is about eight and twelve times more cytotoxic than the extracts of *G. andersonii* and *G. dolichocarpus*, respectively. However, the extract of *G. velutinus* is approximately twice more cytotoxic than the extract of *G. malayanus*.

5.3.5.5 *Goniothalamus macrophyllus*

The LC_{50} value of *Goniothalamus macrophyllus* had dropped from 100.3 μg/ml to 78.2 μg/ml in moving from the ethanol extract to the methanol soluble fractions. The LC_{50} value of this extract is about ten-fold that for *G. malayanus* (LC_{50} 7.1 μg/ml), almost twenty-fold that for *G. velutinus* (LC_{50} 3.8 μg/ml) and about the same range as that for *G. dolichocarpus* (LC_{50} 81.0 μg/ml). The LC_{90} value had also decreased from 209.7 μg/ml to 163.7 μg/ml.

5.3.5.6 *Goniothalamus ridleyi*

The cytotoxicity of *Goniothalamus ridleyi* had improved upon partitioning of the ethanol extract. The LC_{50} value had decreased from 132.2 μg/ml to 54.4 μg/ml which falls within the range of the LC_{50} value for *G. andersonii*. This extract is now about one and a half times more cytotoxic than the extract of *G. dolichocarpus* (LC_{50}

81.0 μg/ml). However, this extract is fourteen times and seven times less cytotoxic than the extracts of *G. velutinus* and *G. malayanus*, respectively.

5.3.5.7 *Goniothalamus gigantifolius*

The cytotoxicity of *Goniothalamus gigantifolius* had not improved even after partitioning into methanol. The LC_{50} value of 160.1 μg/ml was not very different from that of the ethanol extract (LC_{50} 208.7 μg/ml). The LC_{50} value of this extract was three and a half times that of *G. andersonii* and almost four times that of *G. ridleyi*.

5.3.5.8 *Goniothalamus sinclairinius*

The methanol extract of *Goniothalamus sinclairinius* was cytotoxic to the mosquito larvae. An LC_{50} value of 160.1 μg/ml was obtained. This value is two-fold that of *G. dolichocarpus* and three-fold that of *G. andersonii* and *G. ridleyi*.

5.3.5.9 *Goniothalamus montanus*

The methanol fraction of *Goniothalamus montanus* was not cytotoxic to the *Aedes aegypti* mosquito larvae.

5.3.5.10 *Mezzetia umbellata*

The methanol fraction of *Mezzetia umbellata* was very cytotoxic to the mosquito larvae. However, the LC_{50} value (9.2 μg/ml) had increased compared to that of the ethanol extract (LC_{50} 5.5 μg/ml). A higher dosage of 18.0 μg/ml was required to obtain a 90% mortality. The LC_{90} value for the ethanol extract was 10.7 μg/ml. The LC_{50} value for this methanol extract is six times less than that of *Goniothalamus andersonii* and *G. ridleyi*, nine times less than that for *G.*

dolichocarpus and eight and a half times less than that of *G. macrophyllus*. However, the extract of the *Mezzetia umbellata* is three times less cytotoxic than that of *G. velutinus*. For details of probit analysis, see Appendix 3.

4.3.5.11 *Disepalum anomalum*

The methanol extract of *Disepalum anomalum* was extremely cytotoxic with an LC_{50} value of 5.3 μg/ml. This value is approximately four times less than that of the ethanol extract (LC_{50} 19.6 μg/ml). The LC_{90} value also had decreased three-fold from 89.7 μg/ml to 33.7 μg/ml. This extract is ten times more cytotoxic than that of *Goniothalamus andersonii* and *G. ridleyi*.

5.3.6 Conclusions

Most of the methanol soluble fractions of the bark extracts tested were cytotoxic to the mosquito larvae and had LC_{50} values < 100 μg/ml. Among the extracts tested, those of *Disepalum anomalum, Mezzetia umbellata, Goniothalamus velutinus, G. malayanus, G. andersonii, G. ridleyi, G. dolichocarpus* and *G. macrophyllus* had LC_{50} values below 100 μg/ml. Most of these extracts showed a decrease in LC_{50} values compared to their ethanol extracts except for *Mezzetia umbellata* which had an increase in its LC_{50} value and *G. andersonii* whose LC_{50} value remain the same. The most cytotoxic extracts are from the *G. velutinus, D. anomalum, G. malayanus* and *M. umbellata* with LC_{50} values of 3.8 μg/ml, 5.3 μg/ml, 7.0 μg/ml and 9.2 μg/ml, respectively. These LC_{50} values are comparable with that of the *Azadiachta indica* (Zebitz, 1986) and *Asimina triloba* (Mikolajczak *et al.*, 1988) which both had LC_{50} values below and equal to 20 μg/ml. Next in line of natural larvicides are *Goniothalamus andersonii, G. ridleyi, G. macrophyllus* and *G.*

dolichocarpus which had LC_{50} values below 100 μg/ml but > 50 μg/ml. These extracts are comparable to those of *Cinnamomum iners* and *C. zeylancium* (Jantan *et al.*, 1995). The strongly cytotoxic behaviour of the bark extracts of *Mezzetia umbellata, G. velutinus, G. malayanus* and *Disepalum anomalum* is probably contributed by the presence of the very cytotoxic annonaceous acetogenins.

As for the previous two sets of extracts, the LPRL of these extracts all had slopes of more than 1.0. The four most toxic extracts, *Goniothalamus velutinus, G. malayanus, Mezzetia umbellata* and *Disepalum anomalum* had slopes ranging from 1.59 to 4.43. *Disepalum anomalum* and *G. ridleyi* have the smallest slope while *Mezzetia umbellata* has the highest slope. This means that *Aedes aegypti* is very much more sensitive to changes in concentrations of *Mezzetia umbellata* than of *G. ridleyi.*

Preliminary *in vitro* cytotoxicity screening against P388 cell lines for the above plant extracts indicated some bioactivity. The results are as shown in the table below. Among the species screened for *in vitro* cytotoxicity activity, the extract of *Goniothalamus macrophyllus* is the most bioactive with an IC_{50} value of 6.0 μg/ml. Equally as bioactive against the P388 cell lines are bark extracts of *G. andersonii* and *G. ridleyi* with IC_{50} values of 7.0 and 7.5 μg/ml respectively. The bark extract of *G. macrophyllus* was also strongly cytotoxic against the larvae of the *Aedes aegypti.* However, it was not the most cytotoxic. The bark extract of *G. velutinus* which was the most cytotoxic against the larvae of the *Aedes aegypti* is not however, the most bioactive against P388. *G. velutinus* has an IC_{50} value of 9.0 μg/ml. The least bioactive is the extract of *G. dolichocarpus* with an IC_{50} value of 13.0 μg/ml. *G. malayanus*, *G. sinclairinius*, *Mezzetia umbellata* and *Disepalum anomalum* all have similar IC_{50} values of 9.5 μg/ml.

Table 5. 5: *In vitro* cytotoxicity activity of methanol soluble fractions

Plant	IC_{50}(μg/ml)[d]
Goniothalamus dolichocarpus	13.0
Goniothalamus velutinus	9.0
Goniothalamus malayanus	9.5
Goniothalamus ridleyi	7.5
Goniothalamus gigantifolius	15.0
Goniothalamus macrophyllus	6.0
Goniothalamus andersonii	7.0
Goniothalamus sinclairinius	9.5
Mezzetia umbellata	9.5
Disepalum anomalum	9.5

[d] IC = inhibition concentration

5.4 Larvicidal activity of some compounds isolated from the various plant species studied

McLaughlin and co-workers (1991) have reported on the various biological activities of the styrylpyrones and annonaceous acetogenins. However, no reports on their larvicidal activity have been made.

Various styrylpyrones, two flavonoids, two alkaloids and two acetogenins were tested on the larvae of the *Aedes aegypti*. The table that follows summarises the LC_{50} values obtained from these testings.

Table 5. 6: Larvicidal (*Aedes aegypti*) activity of compounds

Compounds	LC_{50} (µg/ml)[a] (95% C.L.)[b]	LC_{90} (µg/ml)[a] (95% C.L.)	Slope ± S.E.[c]
Goniothalamin	15.0 (12.0 - 18.0)	57.7 (44.5 - 85.1)	2.19±0.27
Goniothalamin epoxide	50 - 100	-	-
Isogoniothalamin epoxide	150 - 200	-	-
(-)-Iso-5-deoxygoniopypyrone	15 - 20	-	-
(+)-5β-Hydroxygoniothalamin	100 -150	-	-
Goniodiol diacetate	50 - 100	-	-
Naringenin	3.7 (3.1 - 4.5)	15.1 (11.0 - 24.5)	2.10±0.24
Pinocembrin	33.0 (25.4 - 41.2)	98.4 (66.4 - 335.4)	2.71±0.12
Annonacin	9.5 (7.5 - 12.2)	22.3 (16.4 - 41.0)	3.48±0.69
Ouregidione	10-25	-	-
Goniothalenol	100 - 150	-	-
Aristolactam BII	50 - 100	-	-
Disepalin	27.4 (22.3 - 34.5)	82.0 (58.3 - 152.1)	2.70±0.45
Pyrethrum*	10.0**	N.A.	N.A.
Rotenone (97% pure)*	10.0	N.A.	N.A.
Azadirachtin	1.5 - 2.0	N.A.	N.A
Termephos (synthetic)	0.005 (0.004-0.006)	0.009 (0.008 - 0.01)	5.89±0.81

*Mikolajczak *et al.* (1988), ** At this concentration 100% died, [a] LC = lethal concentration, [b] 95% CL = confidence interval at 95% confidence level, [c] SE = standard error

5.5 Conclusions

The larvae of the *Aedes aegypti* were susceptible to most of the pure compounds tested; the most cytotoxic compound being naringenin with an LC_{50} value of 3.7 µg/ml. The next cytotoxic compound is annonacin with an LC_{50} value of 9.5 µg/ml (Goh *et al.*, 1994) (see Appendix 4 for details of probit analysis results). These LC_{50} values are comparable to that of rotenone (LC_{50} = 10.0 µg/ml). The new natural product (-)-iso-5-deoxygoniopypyrone is cytotoxic to the larvae of the *Aedes aegypti* as well, with an LC_{50} value ranging from 15-20 μg/ml. No exact LC_{50} value is available because of shortage of natural product to perform a complete range of concentration in the experiment. Goniothalamin is very bioactive to the larvae as well and gives an LC_{50} value of 15.0 μg/ml. This compound probably contributes to the very bioactive nature of some of the hexane extracts like *Goniothalamus andersonii*, *G. macrophyllus*, *G. ridleyi* and *G. uvarioides* which contain goniothalamin. Other styrylpyrone derivatives such as goniothalamin epoxide, goniodiol diacetate, 5β-hydroxygoniothalamin, goniothalenol and isogoniothalamin epoxide are moderately cytotoxic with LC_{50} values ranging from 50-100 μg/ml, 100-150 μg/ml and 150-200 μg/ml, respectively. Again, shortage of the natural products hindered the experiment to be carried out to the complete range of concentrations. The other new natural product 5β-hydroxygoniothalamin is not very cytotoxic to the mosquito larvae of the *Aedes aegypti*. The LC_{50} value is between 100 - 150 μg/ml. The dioxoaporphine, ouregidione present in *G. velutinus* and *G. malayanus* is also cytotoxic with an LC_{50} value of 20.3 μg/ml. However, the phenanthrene lactam Aristolactam BII is less cytotoxic and gives an LC_{50} value ranging from 50-100 μg/ml.

Disepalin isolated from *Disepalum anomalum* is also cytotoxic with an LC_{50} value of 27.8 μg/ml. This LC_{50} value is higher than those of the crude ethanol and methanol extracts. This is because, the crude extracts probably contain other bioactive components apart from disepalin. This acetogenin contains an acetate group hence is less bioactive than annonacin (LC_{50} 9.5 μg/ml) which has four hydroxyls.

So far only two natural products (naringenin and annonacin) from the list have LC_{50} values comparable to those of rotenone and pyrethrum but not to azadirachtin (LC_{50} = 1.5 - 2.0 μg/ml).

The preliminary *in vitro* cytotoxicity screening against P388 cell lines of the pure compounds indicated some bioactivity.