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PHYTOCHEMICAL ANALYSIS AND ANTILARVICIDAL ACTIVITY OF AQUEOUS, ACETONE AND ETHANOL EXTRACTS OF SELECTIVE MEDICINAL PLANTS FROM TIRUCHIRAPPALLI DISTRICT AGAINST *ANOPHELES* LARVAE.

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ABSTRACT

Malaria is very dangerous disease mainly caused by *Anopheles* mosquito. Phytochemical compounds present in the medicinal plants which are anti larvicidal activity against this malarial vector. The plants parts were washed, air dried and aqueous, acetone ethanol extracts of plant sample were used for the phytochemical analysis to find out the phytochemical constituents in the plant such as alkaloid, glycoside, phytosterol, flavonoid, phenol, protein and carbohydrate. The plant extracts from 12 medicinal plants species to larvae of *Anopheles* were determined in the laboratory response varied according to plant extracts and mosquito larvae. At the concentration of 50 ppm 100 percent mortality was obtained with a extracts of *Carica papaya, Azadirachta indica, Ocimum sanctum, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota,* and *Allium sativum Carica papaya, Azadirachta indica, Ocimum sanctum, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota,* and *Allium sativum* respectively. The plants described merit further study as potential mosquito larval control agent.

KEYWORDS: Medicinal plants, phytochemical analysis and larvicidal activities.

INTRODUCTION

Malaria is a major parasitic disease in the world. It is responsible millions deaths every year. *Plasmodium falciparum* the most widespread etiological agent for human malaria has become

increasingly resistant to standard antimalarials *e.g.* chloroquine and antifolates. Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use (WHO, 2008).

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of outrageous diseases like Malaria, causing millions of deaths every year. Eliminating the source of infection is an essential step in the control of mosquito-borne diseases. Most of the mosquito control programs target on the larval stage in breeding sites, as adulticides may only reduce the adult population temporarily. In recent times, chemicals derived from plants have been projected as weapons of future mosquito control programs as they are shown to be ecologically friendly(Ghosh, *et.al.*, 2012; Medhi *et. al.*, 2010). Moreover plant based bio products are mostly non-toxic to humans and other mammals and have a high degree of biodegradation. In view of an increasing interest in developing plant based insecticides as an alternative to chemical insecticides, the present study was undertaken to assess the larvicidal potential and phytochemical analysis of the aqueous, acetone and ethanolic extracts of selected medicinal plants against mosquito larvae.

MATERIALS AND METHODS

Study area

We collected the plants from Tiruchirappalli and its surroundings. It is approximately located at 10.7905° N, 78.7047° E and its land mass is 16.72 Km^2 and its population is 11,29,422.

Collection of plant material

The leaves of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Senna auriculata, Manilkara zapota,* bulb of *Allium sativum* and flower of *Targetes erecta* were collected from Tiruchirappalli district in Tamilnadu.the plants parts are washed thoroughly blotted and shade dried.

Extraction

About 10 gm of dry sample of each plant was macerated with sterile water, Acetone and Ethyl alcohol and left to stand at room temperature for 48hrs. Then mixture was filtered through a Whatman no.1 filter paper by suction. Filtrate was evaporated under vacuum for 40°C unit.

Phytochemical screening

Preliminary qualitative Phytochemical examinations were carried out to identified the secondary metabolites present in the various aqueous, acetone and ethanol extract of leaf, bulb and flower all the extracts as per the Harborne (1998) methods.

Collection of Mosquito Larvae

Mosquito larvae were gathered from the water present in the exposed coconut shells in Ramalingam Nagar, Tiruchirappalli. Mosquito larvae were brought to a small room enclosed with a mosquito net and then transferred to a molder with clear tap water. The larvae were kept in a small room which is enclosed with a mosquito net for safety precautions in case of the fast emergence of the larvae into an adult mosquito.

Mosquito Larvae

Larvae of a mosquito can be identified from any other aquatic insects since it has a combination of two characters, they have no legs and the thorax is wider than the head or abdomen. The three divisions of the body part mosquito larvae are head, thorax and abdomen. The structure of three body regions serves as the basis for identifying the mosquito larvae. The mosquito larva was identified using a compound microscope. A small amount of water with a mosquito larvae was drop in a slide to be able to view the specimen in the compound microscope. The target mosquito larva in this study was the third instar larva of dengue carrying mosquito *Anopheles* (Maurya *et.al.,* 2007).

The *Anopheles* larva lacks a respiratory siphon, so it positions itself so that its body is parallel to the surface of the water. The larvae were separated from the other mosquito species and were placed in a water- filled plastic molder.

Mosquito Larvicidal Bioassay

The efficacy of the plant extracts as larvicide against the dengue-vector *Anopheles* was evaluated in accordance with the guidelines of World Health Organization 13. Batches of 10 third-instars larvae of *Anopheles* were placed separately in a small plastic container with 50 ml dechlorinated water and lay in the netted area in the Laboratory room at 30-32^{o}C. For the control group, the mosquito larvae were exposed to 60 mg/mL water since it is the solvent used in the extraction of different plant samples (Mohan *et.al.,* 2006). The experimental group is the Aquous, acetone and Ethyl alcohol extracts of the flower, bulb and leaf of *carica papaya, Azadirachta indica, Ocimum sanctum, Allium sativum, Cymbopogan citratus,*

Mentha spicata, Aloe vera, Vitex negundo, Senna auriculata, Plectranthus amboinicus, Targetes erecta and Manilkara zapota with 10 ppm, 20 ppm, 50 ppm concentrations. These concentrations were chosen after the pre-test//pretreatment conducted. Identification of the mosquito larvae were done by tapping it with a needle in the siphon or cervical area. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting s number of dead larvae after 24 and 48 hours of treatment, and the percentage mortality was computed(WHO 2015).

$$\textbf{Percentage mortality} = \frac{\textbf{Number of dead larvae x 100}}{\textbf{Number of larvae Introduce}}$$

RESULTS AND DISCUSSION

Phytochemical Result

Table-1, shows the phytochemicals present in the Aquous extracts of leaf of *carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota. Ocimum sanctum, Azadirachta indica,* bulb of *Allium sativum and* flower of *Senna auriculata*. leaves contain most of the phyto chemical components. However, in acetone extract of flower, bulb and leaves of *Cymbopogan citratus, Senna auriculata, Plectranthus amboinicus, Targetes erecta* have most of chemical components and in Ethyl alcohol **e**xtract mostly *Ocimum sanctum* gave maximum result. The phytochemicals of the plants serve as huge storage of compounds that have biological action. Alkaloids, saponins, and tannins are known to possess medicinal and larvicidal properties.

In our phytochemical analysis of 12 plants, *Azadirachta indica* in Aqueous extract show the highest result which have Alkaloid, glycoside, saponin, phytosterols, flavonoid, protein and diterpenes.

Larvicidal Activity Result

The larvicidal effects of leaf extracts of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta Manilkara zapota,* Flower extract of *Senna auriculata,* and bulb extract of *Allium sativum* were tested on the larvae of the *Anopheles*. Phytochemical screening of the extracts was conducted to determine the active toxic compounds. Various concentrations (10 ppm, 20 ppm and 50 ppm) of the plant extracts were tested against third instar larvae of *Anopheles*.

Table–2,3,4 shows that the testing the Aqueous, acetone and Ethyl alcohol extract of leaves of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota*, flower of *Senna auriculata* and bulb of *Allium sativum* in the various amount 10 ppm, 20 ppm and 50 ppm separately in *Anopheles larvae* in the container and take the result for 24 and 48 hours. Show the result that in 48 hours of aqueous extract of *Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Senna auriculata, Targetes erecta* and *Aloe vera* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota, Senna auriculata* and *Allium satium* in *Anopheles* show 100% mortality. conclusion, an attempt has been made to evaluate the role of plant extracts in mosquitoes larvicical activity. The results reported in this study open the possibility of further investigation of the efficiency of the larvicidal properties of natural product extracts. The result of this screening show that plant extracts represents a rich source of bioactive molecule with often specific activities which could play an important role in the search for new biocompounds. Secondary metabolites produced in plants for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *Anopheles*. Several studies have focused on natural products for controlling mosquitoes as insecticides and larvicides, but with varied results (Anitha Rajasekaran and Geethapriya Duraikannan., 2012, Mansour *et.al.*, 2000; Trabulsi *et.al.*, 2012).

In the present study the 20 ppm aqueous extract of *Ocimum sanctum*, and 50 ppm of *Ocimum sanctum, Mentha spicata, Azadirachta indica, Cymbopogan citratus, Senna auriculata, Targetes erecta*, and Aloe vera in *aedes* show the 100% motality and in Ethyl acohol of 20 ppm extract of *Ocimum sanctum, Mentha spicata* and 50 ppm extract of *Carica papaya, Azadirachta indica, Ocimum sanctum, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota,* and *Allium sativum* in *Anopheles* show 100% mortality and in 48 hours of aqueous extract of *Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Senna auriculata, Targetes erecta* and *Aloe vera* in *Anopheles* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Vitex negundo, , Plectranthus amboinicus, Targetes erecta, Manilkara zapota, Senna auriculata* and *Allium satium* in *Anopheles* show 100% mortality after 48hrs of incubation.

Moreover behavioural changes were observed in the movement of the larvae. These effects may be due the presence of neurotoxin compounds in plant extracts. No behavioral changes were obtained in control group. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vector of public health importance (Bower *et.al.*, 1995; Rajkumar and Jebanesen, 2009; Sheeran *et.al.*, 2006 and Kassir *et.al.*, 1989).

Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes (Kamaraj and Rahunanan, 2011; Senthinathan, 2017 and Lucis *et al.*, 2012) However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in *Anopheles*. The phytochemicals of medicinal extracts can be well utilized for preparing biocides or insecticidal formulation (Anitha and Geethapriya, 2012 and Medhi *et al*., 2010).

Table 1: Phytochemical analysis of selected medicinal plants.

Test	*Ocimum sanctum*			*Mentha spicata*			*Azadirachta indica*			*Cymbopogan citratus*			*Senna auricata*			*Targetes erecta*			*Vitex negundu*			*Plectranthus auriculata*			*Manilkara. zapota*			*Aloe vera*			*Carica papaya*			*Allium sativum*		
	W	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**	**W**	**A**	**E**
AL	+	-	-	+	+	+	+	+	-	+	+	-	+	+	+	-	+	-	-	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-	+	-
CA	+	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
GL	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GL	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-
SA	-	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-
SA	-	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
PH	+	+	+	+	-	-	+	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+	-	-	-
PH	-	-	-	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	+	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-	-	+
FL	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-	+	+	-
FL	+	-	-	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	-	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	+
PR	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-	-	-	-	-
PR	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-	+	+	+	-	-	-	+	-	-	+	-	-	+	+	-	-	-	+
DI	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-	+	-	-	-

AL	ALKALOIDS
CA	CARBOHYDRATES
GL	GLYCOSIDES
SA	SAPONINS
PH	PHYTOSTEROLS
FL	FLAVONOIDS
PR	PROTEIN
DI	DITERPENES

W	WATER/AQUOUS
A	ACETONE
E	ETHYL ALCHOL

+	PRESENT
-	ABSENT

Table 2: Antilarvicidal activity of selected medicinal against aqueous extract.

Plant name	**Anopheles (Percentage)**					
O.*sanctum*	**24 hours**			**48 hours**		
	10 ppm	**20 ppm**	**50 ppm**	**10 ppm**	**20 ppm**	**50 ppm**
	0	0	20	100	100	100
M.Spicata	40	60	60	70	100	100
A.Indica	70	80	90	90	100	100
C.citratus	80	90	100	80	100	100
S.auriculata	80	80	90	90	90	100
T. erecta	60	60	40	80	90	90
V.negundo	80	80	90	80	90	90
P. amboinicus	80	90	100	90	100	100
M. zapota	40	40	50	80	70	80
A.vera	90	20	80	80	90	100
C.papaya	40	60	60	60	70	80
A.sativum	0	20	80	70	80	90

Table 3: Antilarvicidal activity of selected medicinal against acetone extract.

Plant name	***Anopheles (*** **Percentage)**					
O.sanctum	**24 hours**			**48 hours**		
	10 ppm	**20 ppm**	**50 ppm**	**10 ppm**	**20 ppm**	**50 ppm**
	20	40	80	50	80	100
M. spicata	40	70	80	60	100	100
A.indica	40	70	100	80	100	100
C.citratus	30	50	90	100	100	100
S.auriculata	70	90	100	100	100	100
T. erecta	50	70	80	60	70	80
V.negundo	10	50	70	50	100	100
P. amboinicus	40	60	80	70	80	100
M. zapota	50	70	100	80	90	100
A.vera	10	40	80	40	60	100
C.papaya	0	0	20	50	40	70
A.sativum	0	20	60	30	50	90

Table 4: Antilarvicidal activity of selected medicinal against Ethyl alcohol extract.

Plant name	*Anopheles* (Percentage)					
O.sanctum	24 hours			48 hours		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
	20	60	70	50	100	100
M.spicata	10	40	70	60	90	100
A.indica	30	50	70	50	80	100
C.citratus	30	50	90	70	90	100
S.auriculata	10	60	80	30	50	100
T. erecta	10	50	80	60	80	100
V.negundo	20	50	80	70	90	100
P. amboinicus	0	40	90	40	70	100
M. zapota	20	50	70	40	70	100
A.vera	20	60	70	50	90	90
C.papaya	20	60	80	60	90	100
A.sativum	30	50	90	80	70	100

CONCLUSION

The overall view that, The phytochemicals present in the Aqueous extracts of flower, bulb and leaf of *carica papaya, Azadirachta indica, Ocimum sanctum, Allium sativum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Senna auriculata, Plectranthus amboinicus, Targetes erecta* and *Manilkara zapota. Ocimum sanctum, Azadirachta indica and Senna auriculata* leaves contain most of the phyto chemical components. However, in acetone extract of flower, bulb and leaves of *Cymbopogan citratus, Senna auriculata, Plectranthus amboinicus, Targetes erecta* have most of chemical components and in Ethyl alcohol **e**xtract mostly *Ocimum sanctum* gave maximum result.

Testing the Aqueous, acetone and Ethyl alcohol extract of leaves of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Targetes erecta, Manilkara zapota*, flower of *Senna auriculata* and bulb of *Allium sativum* in the various amount 10 ppm, 20 ppm and 50 ppm in *Anopheles larvae* in the container and take the result for 24 and 48 hours. Show the result that in 48 hours of aqueous extract of *Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Senna auriculata, Targetes erecta* and *Aloe vera* in *Anopheles* show 100% mortality and in 50 ppm acetone extract of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Aloe vera, Vitex negundo, Plectranthus amboinicus, Manilkara zapota, Senna auriculata* and *Allium sativum* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya, Azadirachta indica, Ocimum sanctum, Cymbopogan citratus, Mentha spicata, Vitex negundo, Plectranthus*

amboinicus, Targetes erecta, Manilkara zapota, Senna auriculata and *Allium satium* in *Anopheles* show 100% mortality. The results reported here open the possibility of further investigations of efficacy on their larvicidal of natural products extracts. Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for Mosquito control.

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