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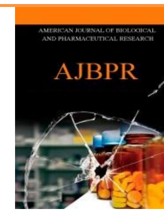

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INVITRO STUDIES ON PHYTOCHEMICALS ANALYSIS AND LARVICIDAL EFFICACY OF MEDICINAL PLANT EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Mosquitoes transmit human diseases, causing millions andmillions of deaths every year Mosquito borne diseases are one of the most serious public health problems in the developing countries. It can be controlled by using repellent, causing larval mortality and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. Medicinalplants extracts of Vitex negundo Azadirachta indica and Eucalyptus tereticornis were tested for their larvicidal activity against Culex quinquefasciatus. There are four different solvents were used (Petroleum ether,Ethanol, Acetone and Hexane extract) for the preparation of crude extracts from the plant leaves. The larval mortality of second and third instar larvae C. quinquefasciatus after 24 hour to 48 hour of treatment were observed separately in control,100, 200,300,400 and 500 ppm concentrations of the leaf extract. The seven different solvent extract of Vitex negundo showed good larvicidal activity.

INTRODUCTION

The mosquito is the principal vector of many of the vector borne diseases affecting human beings and other animals [1]. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever [2]. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations [3]. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of the vectors [4]. This study is concerned with the using of such effective plant source against the larval of Mosquito.

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MATERIALS AND METHODS

The list of plant leaves included in this study was

1. Vitex negundo (Nochi)
2. Azadirachta indica (neem)
3. Eucalyptus tereticornics(Thailam)

COLLECTION OF MOSQUITO LARVAES

The mosquito larvae (Culex quinquefasciatus) were collected from The Kolavai Lake (KolavaiEri), situated near Chennai-Trichy railway line on the eastern side of the Chengalpattu town in the Kancheepuram district of Tamil Nadu.The larvae were collected in a container and transferred to the laboratory immediately. From these larvae, unwanted large size larvae and pupae were collected and discarded From the remaining medium sized larvae second and third instar larvae alone were collected for the larvicidal bioassay. Feed is supplied to the mosquito larvae for its growth [5-7].

Preparation of extract

10 grams of powdered plant leaves was taken and 100ml of solvent was added and kept overnight in the shaker. The extract is filtered again the solvent is added and kept in the shaker for 7 hours. In this way the plant extract is collected and stored in the air tight container for future use.

Test for Carbohydrates

1ml of different crude extract was dissolved in 10ml of distilled water and filtered. The filtrate underwent molish's test to confirm the presence of carbohydrates.

Molish's test

The filtrate was treated with 2ml of concentrated sulphuric acid along the sides of the test tube. Appearance of violet colour ring at the junction of the two liquid shows the presence of carbohydrates.

Test of Glycosides

A small portion of different crude extract were hydrolyzed with hydrochloric acid for few hours on water bath and the hydrolysate was collected.

Fehling's test

2ml of extract was taken in a test tube. 1ml of fehilng's A solution and 1ml of fehling's B solution was added to the extract, mixed well and boiled. Appearance of yellow or red colour precipitate indicates the presence of glycosides (reducing sugar).

Test for Proteins

A small portion of crude was dissolved in few ml of distilled water and it was subjected to Xantho protein test to confirm the presence of protein.

Xantho test

Take 3ml of extract to which 1ml of concentrated nitric acid was added. A white precipitate was obtained. The solution was heated for 1 minute and cooled under the tap water. 40% of NaOH was added to the solution. Appearance of orange colour indicates the presence of protein.

Test for Phenolic compounds and Tannins

A small portion of crude extract was dissolved in few ml of distilled water and subjected to $FeCl_3$ test.

$FeCl_3$ test

Few ml of extract 5% $FeCl_3$ was added. Appearance of violet colour indicates the presence of phenolic compounds and tannins.

Test for Flavonoids

The crude extract was treated with concentrated sulphuric acid. Appearance of yellowish orange colour indicates the presence of anthocyanin, on futher adding yellow turns to orange which indicates the presence of flavones, on futher adding turns to crimson which indicates the presence of flavonones.

Test for terpenoids

2ml of crude extract was dissolved in 2ml of chloroform to which 2ml of sulphuric acid was added and the heated for 2 minutes. Appearance of grayish colour indicates the presence of terpenoids.

Test for Pholbatannins

2ml of extract was taken to which 2ml of 1% HCl was added and boiled. Formation of red precipitate indicates the presence of phlobatannins [8-14].

LARVICIDAL BIOASSAY

Initially 12.5 mg crude extract of petroleum ether solvent of each solvent extract was taken and dissolved in 1ml of acetone in an eppendorf. Then the dissolved crude extract was mixed in container containing 50 larvae in 100ml of water. Every 24 hours the mortality rate was noted and reading was taken. The dead larvae were taken out at every 24 hours since it may leads to contamination of the water. The readings were taken for 2 days (48hours).Then 0.25% (250ppm) concentration from each plant crude extract was introduced into containers containing larvae. Similarly for this reading wastaken for every 24 hours for 2 days. Then 0.50% concentration (500ppm) of crude extract was introduced and reading was noted for every 24hrs for two days [15-19].

Qualitative Analysis of Phytochemicals

The phytoconstituents detected in the plant extract could be responsible for their antioxidant and antimicrobial activity.

RESULT AND DISCUSSION

Larvicidal activities of seven medicinal plant extracts were studied with the control,100, 200, 300, 400 and 500 ppm concentrations .The present study assessed therole of larvicidal activities of Medicinal plants extracts of *Vitex negundo* and *Azadirachta indica* and *Eucalyptus tereticornis* by Solvents Petroleum ether, Hexane, Acetone and Ethanolagainstthe larvae of malaria vector *Culex quinquefasciatus* [20-23]. The activity of crude extracts of the medicinal plant is was noted. The seven different solvents were tested against *Culex quinquefasciatus* and larval mortality percent was observed .through statisticall analysis it was analysed that All plant extracts showed moderate toxic effect on larval. however, the highest mortality rate was found by the *Vitex negundo* plant extracts [24].

Table 1. Qualitative analysis of phytochemicals in various extracts of *Vitex negundo*

Phytochemicals/Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+_	-	-	+
Flavanoids	+	+	+	+
Tannins	-	-	-	+
Phenols	+	+	-	-
Glycosides	+	-	+	+
Saponins	-	-	-	-
Phlobotannins	+	+	+	-
Anthraquinones	+	+	-	+

(+) – Indicates the presence of phytochemicals
(-) – Indicates the absence of phytochemicals

Table 2.Qualitative analysis of phytochemicals in various extracts of *Azadirachta indica*

Phytochemicals/Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+	-	-	+
Flavanoids	+	+	-	+
Tannins	+	-	+	-
Phenols	-	+	-	-
Glycosides	+	-	+	+
Saponins	-	-	+	-
Phlobotannins	+	+	+	-
Anthraquinones	+	+	-	+

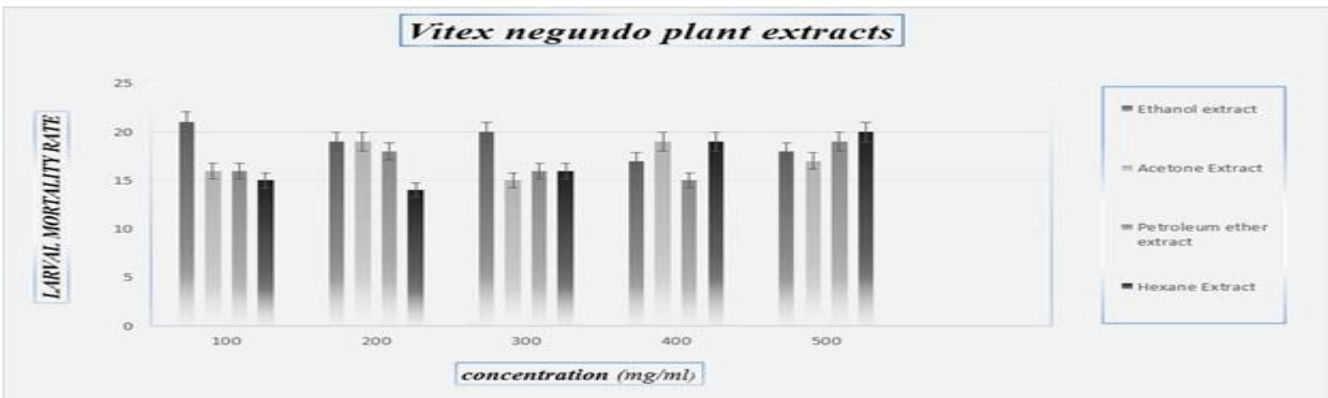
(+) – Indicates the presence of phytochemicals
(-) – Indicates the absence of phytochemicals

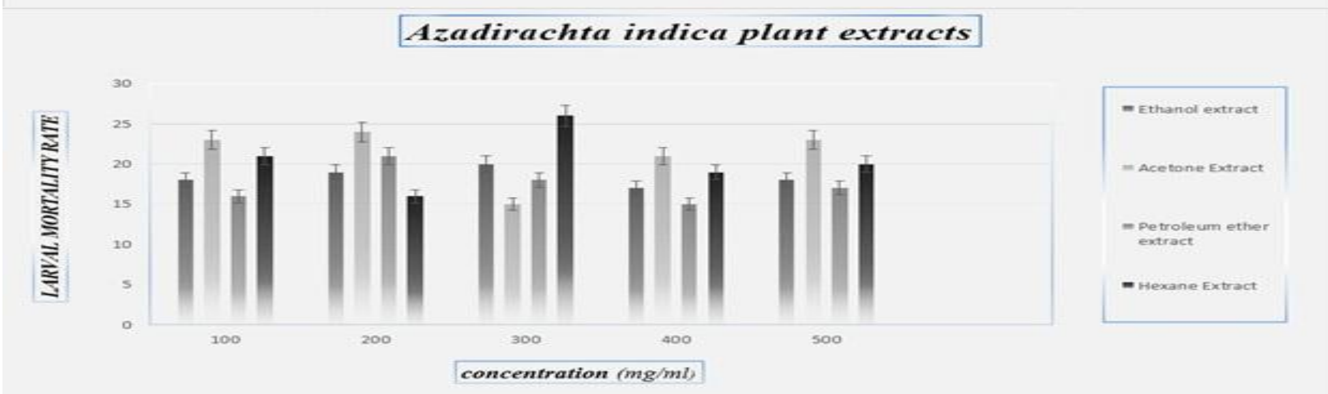
Table 3. Qualitative analysis of phytochemicals in various extracts of *Eucalyptus tereticornis*

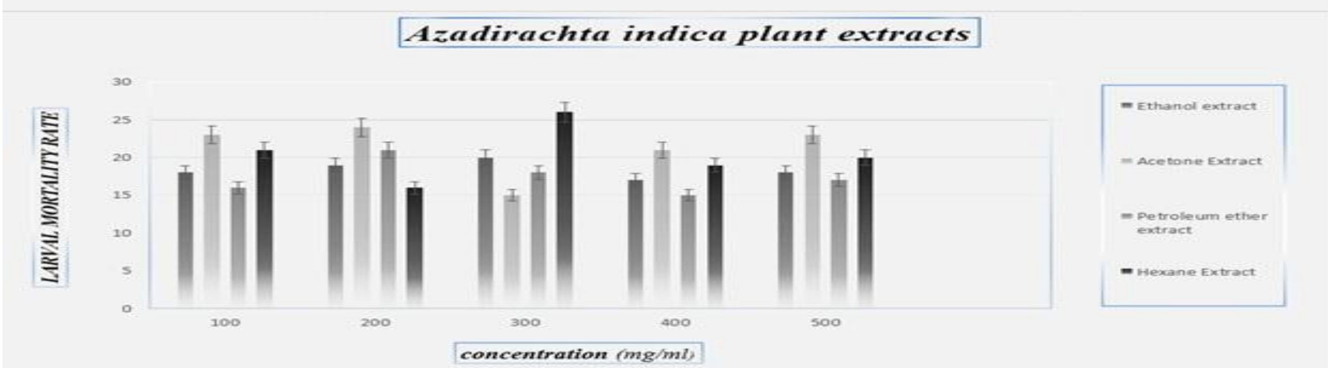
Phytochemicals/Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+_	+	-	-
Flavanoids	+	+	+	+
Tannins	-	-	-	+
Phenols	+	+	-	+
Glycosides	+	+	+	+
Saponins	-	+	-	+
Phlobotannins	+	+	+	-
Anthraquinones	+	-	-	+

(+) – Indicates the presence of phytochemicals

(-) – Indicates the absence of phytochemicals







CONCLUSION

An insecticide does will not cause high mortality on target larvae but may serve medicinal plants extracts as suitable alternatives to synthetic insecticides .plant extract are more safer, inexpensive, reduce dependence on imported products(synthetic) and are readily available in many region of the world. the findings of the present investigation revealed that Medicinal plants extracts of Vitex negundo, Azadirachta indica and Eucalyptus tereticornis good larvicidal activity against Culex quinquefasciatusand Leucas aspera. statistically Vitex negundo had high larvicidal efficacy.These Medicinal plants extracts are the best alternate product against the control of mosquitoes vectorit can be replace the chemical pesticides which cause environmental pollutions and other health problems .

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