PRELIMINARY PHYTOCHEMICAL SCRREENING AND STUDIES ON MOSQUITO LARVACIDAL EFFICACY OF *RICINUS COMMUNIS* L.AND *EUPHORBIA HIRTA* L.

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Abstract

Plants contain many biologically active compounds which have potential for the development as medicinal agents. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment

Key Words: Phytochemical,larvicides,euphorbiaceae.

INTRODUCTION

Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Mosquito borne diseases still remain a major health problem in both human and veterinary sectors. They are one of the most medically significant transmitters as they spread parasites and pathogens, which continue to have devastating effects on human beings . Mosquito borne diseases such as Malaria, Filariasis ,Yellow fever and Dengue cause extensive morbidity and mortality and are a major economic burden within disease endemic countries (Sachs and Malaney 2002 ; Boutayeb 2006). Every year, about 300 million people are estimated to be affected by Malaria, a major killer disease, which threatens 2,400 million of the world's population (Sharma 1999; Snow et al., 2005). Similarly,lymphatic Filariasis caused by Wuchereria bancrofti affects about 106 million people worldwide. About 20 million people are infected every year by Dengue viruses transmitted by Aedes mosquitoes with about 24,000 deaths (Poopathi et al., 2010).These diseases spread globally, causing high levels of human mortality and thereby acting as factors impedimenting the economic development of most of the developing countries across the world .

Mosquitoes adversely affect human health, as they act as vectors for a wide variety of dreadful diseases. Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Mosquitoes are the most important group of bloodsucking arthropods. They not only create nuisance to humans by biting, but also transmit serious diseases with many socioeconomic consequences. The situation has become worse over the last decade due to global climatic changes.

Natural products are generally preferred because of their less harmful nature to nontarget organisms and due to their innate biodegradability (Shaalan et al., 2005). Natural products of

plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and or the environment. Natural pesticides especially those derived from plants, are more promising in this aspect. Many researchers have reported on the effectiveness of plant extracts against mosquito larvae.

Phytochemicals are the bioactive, natural chemical compounds, found in plants. Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes.(Shaalan et al;2005).

One of the methods available for controlling the mosquitoes is use of synthetic insecticides. Mosquitoes develop genetic resistance to synthetic insecticides and even to biopesticides such as Bacillus sphaericus. Also synthetic insecticides adversely affect the environment by contaminating air, water, and soil. There is a urgent need to find alternatives to the synthetic insecticides which is more potent and low-cost (Ghosh et al; 2010).

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly. Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemicals may act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable. Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities. About 2000 species of terrestrial plants have been reported for their insecticidal properties. Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities. Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponines, steroids, isoflavonoids, essential oils, alkaloids and tannins has potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitos (Maurya et al; 2007).

Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae. (Sharma et al., 2006; Rasheed et al., 2005; Siddiqui et al., 2004).

Euphorbiaceae species are commonly used in traditional medicine and has been reported to possess various biological activities.In traditional medicine, there are many natural crude drugs that have the potential to treat various diseases and disorders, one of them is Ricinus communis L. belonging to the Family Euphorbiaceae, popularly known as 'castor plant' and commonly known as 'Palm of Christ'.Castor bean is an evergreen herbaceous or semi-woody, large shrub or small tree. This plant is widespread throughout the tropical regions. (Maman et al., 2005; Lal et al., 2017).

Euphorbia hirta is an important medicinal herb belonging to the family Euphorbiaceae(Akinnmade et al., 2010). It is a small annual herb and it is common to tropical countries. It is a popular herb also called asthma herb and pill bearing spurge (Abu-Sayeed et al.,2005; Anuradha et al., 2008). In East and West Africa extracts of the plant are used in treatment of asthma and respiratory tract inflammations. It is also used for coughs, chronic bronchitis and other pulmonary disorders in Malagasy. The plant is also widely used in Angola against diarrhoea and dysentery, especially amoebic dysentery. In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing.(Chika Ogueke et al., 2007). It possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties.(Kumar et al.,2010).

Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products are generally preferred because of their less harmful nature to nontarget organisms and due to their innate biodegradability. Hence the present study was an attempt to find out the phytochemicals and to determine the larvicidal potential of the selected plants.

MATERIALS AND METHODS

The plants *Ricinus communis* and *Euphorbia hirta* were collected for the present investigation from different localities of Changanacherry. They were identified by comparing the herbarium specimens deposited in the herbarium of Assumption College.

PLANT DESCRIPTION

SL. NO.	PLANT	DESCRIPTION	PHYTOCHEMICAL
1	***Ricinus communis***	Ricinus communis L generally called castor plant is one of tropical flowering plant species of spurge family Euphorbiaceae and has been found to grow widly across the world . it is a tall shrub of about 2 to 4 mtrs heigh, and its leaf are about 15to 45 cm long;long stalked ;palmate of 5to12 lobeswith coarsely toothed segments	Terpens, Phenolics, Flavanoids, Tannins, Reducing Sugar and Protein
2	***Euphorbia hirta***	Euphorbia hirta is herb or shrub. The stem is herbaceous or woody, erect or prostrict. The leaf of members of Euphorbiaceae are usually simple, alternant and stipulate. But it is opposite in Euphorbia hirta . Inflorescence is highly variable in this family. Inflorescences a highly specialized cyathiumin Euphorbia. Presence of milky or watery latex.	Terpens, Phenolics, Flavanoids, Tannins, Reducing Sugarand Protein

PHYTOCHEMICAL SCREENING

PREPARATION OF CRUDE LEAF EXTRACT

The fresh leaf of *Ricinus communis* and *Euphorbia hirta* were collected , brought to the lab , wash thouraghly under tap water and shade dried . Only young and fresh leaf were used for the preparation of wide extract. The leaf were then chopped and grind in motar and pistle to make a paste.it was then made up to 100ml by adding distilled water. The leaf extract was ued to perform phytochemical screening test.

The phytochemical screening test were carried out to conform the probable existence of Terpens, Phenolics, Alkaloids,Saponins, Philobatannins, Tannins,Reducing sugar, Glycosides, Flavanoids, Proteins, and Volatile Oil.

The test was based on the visual observation of colour modification or precipitate formation after the addition of specific reagent. (Talukdar and Choudhary, 2010)

TESTS	PROCEDURE	*Ricinus communis*	*Euphorbia hirta*
Terpenoids	To 2ml of the extract 5ml of chloroform and 2ml acetate anhydride was added followed by conc.sulphuric acid.	+	+
Phenolics	To 2ml of extract 5% Ferric chloride solution was added	+	+
Saponins	To 2ml of the extract was treated with 5ml distilled water shake well and heat it.	+	+
Phlobatannins	To the extract 1% of Hydrochloric acid was added and each plant sample was then boiled with the help of hot plate stirrer.	–	–
Alkaloids	To 2ml of the extract in a test tube a few drops of picric acid solution is added.	+	+
Flavonoid	To the extract add 10ml of distilled water,5ml of dilute Ammonium solution followed by 1ml of concentrated Sulphuric acid.	+	+
Tannins	A protein of the extract is diluted with water 3-4% of 1%ferric chloride was added.	+	+
Glycosides	1ml of dilute sulphuric acid is added to 5ml of the extract in a test tube and boiled for 15 minutes ,cooled and neutralized with 10% Sodium hydroxide then 5ml Fehling solution is added.	+	+
Volatile oils	To 2ml of the extract was shaken with 0.1ml of dilute Sodium hydroxide and a small quantity of dilute Hydrochloric acid is added.	+	+
Reducing sugars	To 0.5ml of plant extract ,1ml of water and 5-8 drops of Fehlings solution were added and heated over water bath	+	+
Proteins	2ml of extract was treated with few drops of concentrated Nitric acid.	+	+

MOSQUITO LARVICIDAL ACTIVITY

MOSQUITO LARVAE

For the present study, the fourth larvae of *Aedes albopictus* mosquitoes were used throughout the investigation. These larvae were chosen because they were abundant in the water bodies. The mosquito larvae's has a well developed head with mouth crushes used for feeding, a large thoraxes with no legs and segmented abdomen. Larvae swim either through population with their mouth brushes or by jerky movement of their enter bodies, giving them the common name of wigglers.

Fourth instar larvae of *Aedes albopitus*

The experiment was conducted using laboratory reared larvae of *Aedes albopictus*, which was grown in a dish that was kept opened. Decaying leaves and yeast granules were added to water and allowed to remain stagnant till larvae appears. Excess of food was avoided to prevent excessive microbial growth which may lead to the non-specific of test organisms. The larvae of different mosquito developed after 8-10 days in the dish and were covered using a mosquito net to prevent their escape. The larvae's of*Aedes albopictus* were distinguishing by the silvery patch on the back of its mesotonum.

PREPARATION OF CRUDE LEAF EXTRACT FOR MOSQUITO LARVACIDAL ACTIVITY

The fresh leaves of *Ricinus communis* and *Euphorbia hirta* were collected, washed thoroughly under the running tap water and shade dried. The leaves were weight to make different concentration. 9g of weight leaf were grinded in a mortar and pestle using distilled water and made up to required concentrated of 0.5%,1%.2.5%,and 5% respectively. The fourth instar larvae were picked from the rearing dish with a rubber teat aided wide month pipette to prevent injury to larvae from sharp glass. In each test tube 10ml of different concentration of aqueous extract was taken for the present study. About 10 number of the fourth instar larvae of *Aedes albopictus* was introduced into each test tube. For each experiment a control with distilled water was also kept. Five replica of experiment were maintained. The percentage of mortality was calculated using formula.

$$\text{Mortality percentage} = \frac{\text{Number of larvae died}}{\text{Number of larvae introduced}} \times 100$$

EXPERIMENTAL SET-UP SHOWING MOSQUITOLARVICIDAL EFFICACY ON DIFFERENT CONCENTRATION OF CRUDE AQUEOUS EXTRACT of *Ricinus Communis*

EXPERIMENTAL SET-UP SHOWING MOSQUITOLARVICIDAL EFFICACY ONDIFFERENT CONCENTRATION OF CRUDE AQUEOUS EXTRACT of *Euphorbia hirta*

MOSQUITOLARVICIDAL ACTIVITY OF DIFFERENT CONCENTRATION OF CRUDE AQUEOUS EXTRACT OF *Ricinus communis*

Concentration of aqueous extract (*Ricinus communis*)	No of larvae introduced	Time in hous	No of larvae died					Average	Percentage of Mortality
0.5%	10	24	0	1	2	2	1	1	10%
		48	2	2	2	2	3	2	20%
		72	3	3	4	3	4	3	30%
1%	10	24	1	3	2	3	3	2	20%
		48	3	3	3	4	4	3	30%
		72	3	4	4	4	6	4	40%
2.5%	10	24	3	3	3	4	4	3	30%
		48	4	3	4	4	6	4	40%
		72	5	5	5	6	6	5	50%
5%	10	24	6	6	6	6	10	6	60%
		48	4	6	6	10	10	7	70%
		72	10	10	10	10	10	10	100%
0% (control)	10	24	0	0	0	0	0	0	0%
		48	0	0	0	0	0	0	0%
		72	0	0	0	0	0	0	0%

The larval mortality was recorded after 24hours, 48hoursand 72hours as in table 2.The larvae were considered as died, if they were not responsive to a gentile prodding with a fine needle. The number of larvae introduced in each test solution is ten.

In 0.5%concentration of leaf extract at 24 hours the percentage of mortality was 10%. In 48 hours the percentage of mortality was20%. In 72 hours the percentage of mortality was 30%

In 1% concentration of leaf extract at 24 hours the percentage of mortality was20%. In 48 hours the percentage of mortality was30%. In 72 hours the percentage of mortality was 40%.

1n 2.5% concentration of leaf extract at 24 hours the percentage of mortality was 30%. In 48 hours the percentage of mortality was40%. In 72 hours the percentage of mortality was 50%.

In 5% concentration of leaf extract at 24 hours the percentage of mortality was 60%. In 48 hours the percentage of mortality was70%. In 72 hours the percentage of mortality was 100%. All mosquitoes survived in controlled experiment.

MOSQUITOLARVICIDAL ACTIVITY OF DIFFERENT CONCENTRATION OF CRUDE AQUEOUS EXTRACT OF

Euphorbia hirta

Concentration of aqueous extract (Euphorbia hirta)	No of larvae introduced	Time In Hous	No of larvae died					Average	Percentage of Mortality
0.5%	10	24	0	0	0	1	2	1	10%
		48	0	0	1	2	2	2	20%
		72	0	2	2	3	3	3	30%
1%	10	24	0	1	2	2	2	2	20%
		48	1	3	3	3	1	3	30%
		72	2	3	4	4	4	4	40%
2.5%	10	24	4	3	2	4	4	4	40%
		48	1	3	5	5	5	5	50%
		72	3	6	6	7	6	6	60%
5%	10	24	5	4	6	10	10	7	70%
		48	4	6	8	8	6	8	80%

		72	10	10	10	10	10	10	100%
0% (control)	10	24	0	0	0	0	0	0	0%
		48	0	0	0	0	0	0	0%
		72	0	0	0	0	0	0	0%

The larval mortality was recorded after 24hours, 48hoursand 72hours as in table 3. The larvae were considered as dead, if they were not responsive to a gentile prodding with a needle. The number of larvae introduced in each test solution is ten.

In 0.5%concentration of leaf extract at 24 hours the percentage of mortality was 10%. In 48 hours the percentage of mortality was20%. In 72 hours the percentage of mortality was 30%

In 1% concentration of leaf extract at 24 hours the percentage of mortality was20%. In 48 hours the percentage of mortality was30%. In 72 hours the percentage of mortality was 40%.

1n 2.5% concentration of leaf extract at 24 hours the percentage of mortality was 40%. In 48 hours the percentage of mortality was50%. In 72 hours the percentage of mortality was 60%.

In 5% concentration of leaf extract at 24 hours the percentage of mortality was 70%. In 48 hours the percentage of mortality was80%. In 72 hours the percentage of mortality was 100%.All mosquitoes survived in controlled experiment.

DISCUSSION

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases. Repeated use of synthetic insecticides for their control has disrupted natural biological control systems and lead to resurgences in mosquito populations. 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. (Deshmukh et al., 1982; Mittal et al., 2003; Das, 2007). In the past the use of chemical insecticides, have resulted in the development of insecticide resistance in some important vectors of Malaria, Filariasis and Dengue fever (WHO 1992).

Plants are considered as a rich source of bioactive chemicals and may be an alternative source of mosquito control. The secondary metabolite of plant origins makes up a vast repository compounds with a wide range of biological activities. David et al., (2000) found that phytochemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Maurya et al., 2007).

Based on the results of the phytochemicals screening, both the plants contained tannins, alkaloids, phenols and steroids (Table 1) Alkaloids, tannins and Phenols have been shown to possess toxic activities against insects; hence,

their presence in the extracts can be the cause of their larvicidal activity (Liu et al., 2012). The qualitative screening of phytochemical compounds in *E. hirta* revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavanoids and phenolic compounds (Basma 2011).This was in concordance with the results of our analysis of the plant extracts which revealed the presence of Phytochemicals such as Phenols,Tannins, Flavonoids, Saponins, Glycosides, Steroids, Terpenoids and Alkaloids.

Waseem Ahmad, et al; (2017) reportedthat in ethanol leaf extract of E. hirta showed the presence of alkaloid, flavanoid, sponins, carbohydrate and terpenoids and the hexane extract exhibited flavonoid . The ethanol extract of flower showed the presence of alkaloid, flavanoid terpenoid, tannin and carbohydrate. The chloroform extract of the flower of E. hirta showed the presence of alkaloid, flavonoid, terpenoid, tannin and carbohydrate and the hexane extract exhibited the presence of flavonoid.

Significant Mosquito larvicidal mortality rate was shown by both the plants *E. hirta*and *R. communis*. Panneerselvam et al., (2013) suggested that methanol leaf extracts of *E. hirta* and *B. sphaericus* have potential to be used as an ideal ecofriendly approach for the control of the malarial vector, *An. stephensi* as target species of vector control programs

In search of a natural larvicide, Batabyal Lata, et al; (2009) petroleum ether, carbon tetrachloride, and methanol extracts of R. communis (Castor) was tested for larvicidal activity against Cx. quinquefasciatus. Among the extracts tested, the carbon tetrachloride extract of R. communis was observed the most potent with LC50 at 144.11 ppm after 24 hours and 92.44 ppm after 48 hours and LC90 at 432.42 ppm after 24 hours and 352.89 ppm after 48 hours; the extract exhibited potential results and can be exploited as a preferred natural larvicide for the control of mosquitoes. This research aims to introduce a method for controlling Anopheles arabiensis Patton in their larval stages, using different chemical extracts of R. communis; which will hopefully eventually results in reduction of the disease.

Insecticide resistance is now a major problem facing malaria vector control programs in most African countries (Abdalla et al., 2008). Repeated use of chemical insecticides has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern this initiated a search for alternative control measures. (Brown et al., 1986; Hayes et al., 1991). In recent years, the emphasis to control mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, of botanical origin. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent activities against mosquitoes. (Ciccia et al., 2000; Ansari et al., 2000).

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to chemical insecticides (Kumuda et al., 1991). The plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment (Wattal et al., 1981). The recent increased interest in developing insecticides of plant origin as an alternative to chemical insecticides, provided the basis for many studies.

The plant-derived natural products as larvicides have the advantage of being harmless to beneficial nontarget organisms and environment when compared to synthetic ones. (Pitasawat et al; 2007). In recent years, the emphasis to control the mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials (Heal et al., 1950). For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes (Ciccia et al., 2000; Ansari et al., 2000). Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Although several plants have been reported for mosquitocidal activity, only a few botanicals have moved from the laboratory to field use most of the works are restricted to preliminary screening and determination of active principals is poorly characterized.

CONCLUSION

Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products are generally preferred because of their less harmful nature to nontarget organisms and due to their innate biodegradability.

The present investigation showed that the aqueous extract of *Ricinus communis* and *Euphorbia hirta* contain potent larvicidal bioactive principles which may need further purifications to be carried out in the future research. The results of the present study suggest that the biological activity of these plant extracts might be due to the phytochemicals, that exist in plants. These compounds may contribute jointly or independently to larvicidal and adult emergence inhibition activity. The results reported in this study open the possibility of further investigations on the efficacy of the larvicidal properties of natural product extracts.

Resistance to synthetic Insecticides and polluting the environment are challenges facing vector control; therefore botanical insecticides may serve as efficient, low cost powerful larvicides and a suitable alternative biocontrol techniques in the future.

For successful application of these phytochemical ingredients in insect biocontrol, it is obligatory to understand the mechanisms of their action in the target insects as well as the spectrum of insects effected by them. Further work on these plant derived derivatives is needed for developing them into effective formations to be utilised in integrated vector control. Further research should concentrate to identify the biologically active constituents of these plants to develop a new potential eco-friendly larvicide.

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