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Nano-Phytochemicals from the Leaves of *Plumbago Zeylanica* for Mosquito Control

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Abstract: Mosquito bites transmit several diseases like malaria, filaria, dengue, encephalitis and yellow fever that afflict humans. At present there were many remedies for mosquito menace such as coils, repellent creams, liquidator etc. But majority of them were chemical-based and lead to many harmful side-effects on human health, especially respiratory problems such as asthma, allergies etc. In our studies we have aimed to control the population of mosquitoes by destruction of mosquito larvae at the place of their generation by using nano-size phytochemicals. Since it is herbal-based there were no side effects and the raw materials were cheaper than conventional chemicals used for mosquito control. The leaves of *Plumbago zeylanica* (Doctor Bush) were extracted in Soxhlet apparatus using methanol as solvent. The obtained phytochemical extracts were analysed for the presence of functional groups using FTIR analysis and it was confirmed as alkanes. The extracts were converted into solid form by addition of inert filler and then reduced to nano-size using planetary ball mill and confirmed using particle size analyzer. The mortality test for mosquito larvae was done at Zonal Entomological Research Centre, Vellore. From the mortality tests, it was found that *Plumbago zeylanica* gives 76% mortality when 1gm per 250 ml was used in 24 hour.
Keywords: Mosquito, mortality, doctor bush, nanopowder.

Introduction

In the history of the world, more people would have died from diseases transmitted by mosquitoes than from all the fighting in the wars. The world's most dangerous creature is in fact the mosquito. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one". Several mosquito species belonging to genera *Anopheles, Culex,* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever, chikungunya, etc.[1].

Extensive use of chemical insecticides against vector mosquitoes for the control of mosquito borne diseases has caused development of resistance in mosquitoes to these insecticides and hazards to the environment. In spite of the sustained and prolonged use of chemical insecticides, these diseases are not only still prevalent but also outbreak into epidemics[2]. The introduction of new and more toxic mosquito repellents into the environment may become potential hazards to human health. Mosquitoes in the larval stage are attractive targets for pesticides, in general, because they breed in water and, thus, are easy to deal with them in the aquatic habitat[3]. Many of the chemical larvicides are extremely toxic to non-target organisms such as fishes. Thus one realizes the need for a safe and eco-friendly mosquito control agent based on natural herbs. Therefore, to minimize the dependency on chemical insecticides, efforts have been made worldwide for the search and development of alternative methods for the control of vector mosquitoes[2]. In this respect various biological control agents are under investigation worldwide.

The plant world comprises a rich storehouse of a variety of phytochemicals that could be tapped for use as mosquito larvicides. The leaf of repellent plant is one of the commonly and extensively used plant parts to repel the insects and mosquitoes, followed by root, flower and remaining parts of repellent plants[4]. One such plant treasure for mosquito control that was identified for this study is *Plumbago zeylanica* leaves.

In India *Plumbago zeylanica* grows in different parts as wild species but it is also cultivated due to its wide therapeutic applications. It is used in indigenous system of medicine, and commonly known as "Doctor bush" in English. The plant is an evergreen small perennial shrub which grows to a height of about 3-4 feet. The leaves are simple, alternate, oblong, spirally arranged, hairy margin, thick, and flashy, 4-10 cm long, pointed at the tip[5]. Plant extracts of *Plumbago zeylanica* have shown potent mosquito larvicidal activity against the larvae of *Aedes aegypti* while showing no toxicity to fish[6]. The different parts of the *Plumbago zeylanica* plant have been studied for various medicinal properties[7]. The napthoquinone, plumbagin was isolated from the plant reported having chitin synthetase inhibiting activity in organisms[8]. This suggests that the phytochemical constituents present in the plant extract may arrest the metabolic activities of the mosquito larvae.A study by Patel *et al.,* reflects the larvicidal potency of crude extracts obtained from *Plumbago zeylanica* against *Aedes aegypti*, *Anopheles stephensi* larvae which is the basic and most important step in the development of an insecticide of botanical source. There are probabilities that the active principle contained in these plant extracts, especially the methanol extracted fractions will be further more potent as mosquito larvicides as compared with their crude forms. The identification and isolation of these active components is a part of further search for an efficient, eco-friendly, biodegradable insecticide of plant origin[9].

In this work, the methanolic extract of *Plumbago zeylanica* leaves had been taken for study. They were converted as nano-phytochemical powder and used for mortality testing on mosquito larvae belonging to species *Culex pipiens* and the results were found to be fruitful as detailed in the following sections.

Experimental

Plant material collection and preparation:

The leaves of the plant *Plumbago zeylanica* were collected from Vandavasi and Ramaapuram in Kancheepuram District, Tamilnadu, India in January 2014. The leaves were shade dried (27±2°C) for about three days. The dried leaves were ground using a commercial stainless steel electrical blender. The ground material was sieved to get a fine powder. About 100 g of the leaf powder was packed in a thin cotton cloth and loaded in a Soxhlet apparatus. It was extracted using 250 ml of methanol in the apparatus until exhaustion. The extract was concentrated in a rotary vacuum evaporator and residues obtained were stored at 4°C. The obtained phytochemical extracts were analyzed for the presence of functional groups using FTIR analysis. An inert filler material was added to the concentrated and cooled extract to aid solidification of the extract. Initially slurry was obtained which when spread in thin layers and tray-dried yields a solid cake. The cake was crushed to powder form using a blender followed by further size reduction in an ordinary ball mill. The powder obtained from ball mill was sieved and reduced to nano-size in a planetary ball mill and confirmed using particle size analyzer. The final product was packed and stored in an airtight container to avoid any possible agglomeration of the particles.

Test organisms rearing and bioassay:

For the laboratory trial, larvae of *Culex pipiens* (common household mosquito in India) were collected from the stagnant water ponds in and around Vellore, Tamilnadu, India. The mosquito species were identified by an entomologist at the Zonal Entomological Research Centre, Vellore. The collected larvae were reared in plastic trays containing dechlorinated tap water. Larvae were fed with a diet of finely ground yeast powder and dog biscuits (3:1 ratio). All the experiments were carried out at 28+2°C and 75-85% relative humidity under 14:10 light and dark cycles. Four numbers of 500 ml glass beakers were taken, each containing 250 ml of dechlorinated tap water having different concentrations of *Plumbago zeylanica* nanopowder viz., 0 g, 0.1 g, 0.5 g and 1 g respectively. Twenty five numbers of fourth-instar larvae of C*ulex pipiens* were counted and placed in each one of the four test beakers. The beaker with no powder added to it was termed the control beaker and the control larvae were thus reared in that beaker containing only water. Exposure time was 24 hours for all the treatments after which suspensions, and control water were replaced with fresh water. The effects of *Plumbago zeylanica* nanopowder were assessed by counting the number of dead larvae at an interval of every two hours for 24 hours.

Results and Discussion

FTIR Analysis:

The functional groups present in the extract were obtained from FTIR analysis and interpreted using the IR chart. The FTIR analysis for *Plumbago zeylanica* is as shown in figure 1.

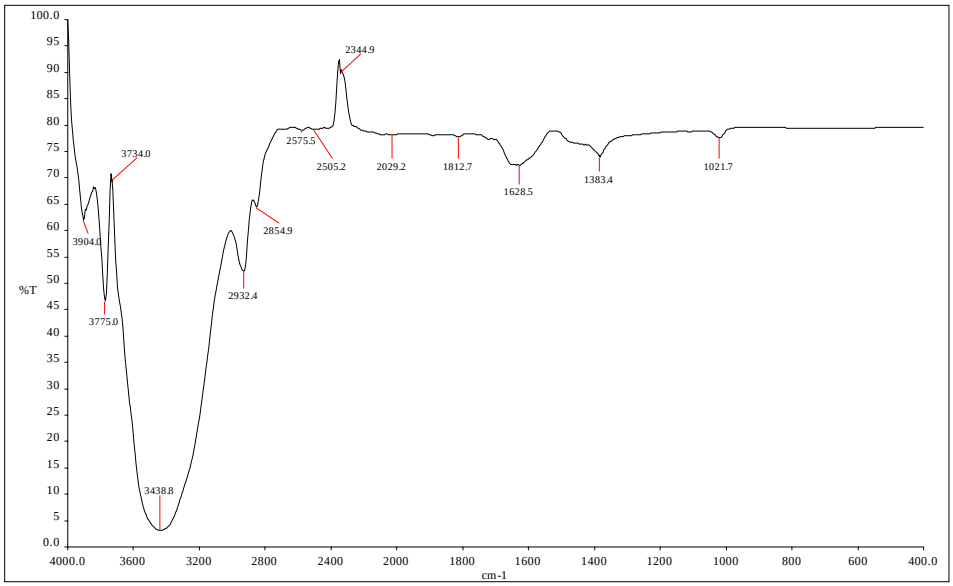

Figure 1. FTIR analysis of *Plumbago zeylanica* extract

The functional groups present in *Plumbago zeylanica* extract were interpreted from the graph which is listed in table 1.

Table 1.Intepretation of FTIR analysis for *Plumbago zeylanica*

S.No.	Time (Hours)	No. of larvae dead in each beaker (out of a total of 25 taken in each beaker for analysis)			
		T_0 0g/250 ml	T_1 0.1g/250 ml	T_2 0.5g/250 ml	T_3 1g/250 ml
1	2	0	0	0	0
2	4	0	0	0	1
3	6	0	0	0	2
4	8	0	0	1	4
5	10	0	0	2	6
6	12	0	1	3	9
7	14	0	1	4	11
8	16	0	1	5	13
9	18	0	2	7	15
10	20	0	2	8	16
11	22	0	2	9	18
12	24	1	3	10	19

Thus the presence of alkane functional group is confirmed from table 1 for *Plumbago zeylanica* extract.

Mortality test results:

The control test beaker was denoted as T_0. The other three test beakers containing concentrations of 0.1g, 0.5g and 1g of *Plumbago zeylanica* nanopowder were designated as T_1, T_2 and T_3 respectively. The average mortality test results of five replicates using various concentrations of *Plumbago zeylanica* are given in table 2. The mortality percentage is calculated based on the above data and the trend is illustrated in figure 2.

Table 2. Mortality test results for *Plumbago zeylanica*

Frequency, cm^{-1}	Functional group
3438.8	Alcohols, Phenols (O–H stretch, H–bonded)
2932.4	Alkanes (C–H stretch)
1628.5	1° Amines (N–H bend)
1021.7	Aliphatic Amines (C–N stretch)

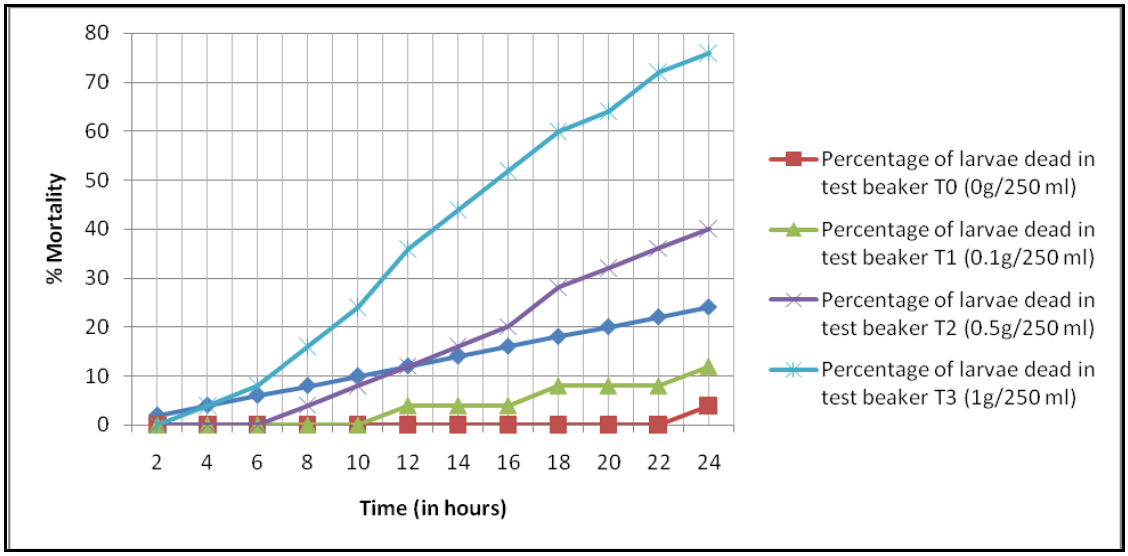

Figure 2. Mortality % of larvae in each test beaker Vs. Time (in hours)

Conclusion

This study briefly presents the mosquito larvicidal potential of the leaves of the plant *Plumbago zeylanica*. This is an attempt to highlight the need for research and development in biological larvicides. It is evident from the study that *Plumbago zeylanica* has great potential to be integrated into mosquito control programs. Future work on *Plumbago zeylanica* would offer a noticeable socioeconomic impact in turning a common plant species into a beneficial mosquito control product.

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