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STUDIES ON THE EFFECT OF PLANT AQUEOUS EXTRACTS ON THE CONTROL OF MOSQUITO VECTORS

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Abstract: In epidemiological terms, vectors are organisms that transmit infections from one host to another. Nearly half of the world's population is infected by vector-borne diseases every year, resulting in high morbidity and mortality. Among diverse types of vectors, mosquitoes are the principal vector of many vector borne diseases like Malaria, Dengue fever, Yellow fever, Filariasis, Schistosomiasis and Japanese encephalitis affecting human beings and animals, in addition to nuisance. Mosquitoes are predominant in all countries, where not much importance is given to sanitation and hygiene. It is estimated that worldwide over one million people die annually due to the diseases caused by this haematophagous vector. Since they are acting as primary vectors for various pathogens causing diverse types of fatal infections, their eradication is becoming a pre requisite for establishing proper health and hygiene. Vector control envisages various physical, chemical and biological means of controlling vector populations. Even chemical control measures are facing setbacks as these vectors are gaining resistance. Moreover, such chemicals have given rise to serious environmental issues. This has led to the search for bio pesticides, which are having several advantages over conventional chemical pesticides / insecticides. In light of the above, the present study has been outlined to assess the feasibility of using phytochemicals in the control of mosquito vectors belonging to five taxonomic groups. The objective of the study includes s screening of plants, preparation of aqueous extracts, assessment of the lethal effect of plant extracts on the survival of mosquito larvae and Confirmation of lethality. Fifteen species of plants were screened for this purpose. Aqueous extracts of selected plants were prepared and tested against mosquito larvae collected from breeding sites and reared from eggs under laboratory conditions. Different concentrations of the extracts were prepared (0.5, 1.0, 2.0, 4.0 and 8.0 mg/ ml) and were then applied to the treatment sets, each containing 20 larvae and were monitored for a period of 96hours. Mortality percentages and LC_{50} were calculated. Out of 15 plant species attempted, 10 could induce 100% mortality to mosquito larvae at varying concentrations and retention times. The LC_{50} estimate for the promising plants like *Zingiber officinalis* (rhizome), *Adenocalymma alliaceaum* (leaf), and *Saritea magnifra* (leaf) were noted to be 1.1, 3.6, and 1.2mg/ml respectively. In the present study, it has been noted that aqueous extracts of the rhizome of *Zingiber officinalis*, leaf of *Saritea magnifra* and leaf of *Adenocalymma alliaceum* have the potential to be used as effective insecticides thus signifying an ecofriendly approach for the control of the dreadful mosquito vectors. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed.

Key words: Aqueous extract, Larval mortality, Phytochemicals, Bioassay, LC_{50}.

INTRODUCTION

Among the various groups of invertebrate animals, insects have a very close relationship with life and existence of mankind. In the insect group, many insects of the Order Diptera act as vectors and play a role in spreading diseases among human beings. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases such as Malaria, Filariasis, Dengue, Japanese encephalitis, etc., causing millions of deaths every year (Arivoli *et al*., 2011). One of the strategies of the WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. The only efficacious approach in controlling mosquito vectors is by application of insecticides to larval habitats.

In the past few decades, synthetic insecticides were used as mosquito controlling agents, but have produced a negative feedback of environment ill effect, evidenced by non-target organisms being affected and most of the mosquito species becoming physiologically resistant (Lokesh *et al*., 2008). These factors have created a search for eco-friendly, biodegradable and target specific insecticides against mosquito vectors. In recent years, the emphasis to control mosquito population has shifted steadily from the use of conventional chemicals towards more specific and eco friendly materials, which are generally of botanical origin. Plant products have been used traditionally by human communities in many parts of the world against the vector and pest species of insects. The plant derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal actions against mosquitoes (Omena *et al*., 2007).

In recent times, there is a resurgence of interest in the utilization of plant extracts in controlling mosquito larvae, since a control over the larvae will terminate the life cycle and thus further multiplication. Many researchers have been oriented in this direction with different types of extracts from various plant categories. Since bio active compounds varies with plant types, the present study has been outlined to assess the feasibility of using phytochemicals in the control of mosquito vectors belonging to various taxonomic groups.

MATERIALS AND METHODS

The present study has been carried out to assess the larvicidal activity of aqueous extracts of plants belonging to varied families. The experimentation has been carried out in the following steps:

Plant collection and processing

The selection of plants were carried out based on their local availability and reported pharmacological and aromatic properties. The materials were collected from healthy plants, free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing.

Preparation of extracts

The washed plant materials were chopped properly and kept in clean trays. For the preparation of extracts, a known weight of plant was taken, ground in a homogenizer using distilled water. The extract was filtered and the filtrate was used as stock solution for further experimentation. Serial dilutions of the stock solutions were prepared for assessing treatment efficiencies.

Larval screening and experimentation

Mosquito larvae, collected from controlled breeding sites were used in the present study. Collected larvae were pooled in the laboratory and were subjected to species level identification using a microscope. For experimentation, 20 larvae, each were introduced into 500ml beaker containing 250 ml of natural growth media. To the treatment set, respective concentrations of the plant extracts (0.5, 1.0, 2.0, 4.0 and 8.0 ml) were added. A control was maintained, containing only larvae and natural growth media. Mortality counts of larvae were monitored at regular intervals i.e. 6, 12, 24, 48, 72 and 96 Hours after treatment. Larvae were considered dead if they settled and remained motionless at the bottom of the test beaker with no response to light or mechanical stimulus or not recovering life functions even after being transferred to a control water solution. Mortality percentages and LC_{50} were calculated using regression method.

RESULTS

The present study has been carried out to assess the lethal properties of 15 spp. of plants belonging to 5 families on mosquito larvae. Details of plants used for the present study, together with the rate of mortality of mosquito larvae are depicted in Table 1. The percentage mortality of mosquito larvae exposed to aqueous extracts of plants at 96 hours after treatment is given in Table 2.

Systematic analysis of the larvae using standard manual "The Fauna of British India" (Barraud, 1934) revealed that they are falling in one genus, *Aedes*. Significant differences were observed in the toxicity of the aqueous extracts of plants consisting of flower (3), leaf (11), rhizome (2), seed (3), leaf twig (2), and fruit (1) against mosquito

Table 1. List of plant species used for the preparation of aqueous extracts and their impact on Mosquito larvae

No.	Name of plant	Family	Part used	Larval mortality	Mosquito species
1a	*Adenocalymma alliaceaum*	Bignoniaceae	Leaf	+	Aedes
b	*Adenocalymma alliaceaum*	Bignoniaceae	Flower	+	Aedes
2	*Saritea magnifra*	Bignoniaceae	Leaf	+	Aedes
3a	*Calycopteris floribunda*	Combretaceae	Leaf	Nil	Aedes
b	*Calycopteris floribunda*	Combretaceae	Flower	Nil	Aedes
4	*Terminalia chebula*	Combretaceae	Dry seed	+	Aedes
5	*Terminallia bellarica*	Combretaceae	Dry seed	+	Aedes
6	*Eucalyptus globules*	Myrtaceae	Leaf	+	Aedes
7	*Pimenia diocca*	Myrtaceae	Leaf twig	+	Aedes
8	*Psidium gujava*	Myrtaceae	Leaf	Nil	Aedes
9a	*Syzygium cumini*	Myrtaceae	Leaf	Nil	Aedes
b	*Syzygium cumini*	Myrtaceae	Seed	+	Aedes
10a	*Datura stramonium*	Solanaceae	Fruit	+	Aedes
b	*Datura stramonium*	Solanaceae	Leaf	+	Aedes
11	*Physalis minima*	Solanaceae	Leaf	+	Aedes
12	*Solanum nigrum*	Solanaceae	Leaf twig	+	Aedes
13a	*Alpinia galangal*	Zingiberaceae	Leaf	+	Aedes
b	*Alpinia galangal*	Zingiberaceae	Flower	+	Aedes
14a	*Curcuma longa*	Zingiberaceae	Rhizome	+	Aedes
b	*Curcuma longa*	Zingiberaceae	Leaf	+	Aedes
15a	*Zingiber officinalis*	Zingiberaceae	Rhizome	+	Aedes
b	*Zingiber officinalis*	Zingiberaceae	Leaf	+	Aedes

larvae. Out of 15 plant species attempted, 10 could induce 100% mortality to mosquito larvae at varying concentrations and retention times, but little or no mortality has been observed with other concentrations. Extracts from the leaves of *Psidium gujava, Syzygium cumini,* and leaf and flower extract of *Calycopteris floribunda* were found to have no larvicidal property. But the extracts of *Terminalia chebula,* (Dry seed), *Syzygium cumini,* (Seed) *Datura album,* (Leaf) *Solanum nigrum* (Leaf twig) have exhibited mortality % ranging from 65-80 at 8ml concentration at varying retention time.

Of the 15 plants studied, extracts from *Zingiber officinalis* (rhizome) *Adenocalymma alliaceaum* (leaf), and *Saritea magnifra* (leaf) showed 100% larvicidal property at 0.5ml of the extract at varying retention time. In the present study, it has been noted that aqueous extracts of the rhizome of *Zingiber officinalis*, leaf of *Saritea magnifra* and leaf of Adenocalymma alliaceum can be utilized for controlling mosquito larvae. The LC_{50} estimate for the promising plants like *Zingiber officinalis* (rhizome), *Adenocalymma alliaceaum* (leaf), and *Saritea magnifra* (leaf) were noted to be 1.1, 3.6, and 1.2mg/ml respectively.

DISCUSSION

Aqueous extracts of 15 plants belonging to the family Bignoniaceae (2), Combretaceae (3), Myrtaceae (4), Solanaceae (3), and Zingiberaceae (3) have strong larvicidal activity against third instar larvae of Aedes mosquitoes. In the present study, it has been noted that aqueous extracts of Plants exhibitting100% mortality to mosquito larvae at varying time intervals after treatment (Table 3). Many plant extracts and phytochemicals possess larvicidal activity against various mosquito species (Yang

Table 2. The percentage mortality of mosquito larvae exposed to aqueous extracts of plants at 96 hours after treatment

No.	Plants	Part used	Concentration of the extract mg/ml					
			Control	0.5	1	2	4	8
1a	*Adenocalymma alliaceaum*	Leaf	0	100	100	100	100	100
b	*Adenocalymma alliaceaum*	Flower	0	85	90	100	100	100
2	*Saritea magnifra*	Leaf	0	100	100	100	100	100
3a	*Calycopteris floribunda*	Leaf	0	0	0	0	0	0
b	*Calycopteris floribunda*	Flower	0	0	0	0	0	0
4	*Terminalia chebula*	Dry seed	0	0	0	0	55	75
5	*Terminallia bellarica*	Dry seed	0	0	0	15	100	100
6	*Eucalyptus globules*	leaf	0	10	35	75	100	100
7	*Pimenia diocca*	Leaf twig	0	0	0	20	65	100
8	*Psidium gujava*	Leaf	0	0	0	0	0	0
9a	*Syzygium cumini*	Leaf	0	0	0	0	0	0
b	*Syzygium cumini*	Seed	0	0	0	10	60	80
10a	*Datura stramonium*	Fruit	0	0	0	30	55	100
b	*Datura stramonium*	Leaf	0	0	0	0	35	65
11	*Physalis minima*	Leaf	0	0	0	40	60	100
12	*Solanum nigrum*	Leaf twig	0	0	0	50	65	80
13a	*Alpinia galangal*	Leaf	0	100	100	100	100	100
b	*Alpinia galangal*	Flower	0	100	100	100	100	100
14a	*Curcuma longa*	Rhizome	0	30	60	100	100	100
b	*Curcuma longa*	Leaf	0	0	0	0	40	75
15a	*Zingiber officinalis*	Rhizome	0	100	100	100	100	100
b	*Zingiber officinalis*	Leaf	0	75	75	90	100	100

Table 3. Plants exhibitting100% mortality to mosquito larvae at varying concentration and retention time

Sl. No.	Name of plant	Part used	% of larval mortality	Concentration of extracts mg/ml	Hours
1	*Adenocalymma alliaceaum*	Leaf	100	0.5	12
a	*Adenocalymma alliaceaum*	Flower	100	2	24
2	*Saritea magnifra*	Leaf	100	0.5	24
3	*Terminallia bellarica*	Dry seed	100	4	72
4	*Eucalyptus globules*	leaf	100	4	12
5	*Pimenia diocca*	Leaf twig	100	8	24
6	*Datura stramonium*	Fruit	100	8	24
7	*Physalis minima*	Leaf	100	8	72
8	*Alpinia galangal*	Leaf	100	2	24
a	*Alpinia galangal*	Flower	100	2	24
9	*Curcuma longa*	Rhizome	100	4	12
10	*Zingiber officinalis*	Rhizome	100	0.5	6
a	*Zingiber officinalis*	Leaf	100	4	24

et al., 2002). They reported that the most promising plant based mosquito larvicides are in the families Apiaceae, Araceae, Magnoliaceae, Piperaceae, Rutaceae, Zingeberaceae and Annonaceae plant species can be employed as economical and environment friendly mosquito larvicides. Sukumar *et al*. (1991), examined that the larvicidal activities of plant extracts can be observed by plant types, parts used, native geographical locations and their methods of application. The present finding is a new addition to the list of plants having larvicidal property, being reported by EI Kamali (2001), Narayan and Narayanapillai (1996), Kalyanasundaram and Das (1985),Pushpalatha and Muthukrishnan (1999). The present study also gains significance as the plant / plant parts which are found to be effective in the present study are either perennially available in large quantities or available with ease and little coast. The present study has opened up prospects for large scale extraction of active ingredients of plant origin for effective mosquito control. The study gains more significance as non target organisms in the ecosystems have been little affected by the application of these plant extracts.

CONCLUSIONS

The primary objective of this work is to find out effective means of control of mosquito larvae using plant extracts. In the present study, it has been noted that aqueous extracts of the rhizome of *Zingiber officinalis*, leaf of *Saritea magnifra* and leaf of *Adenocalymma alliaceum* can be utilized for controlling mosquito larvae. The findings of the present study suggest that further purification of the active fractions of the selected plant extracts and isolation of the active principle could lead to the development of more potent and ecofriendly biocontrol agents for the control of dreadful mosquito vectors.

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