Production and Application of Mosquito Toxin from - *Andrographis paniculata* ((Burm.f.) Wall. ex Nees)) and *Acorus calamus* ((L., 1753))

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Abstract

In this present study the mosquito larvae is controlled by bioactive compound from the selected plant Andrographis paniculata and Acorus calamus. Although this plant is reported for its ethno botanical uses and clinical uses, and there was no study is so far reported for its mosquito larvicidal activity. Hence, this work is attempted to verify the mosquito Larvicidal efficiency of bioactive compound from Andrographis paniculata and Acorus calamus.

Key words : *Andrographis paniculata , Acorus calamus and Larvicidal activity.*

INTRODUCTION

Mosquitoes are a family of small, midge-like flies, the *Culicidae*. Although a few species are harmless, most cause a nuisance by sucking blood from vertebrates, including human beings [1]. Several of the most harmful human and livestock diseases are transmitted by mosquitoes during feeding. Scientists suggest complete eradication of mosquitos would not generate serious ecological consequences. In practice, however, control measures focus on small group of mosquito species which are vectors of human or livestock diseases. Mosquitoes of the genus Toxorhynchites predate on other species of mosquitoes that can be used as natural biological control organisms.

Mosquitoes are estimated to transmit disease to more than 700 million people annually in Africa, South America, Central America, Mexico, Russia and much of Asia, with millions of resulting deaths. At least two million people annually die of these diseases. Mosquitoes can act as a vector for many disease-causing viruses and parasites. Infected mosquitoes carry these organisms from person to person without exhibiting symptoms themselves. Monitoring mosquito larval populations is done by providing artificial breeding spots called ovitraps and collecting and counting the developing larvae in fixed intervals[2].

Andrographis paniculata is an annual herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it has been traditionally used to treat infections and some diseases. Mostly the leaves and roots were used for medicinal purposes.

Taxonomical classification

Kingdom	:	Plantae
Order	:	Lamiales
Family	:	Acanthaceae
Genus	:	*Andrographis*
Species	:	*A. paniculata* (Burm.f.) Wall. ex Nees)

Andrographis paniculata grows erect to a height of 30–110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 centimeters long by 2.5 wide. *A. paniculata* is distributed in tropical Asian countries, often in isolated patches. It can be found in a variety of habitats, such as plains, hillsides, coastlines, and disturbed and cultivated areas such as roadsides, farms, and wastelands[3].

In one Chilean study from 1999, the herb had a significant drying effect on the nasal secretions of cold sufferers who took 1,200 milligrams of andrographis extract daily for five days[4]. A 2012 study suggested that Andrographis paniculata extracts may have the potential to be used as a mosquito repellant. A 2006 study reported that extracts of Andrographis exhibited potent anti-inflammatory effects and antioxidant actions in mice[5].

Acorus caramus

Taxonomical classification

Kingdom	: Plantae
(unranked)	: Angiosperms
(unranked)	: Monocots
Order	: Acorales

Family : Acoraceae
Genus : *Acorus*
Species : *calamus* (L., 1753)

This species inhabits perpetually wet areas like the edges of streams and around ponds and lakes, in ditches and seeps. It often shares habitat with the common cat-tail. Grass-like, rhizome forming, perennial that can grow to 2 meters high, resembling an irrs. Long creeping roots that spread out just below the surface of the soil. These roots spread horizontally and can grow to almost 2 meters in length for old, well-established specimens. The thick, erect leaves are very similar in appearance to those of an iris, but with edges that are crimped. Plants very rarely flower or set fruit, but when they do, the flowers are 3-8 cm long, cylindrical in shape, greenish brown and covered in a multitude of rounded spikes. The fruits are small and berry-like, containing few seeds.

Medicinal Uses

The Cree Indians of Northern Alberta use Calamus for a number of medicinal reasons including: as an analgesic for the relief of toothache or headache, for oral hygiene to cleanse and disinfect the teeth, the fight the effects of exhaustion or fatigue, and to help cure/prevent a hangover. It's also used it to treat a cough, made a decoction as a carminative and as an infusion for cholic.

MATERIALS AND METHODS

Collection of plant

The leaves of *Acorus calamus*, and *Andrographis paniculata* were collected from Thanjavur district, Tamil Nadu. The samples were allowed to extraction process to identify the bioactive compounds against plant infection by following methods.

Plant powder and extract preparation

The collected plant leaves were taken and dried under shade for 15 days. The dried plant material was crushed into fine powder With the help of grinder and stored for required purpose. 5gm of the plant powder was dissolved in 45ml of solvent (Ethanol) to prepare extract in clean flask. The flask was covered with the aluminum foil and kept on rotating shaker (120 rpm) for 2 days. The solution was filtered twice, firstly with cheese cloth (four fold) and then with Whatman No 1 filter paper. The filtrates were collected in clean test tubes and were concentrated upto dryness by keeping it in incubator at 35ºC.

PHYTOCHEMICAL SCREENING OF THE EXPERIMENTAL PLANTS

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods [6].

LARVICIDAL ACTIVITY

Testing of the plant extract for Larvicidal activity was carried out at different concentration by preparing the required stock solutions by following the standard procedure. The desired concentrations of the test solution were achieved by adding 0.1 %, 0.5 %, and 1 %, of an appropriate plant extract and alkaloid. Six replicates for each concentration were maintained. Ten numbers of larvae were introduced into the Petri plates, were obtained from the laboratory colony. Ethanol was used as control. The larval mortality in both treated and control was recorded after 24 hrs – 72 hrs and the percentage of mortality was calculated using Abbott's formula [7].

RESULTS AND DISCUSSION

The present study of phytochemical investigation qualitatively and Isolation, Identification of alkaloid from *Andrographis paniculata*, and *Acorus calamus* leaves extract have been presented and discussed here.

Qualitative Phytochemical analysis

There is a growing focus on the medicinal plants use as a therapeutic agent because of their limited side effect and retention of appropriate period of activity. The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, tannins, pseudo tannin, steroidal glycoside, Phenol, Terpenoid, carbohydrate and Flavonoid in Ethanolic extract of *Andrographis paniculata* were detected. Thus the preliminary screening test may be useful in the detection of the bioactive compounds. The results are tabulated in Table – 1.

The Phytochemical character of the ethanol extract of *Acorus calamus* was investigated and the phytochemical constituents such as alkaloids, tannins, pseudo tannin, terpenoid, steroidal glycoside, and Carbohydrate were recorded. It was observed that phenol was absent in ethanol extract of *Acorus calamus* (Table 1).

Alkaloids are important defense of the plant against pathogenic organism and herbivores. It is also toxin for insects. The preliminary qualitative phytochemical investigation revealed the presence of medicinally active constituents in the selected plant extracts.

In modern medicine new trends to use the active ingredients of plants rather than the whole plants. The phytochemicals may be synthesized,

compounded or otherwise transformed to make pharmaceuticals.

Isolation Alkaloid by TLC

The TLC profile of Alkaloid is isolated and identified from *Acorus calamus* and *Andrographis paniculata*. Among the chemical constituents the alkaloid compound has been determined from the sample of Ethanolic extract of *Acorus calamus* and *Andrographis paniculata* that is Larvicidal compound were found to be the most abundant depicted by the Rf value of 0.93 and 1.0 respectively. The present investigation has been undertaken to find out the effectiveness of the alkaloid compound isolated by TLC.

Hence, we concluded that the isolated alkaloid compound was one of the Insecticidal and Larvicidal compounds which is used to control the mosquitoes.

In order to quantify and identify the alkaloid compound from *Acorus calamus* and *Andrographis paniculata* which is allowed to purification. The purified compound condensed at normal temperature that is used to a controlling agent of mosquitoes in larval stages and also the raw extract of plant is used at various concentrations to observe the results.

LARVICIDAL ACTIVITY

The present study shows Larvicidal activity of alkaloid which is isolated from *Acorus calamus* and *Andrographis paniculata* and raw extract also used to investigate the comparative study. The mosquito larvae were collected from ditches around Rajeshwari nagar at Thanjavur district.

The isolated compound and raw extract of *Acorus calamus* and *Andrographis paniculata* was taken at various concentration such as 0.1 %, 0.5 %, 1 % and pure extract were taken to evaluate the Larvicidal activity. The inhibitory rate is vary from once to another depends upon the dilutions of extract.

The results are taken up to 24 -72 hours. The cent percent mortality was occurring in 1 % of isolated compound within 24 hours. The mortality rate was observed in70%, 10% and 100% which was treated with 0.1%, 0.5% concentrations of isolated compound and raw extract of *Acorus calamus* and *Andrographis paniculata*. 20 % of adults are formed in 0.1% and 0.5 % concentration of isolated compound at 72 hours.

The present study shows 100 % mortality was observed in 24 hrs which was treated with 1% of compound isolated from both selected plants whereas the raw extracts of both samples shows very low effect of Larvicidal activity.

The maximum larvae were controlled in 24 hours whereas the gradually increased the mortality rate at 48 hrs to 72 hrs. The inhibitory value was gradually decreased in least dilutions. So we conclude that the 1% concentration was suitable for mosquito larvae control and also the alkaloid was effective for control the mosquito larvae.

Table 1: Preliminary phytochemical analysis in Sample *Acorus calamus* and *Andrographis paniculata*

S.No.	Name of the Test	Phytochemical constituents	*Andrographis paniculata*	*Acorus calamus*
1	Mayer's test Dragondraff test Wagner Test	Alkaloids	+ + +	+ + +
2	Molish Test Fehling Test Benedicts Test	Carbohydrates	+ + +	+ + +
3	Foam Test	Saponins	-	-
4	Lead Acetate	Tannins	+	+
5	Ferric chloride.	Pseudo tannins	+	+
6	Salkowaski	Steroidal Glycosides	+	+
7	H_2So_4	Anthocyanin	-	-

8	Liebermann's Burchard Test	Steroidal Glycosides	+	+
9	H_2So_4	Saponins glycosides	-	-
10	Ammonia	Flavonoids	+	-
11	Shinoda's Test	Flavones	-	-
12	Sodium chloride	Coumarin	-	-
13	Phenol reagent	Phenol	+	-

+ : Present **- : Absent**

Table: 2 Shows Larvicidal Activity 0f *Andrographis paniculata*

S. No	**Concentration of Sample**	**% of Mortality**		
		24 hrs	**48 hrs**	**72 hrs**
1	**Control (EtOAc)**	80	20	-
2	**Raw Extract**	20	60	20% Adults
3	**0.1%**	70	20	10 % Adults
4	**0.5 %**	10	-	-
5	**1 %**	100	-	-

Table: 3 Shows Larvicidal Activity 0f *Acorus calamus*

S. No	**Concentration of Sample**	**% of Mortality**		
		24 hrs	**48 hrs**	**72 hrs**
1	**Control (EtOAc)**	90	10	-
2	**Raw Extract**	10	50	30% Adults
3	**0.1%**	50	30	20 % Adults
4	**0.5 %**	40	50	10 % Adults
5	**1 %**	100	-	-

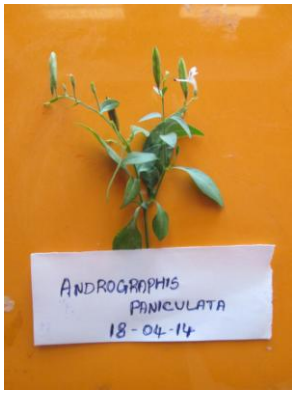

Fig A

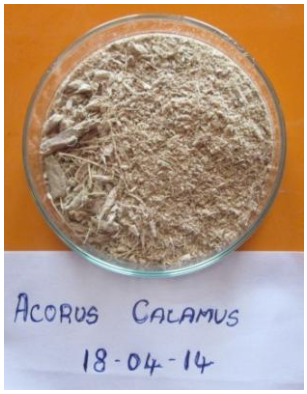

Fig B

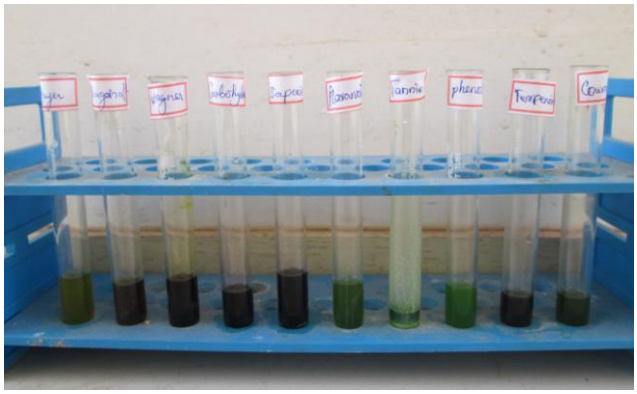

Fig C

Fig D

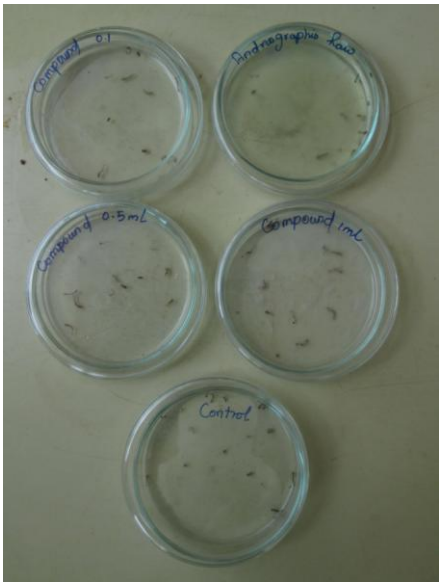

Fig E

Fig: (A & B) – Sample collection *Acorus calamus* and *Andrographis paniculata*

Fig: (C) – Qualitative analysis, Fig (D) – Compound isolation, Fig (E)- Larvicidal activity

SUMARRY AND CONCLUSION

Studies carried out so far have shown that some phytochemicals act as general toxicant (insecticide/ larvicide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and development (growth inhibitors) or with reproduction (chemosterilant) or produce olfactory stimuli thus acting as repellent or attractant.

The plant extract have also shown Larvicidal and/or growth inhibition activity against mosquitoes but their potential for mosquito control under field conditions needs to be evaluated.

These plant derivatives are probable sources of some biologically active agents for mosquito control in the future. Since most of the plant based products are not as effective as synthetic insecticides and do not produce fast results, their use for mosquito control in a large scale.

We conclude that this present study showed some indigenous plant based products are very promising against mosquitoes and can be used as insecticides and/or repellents. They offer a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. The present study suggested that the selected plants such as *Andrographis paniculata and Acorus calamus* and other derivatives can be used alone or in combination with other products for effective protection against mosquitoes.

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