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STUDIES ON MOSQUITO LARVICIDAL ACTIVITY OF *Chloroxylon swietenia* DC.

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Abstract- The laboratory bioassay of the essential oil and the isolated compounds from *Chloroxylon swietenia* against *Aedes aegypti* and *Anopheles stephensi* was carried out to evaluate the Larvicidal activity of leaf extract and the isolated coumarins, xylotenin and heliettin. All the bioassays were run with different concentrations ranging from 1-200 μg/ml and in quintuplicate. LC_{50} value estimated for *Aedes aegypti* and *Anopheles stephensi* were 55.1 & 49.3 μg/ml and 74.5 & 67.5 μg/ml for xylotenin and heliettin respectively. The results obtained are remarkable and the present study thus indicates that the extracts and isolated compounds of *C. swietenia* have potent larvicidal properties and compared favorably with the commercially available insecticide Malathion as a positive control which can be a promising larvicidal agent as an alternative to the synthetic compounds.
Keywords- Mosquito larvicidal activity, Heliettin, Xylotenin, *Chloroxylon swietenia*.

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Introduction

Mosquitoes, are vectors of several diseases like malaria, filariasis, encephalitis, dengue fever, yellow fever etc. which alone transmit diseases to more than 700 million persons annually [14]. These diseases are one of the major health problems in many countries. The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further the indiscriminate use of synthetic compounds for mosquito control resulted in multifarious problems like environmental pollution, insecticide resistance, disruption of the natural biological control system and toxic hazards to humans. Globally there has been a conscientious effort by scientists to overcome these problems and great emphasis has been placed recently on green chemistry for mosquito control using natural plant products. It is well known that natural products derived from plants are extensively used as biologically active compounds particularly in the area of infectious diseases [4]. Several studies have focussed on the plant products as effective insecticides and larvicides for controlling different species of mosquitoes [3,7,10]. Botanical pesticides and insecticides have long been used in the traditional Indian communities as they are effective, safe, natural and promising biodegradable compounds.

Chloroxylon swietenia DC. is a tropical aromatic tree of dry deciduous forests belongs to the family Rutaceae. It is popularly known as East Indian Satin Wood. The tree has immense applications for therapeutic use, where it is a common sight in the tribal areas to hang the leaf garlands in their houses to eradicate mosquitoes and insects. As a part of screening program of botanicals that grow in south India, we set to isolate two coumarins, Xylotenin and Heliettin to evaluate their efficacy on the mosquito larvae and therefore, we here in present the results of our investigative study.

Experimental

Plant Material

Chloroxylon swietenia DC. (*C. swietenia*) leaves were collected from the forests of Kinnerasani region, Andhra Pradesh, India and a voucher specimen has been deposited in the Department of Botany, Osmania University (OU BOT 4784).

Extraction and Isolation

The leaves of *C. swietenia* were air dried, powdered and extracted with cold acetone. The extract was concentrated using rotary evaporatory and re-extracted successively with hexane, ether and chloroform. The insoluble materials like fats, waxes and terpenes were separated by filtration. The hexane extract (20g) and Chloroform extract (20g) were chromatographed over silica gel and fractionated with hexane : ethyl acetate, benzene : acetone mixture and chloroform : MeOH respectively in a usual way. A total of 150 fractions (each 100 ml) were collected.

Mosquito larvicidal activity

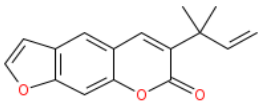
Third instar larvae of *Aedes aegypti* and *Anopheles stephensi* (obtained from Toxicology Division, Vimta Laboratories, Hyderabad, India). All bioassays were performed according to the standard WHO larval susceptibility test method [15]. Third instar larvae were exposed to the crude extract and isolated compounds at different concentrations ranging from 5-200 μg/ml. Control tests were carried out in parallel using DMSO and water for comparison. Malathion obtained from Hindustan Insecticides Ltd, New Delhi, India was used as a positive control. Mortality rate was recorded after 24 hr of exposing and the percentage of mortality was corrected if necessary using the formula [1]. Probit analysis [11] was conducted to determine the LC_{50} and LC_{90} representing the concentrations in μg/ml that caused 50% and 90% mortality along with 95% confidence limits. Subsequently studies were also conducted on the behavior of the larvae.

Results and Discussion

The extracts of *C. swietenia* were investigated and this resulted in three known compounds which were isolated as per the literature. The chloroform extract (20g) was chromatographed over silica gel and fractionated with chloroform and MeOH as eluent. It yielded the compound heliettin. The fractions collected were subjected to TLC, NMR and Mass spectroscopic studies which gave the compounds Xylotenin and isopimpienllin respectively.

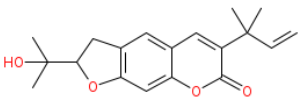
Xylotenin

5. 150 (1, 1H, dd, J=10. 920, J=1. 356), 5. 149 (1, 1H, dd, J=16. 706, J=1. 356), 5. 714 (2, 1H, dd, J=16. 706, J=10. 920), 1. 346 (4, 3H), 1. 346 (5, 3H), 7. 615 (7, 1H), 8. 110 (9, 1H, ddd, J=2. 076, J=1. 711, J=0. 470), 6. 471 (11, 1H, ddd, J=3. 120, J=1. 753, J=1. 711), 7. 825 (12, 1H, ddd, J=3. 031, J=2. 076, J=1. 753), 7. 399 (15, 1H, ddd, J=3. 120, J=3. 031, J=0. 470)



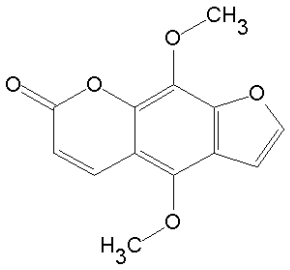
Heliettin

5. 149 (1, 1H, dd, J=10. 920, J=0. 000), 5. 148 (1, 1H, dd, J=16. 702, J=0. 000), 5. 712 (2, 1H, dd, J=16. 702, J=10. 920), 1. 345 (4, 3H), 1. 345 (5, 3H), 7. 589 (7, 1H), 7. 425 (9, 1H, d, J=0. 457), 3. 096 (11, 1H, dd, J=15. 527, J=8. 040), 3. 208 (11, 1H, dd, J=15. 527, J=6. 750), 4. 581 (12, 1H, dd, J=8. 040, J=6. 750), 6. 576 (15, 1H, d, J=0. 457), 1. 207 (21, 3H), 1. 207 (22, 3H)



Isopimpinellin

6. 873 (5, 1H, d, J=1. 754), 7. 474 (6, 1H, d, J=1. 754), 8. 238 (10, 1H, d, J=9. 534), 6. 434 (11, 1H, d, J=9. 534), 3. 788 (16, 3H), 3. 679 (18, 3H)



Laboratory bioassay tests were carried out using the crude extracts and the isolated coumarins against two mosquito vector species. The LC_{50} and LC_{90} values estimated for *A. aegypti* and *An. stephensi* for crude extract, xylotenin and heliettin are presented (Table-1 & 2).

Table 1- Larvicidal activity of leaf extract and coumarins from Chloroxylon swietenia against against Aedes aegypti

Compound	LC_{50} (μg/ml)	95%cl	LC_{90} (μg/ml)	95%cl	Relative[a] Potency
Crude extract	55.1	43.519-66.681	98.4	84.023-112.824	0.243
Xylotenin	74.5	62.689-86.351	131.6	113 750-149.494	0.180
Heliettin	92.0	79.557- 104.561	179.4	159 550-199.652	0.145
Isopimpinellin	100.4	86.442-109.675	192.8	175 264-203.814	0.133
Malathion	13.42	10.435-15.045	18.10	13.193-21.047	1

[a]*Relative potency - LC_{50} standard / LC_{50} test substance*

All the compounds tested demonstrated significant larvicidal effect on both vector species (LC_{50} < 100 μg/ml). Among the three coumarins tested, xylotenin was more active (LC_{50}-74.5 and 67.5 μg/ml for *A. aegypti* and *An. stephensi*) followed by heliettin (LC_{50} - 92.0 and 78.9 μg/ml) and isopimpinellin (LC_{50}-100.4 and 90.7 μg/ml). The tests revealed that at concentrations of 112.3 and 102.5 μg/ml of crude extract could induce 100% mortality in the larvae of *A. aegypti* and *An. stephensi* respectively, while with xylotenin and heliettin 100% mortality is observed at concentrations of 147.6 & 124.9 μg/ml and 191.7 & 168.8 μg/ml respectively for both the vector species. On the other hand, 6. 81 μg/ml and 4.53 μg/ml concentration of Malathion caused 100% mortality of *A. aegypti* and *An. stephensi* respectively.

Table 2- Larvicidal activity of leaf extract and coumarins from Chloroxylon swietenia against Anopheles stephensi

Compound	LC_{50} (μg/ml)	95%cl	LC_{90} (μg/ml)	95%cl	Relative[a] Potency
Crude extract	49.3	33.673-64.927	93.1	75.822-110.381	0.247
Xylotenin	67.5	50.283-84.717	112.2	98.579-125.942	0.181
Heliettin	78.9	64.517-93.283	161.6	144.612-178.591	0.154
Isopimpinellin	90.7	78.317-101.564	189.9	174.283-200.872	0.134
Malathion	12.19	9.352-15.012	16.2	12.993-19.407	1

aRelative potency - LC50 standard / LC50 test substance

Results on percentage mortality of larvae of *A. aegypti* and *An. stephensi* with increase in concentration of crude extract and the

isolated compounds are shown in fig. 1 and 2.

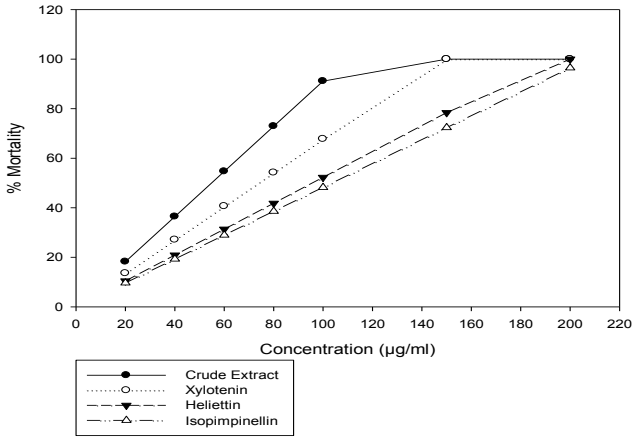


Fig. 1- Percentage mortality of *Aedes aegypti* at different concentrations of crude extract and isolated compounds from leaves of *Chloroxylon swietenia*

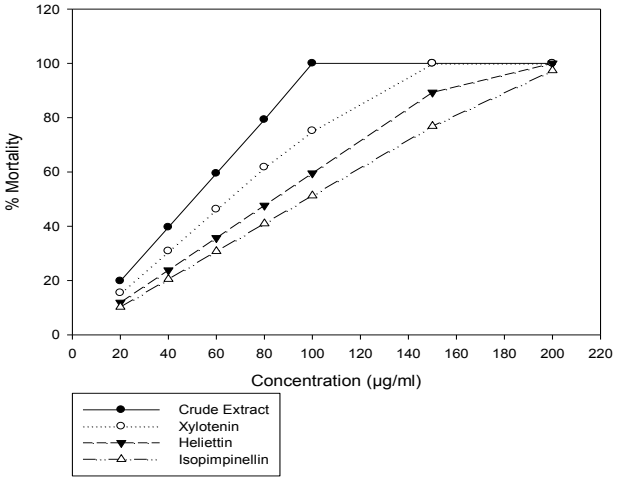


Fig. 2- Percentage mortality of *Anopheles stephensi* at different concentrations of crude extract and isolated compounds from leaves of *C. swietenia*

As there is increase in the concentration of extracts and isolated compounds there is subtle increase in the percentage mortality. At higher concentrations (>200 µg/ml) the larvae were incapacitated and settled at the bottom of beaker with abnormal wagging and died slowly while no mortality is observed with control. However, the larvae did not developed into adults even at low concentrations of extracts and compounds. During the experiments several larvae displayed morphological deformities which impeded their development. The efficacy of *C. swietenia* extracts and coumarins as potent mosquito repellent and larvicidal activity is comparable to earlier results.

Previous studies involving the Larvicidal activity of extracts of various medicinal and aromatic plants, the LC_{50} values ranging from 14 µg/ml to 500 µg/ml against the various mosquito larvae [2,5,13]. However, the present study revealed that the extracts and coumarins from the leaves of *C. swietenia* could induce 50% mortality in the larvae of *An. stephensi* at a low concentration of 25-60 µg/ml which is 1. 2 to 8 times lower than those of the plants studied earlier. The activity of *C. swietenia* extracts can be attributed to the presence of larger amounts of coumarins and this is in agreement with the published reports [8,9,16].

Conclusions

The present study thus indicates that the leaf extract and the compounds of *C. swietenia* have remarkable larvicidal properties and compared favorably with the commercially available insecticide Malathion. Further studies are underway on the synergistic combination of compounds and the potential use of the plant as larvicides against mosquitoes in various control programmes.

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