Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(12)60046-2

Mosquito larvicidal and biting deterrency activity of bud of *Polianthes tuberosa* plants extract against *Anopheles stephensi* and *Culex quinquefasciatus*

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ARTIChloroform INFO

Artichloroform history:
Received 25 March 2012
Received in revised form 5 April 2012
Accepted 27 May 2012
Available online 28 June 2012

Keywords:
Polianthes tuberosa
Culex quinquefasciatus
Biting deterrency
Larvicidal activity
Non-target

ABSTRACT

Objective: To evaluate the larvicide and biting deterrency activity of bud of *Polianthes tuberosa (P. tuberosa)* against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and *Anopheles stephensi (An. stephensi)*. **Methods:** Crude and solvent extract [ethyl acetate, chloroform: methanol (1:1, v/v), acetone] of fresh, mature, bud of *P. tuberosa* was tested against *Cx. quinquefasciatus* and *An. stephensi*. The repellent activity tested by chloroform: methanol (1:1, v/v) solvent extract against both mosquito species. The appropriate lethal concentrations at 24 h for chloroform: methanol (1:1, v/v) extract was also studied on non target organisms such as *Toxorhynchites larvae*, *Diplonychus annulatum* and *Chironomus circumdatus*. **Results:** In a 72 h bioassay experiment, 0.5 % crude extract showed the highest mortality and chloroform: methanol (1:1, v/v) solvent extract showed the highest mortality, the maximum ($P < 0.05$) mortality was recorded at a concentration of 60 mg/L. The chloroform: methanol (1:1, v/v) solvent extract provide 4 h protection against *Cx. quinquefasciatus* and 5 h against *An. stephensi* from biting. **Conclusions:** Both crude and chloroform: methanol (1:1, v/v) extract showed efficient activity against *Cx. quinquefasciatus*, so it could be used as a mosquito larvicide agent. There is no change in the activity of non-target organism so, it is safe to use.

1. Introduction

Mosquitoes are the vectors of the major public health problem. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. The mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*) act as a vector for *Wuchereria bancrofti* responsible for filariasis, while *Anopheles stephensi* (*An. stephensi*) act as a vector of malaria in urban area. One of the methods available for the control of mosquitoes is the use of larvicides, which is one of the oldest methods of controlling malaria[1]. Among other advantages, use of larvicides controls mosquitoes before they are able to spread and transmit diseases[2]. While other methods like adult spraying may have direct effects like visible protection of populations and may show quick results, larval control has yielded several successes than adult mosquito control. Due to the disadvantages associated with synthetic pesticides, including development of pesticide resistant strains, ecological imbalances and harm to non-target organisms, there is a renewed effort to develop substances of plant origin which are considered to be more environmentally friendly due to their innate biodegradability and lower toxicity to most organisms[3]. Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents, which accounted for various uses by man. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins[4]. Mosquito control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence, crude plant extracts have played an important role in this aspect. A large number of plant products have been reported to have repellent activity[5-7].

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Foundation project: financially supported by the DST, New Delhi, India (D.O. NO.SR/SO/HS/84/2007 dated 08/02/08)

Repellents have an important role in protecting man from mosquito bite and in interrupting disease transmission. Repellents from the plant source are free from harmful effects. Protection against mosquito bites was reported for the *Ocimum suave* and *Ocimum kilimandscharicum*[8] and Mentha[9]. Citronella provide the active ingredient of repellents that are commercially sold under several brand names.

P. tuberosa is a perennial plant of the agave family Agavaceae, extracts of which are used as a middle note in perfumery. Its common name is "Rajnigandha". It consists of about 12 species. The flowers are used in wedding ceremonies, garlands, decoration and various traditional rituals. They have tall stems (2-3 ft.) and rather sparse, grass-like foliage. Since the tuberose essential oil has a calming, refreshing and sedative aroma, many aroma therapists around the word use this oil for the treatment of emotional stress, anxiety, and sleep disorders. The constituents of tuberose oil result in the release of certain hormones in the brain, which are responsible for libido and desire. It is therefore, widely used for the treatment of frigidity and loss of libido among women. As an aphrodisiac, it is equally good for men too[10]. A massage with this oil is said to cure the problem of erectile dysfunction and even impotency. Flowers and bulb are diuretic. Externally used for skin eruptions. The bulbs are rubbed with turmeric and butter and applied over red pimples of infants. Dried and powdered bulbs are used for gonorrhoea[11].

The present study was an attempt to find new larvicidal and repellent products from the extracts of plants to control the malarial vector *An. stephensi* and filarial vector *Cx. quinquefasciatus*.

2. Materials and Methods

2.1. Test mosquitoes

The present study was conducted at Burdwan (23°16′N, 87°54′E) West Bengal, India, in the Mosquito Research Unit, Department of Zoology, the University of Burdwan. *Cx. quinquefasciatus* larvae were collected from drains surrounding the university campus and *An. stephensi* larvae were collected from nearer rice field. Larvae of both mosquito species were kept separately in different plastic trays and fed with artificial food *i.e.* mixture of dog biscuits and dried yeast powder at the ratio of 3:1. Colonies were kept free from exposure to pathogen, insecticides or repellents.

2.2. Preparation of crude extracts

Fresh mature sample of *P. tuberosa* was collected from gardens of Burdwan, West Bengal, India. The samples were initially rinsed with tap water and dried on paper towel. Finally the samples were chapped into small pieces of approximately 1 cm size by sharp razor and crushed with a mixer-grinder machine and the juice was filtered by Whatman no-1 filter paper. The filtrate of each sample was used as stock solution for further bioassay experiment and required concentration *i.e.* from 0.1% to 0.5% were prepared through mixing up of stock solution with variable amount of distilled water.

2.3. Preparation of solvent extracts

We harvested 25 g of fresh bud of *P. tuberosa* which were rinsed with distilled water and dried in a shed. The dried buds were put in a Soxhlet apparatus and the plant extracts were prepared using ethyl acetate, chloroform: methanol (1:1, v/v) and acetone (extraction period 72 h and the temperature was < 40°C). The extract was collected separately. The eluted materials and extract was concentrated in combination at 40°C to 100 mL of extract by evaporation in a rotary evaporator. Then extract was filtered, solvent was evaporated and the solid residues were weighed and then dissolved in a suitable amount of sterilized distilled water for the formulation of graded concentrations. The total yield of ethyl acetate, chloroform: methanol (1:1, v/v) and acetone extract was noted.

2.4. Larvicidal bioassay

The larvicidal bioassay followed the World Health Organization[12] standard protocols with slight modifications. Each of the concentrations of crude bud extract (0.1 to 0.5 %) was transferred into sterile glass Petri dishes (9 cm diameter, 150 mL capacity). Ten 1st to 4th instar larval form of *Cx. quinquefasciatus* and *An. stephensi* were separately introduced into different Petri dishes containing appropriate graded concentrations and the mortalities were recorded after 24, 48 and 72 h of exposure periods. The data of mortality at 48 and 72 h were expressed by the addition of the mortality at 24 and 48 h, respectively. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times on separate three days and conducted under laboratory conditions at 25-30°C and 80-90% relative humidity, and the bioassay experiment with solvent extract was carried out on 3rd instar larval forms of both mosquito species as they exhibited highest mortality in bioassay experiment with crude plant extract.

2.5. Biting deterrency activity

Repellent activity of plant compounds was tested with human volunteers. For the repellent activity of plant extract percentage protection in relation to dose method was adopted[13]. Three to four days 100 old blood starved female adult mosquitoes were kept in a net cage. The arms of the tested person were chloroformaned with isopropanol. After air- drying the arm, only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves.

The plant extract was dissolved in isopropanol and this alcohol served as control. The plant extract at 1.0, 1.5, 2.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The number of bites were counted over 5 min, every 30 min, from 18:00

h to 06:00 h. The experiment was conducted five times. The percentage protection was calculated by using the following formula.

$$\% \text{ Protection} = \frac{\text{Number of bites received by control arm} - \text{number of bites received by treated arm}}{\text{Number of bites received by control arm}} \times 100$$

2.6. Effect on non target organisms

The effect of the crude and chloroform: methanol extract (1:1) solvent extract of bud of *P. tuberose* were tested against non-target organisms like Toxorhynchites larvae (mosquito predator), *Diplonychus annulatum* (predatory water-bug) and *Chironomus circumdatus* larvae (insect) as they share the common habitats of target mosquito larvae and some of them were natural predators of mosquito larvae. The predators were exposed to appropriate lethal concentration of crude and chloroform: methanol (1:1) solvent extracts at 24 h to observe the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h of exposure.

2.7. Statistical analysis

The percentage mortality observed was corrected using Abbott's formula[14] during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed using the computer software Statplus 2007 and MS EXCEL 2003 to find the LC_{50}, regression equations (Y=mortality; X= concentrations) and regression coefficient values.

3. Result

Data of larvicidal activity of crude extract of bud of *P. tuberose* against *Cx. quinquefasciatus* and *An. stephensi* were presented in table 1. The mortality rate of all larval instars of *Cx. quinquefasciatus* and *An. stephensi* at 0.5% concentration was significantly higher ($P< 0.05$) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentrations of crude plant extract at 24, 48 and 72 h of exposure. Higher mortality rate was also recorded at 72 h bioassay than those at 24 and 48 h. The LC_{50} and LC_{90} values and regression equation and regression coefficient values of crude extract of bud of *P. tuberosa* against *Cx. quinquefasciatus* and *An. stephensi* were presented in table 2. From table 2 revealed that LC_{50} and LC_{90} values were gradually decreased from exposure period and there is positive relation between mortality(Y) and concentration(X) having regression coefficient value close to one in each case. The result of third-instar larval mortality with ethyl acetate, chloroform: methanol extract (1:1) and acetone solvent extract bud of *P. tuberose* against larvae of both the mosquito species was presented in table 3. LC_{50} value of ethyl acetate, chloroform: methanol extract (1:1) and acetone solvent extract bud of *P. tuberose* were 195.33, 27.28 and 74.19 mg/L respectively, after 24 h of exposure. Table 4 shows the repellent activity of Chloroform: methanol extract (1:1) against the both the mosquito species. No change in the swimming behaviors and survivality were observed when crude and solvent extracts were studied on non-target organisms at appropriate lethal concentration of 24 h and the observation were continued up to 72 h. The total yield of ethyl acetate, chloroform: methanol (1:1, v/v) and acetone extract was 1.75, 2.25 and 2.90 g respectively.

Table 1

Mean larval mortality of mosquito larvae of different instars of *Cx. quinquefaciatus* and *An. stephensi* exposed to different concentration of crude extracts of bud of *P. tuberosa* (mean± SE)(%).

Instar	Concerntration	*Cx. quinquefaciatus*			*An. stephensi*		
	(%)	24 h	48 h	72 h	24 h	48 h	72 h
1st	0.1	43.30±0.33	50.00±0.58	66.70±0.33	40.00±0.00	46.70±0.33	56.70±0.33
	0.2	53.30±0.33	60.00±0.58	73.30±0.33	46.70±0.33	53.30±0.33	60.00±0.00
	0.3	66.70±0.33	76.70±0.33	90.00±0.00	63.30±0.33	70.00±0.00	73.30±0.33
	0.4	60.00±0.58	70.00±0.00	86.70±0.33	66.70±0.33	73.30±0.33	80.00±0.00
	0.5	83.30±0.33	86.70±0.33	96.70±0.33	76.70±0.67	83.30±0.33	86.00±0.33
2nd	0.1	73.30±0.33	80.00±0.00	93.30±0.33	63.30±0.33	60.00±0.58	66.70±0.33
	0.2	76.70±0.33	83.30±0.67	93.30±0.33	73.30±0.33	80.00±0.00	83.30±0.33
	0.3	83.30±0.33	93.30±0.33	96.70±0.33	76.70±0.33	76.70±0.33	83.30±0.33
	0.4	86.70±0.33	93.30±0.33	96.70±0.33	80.00±0..00	86.70±0.33	90.00±0.00
	0.5	83.30±0.33	96.70±0.33	100.00±0.00	93.30±0.33	96.70±0.33	100.00±0.00
3rd	0.1	63.30±0.33	73.30±0.33	83.30±0.33	73.30±0.33	76.70±0.33	83.30±0.00
	0.2	73.30±0.33	80.00±0.58	90.00±0.58	76.70±0.33	83.30±0.33	86.70±0.33
	0.3	83.30±0.33	90.00±0.00	96.70±0.33	80.00±0..00	86.70±0.33	90.00±0.00
	0.4	90.00±0.58	93.30±0.33	100.00±0.00	93.30±0.33	96.70±0.33	100.00±0.00
	0.5	93.30±0.33	96.70±0.33	100.00±0.00	96.70±0.33	100.00±0.00	100.00±0.00
4th	0.1	50.00±0.58	66.70±0.67	66.70±0.33	36.70±0.33	40.00±0.00	50.00±0.58
	0.2	66.70±0.67	73.30±0.33	83.30±0.33	43.30±0.33	53.30±0.33	60.00±0.00
	0.3	66.70±0.33	76.70±0.33	86.70±0.33	46.70±0.33	60.00±0.58	66.70±0.33
	0.4	80.00±0.58	86.70±0.33	90.00±0.00	56.70±0.33	60.00±0.33	73.30±0.33
	0.5	80.00±0.00	86.70±0.33	90.00±0.00	60.00±0.33	66.70±0.33	76.70±0.33

Table 2
Log-probit analysis and regression analysis of larvicidal activity of crude extracts of bud of *P. tuberosa* against different instar larval forms of *Cx. quinquefasciatus* and *An. stephensi*.

Instar	Hours	*Cx. quinquefasciatus*				*An. stephensi*			
		LC_{50}	LC_{90}	Regression equation	R value	LC_{50}	LC_{90}	Regression equation	R value
1st	24 h	0.15	1.29	Y= 8.67x + 3.53	0.91	0.18	1.38	Y= 9.34x + 3.07	0.98
	48 h	0.10	0.89	Y= 8.34x +4.37	0.92	0.14	0.73	Y= 9.32x + 3.73	0.98
	72 h	0.06	0.31	Y= 7.34x + 6.07	0.94	0.09	0.73	Y= 8.00x + 4.73	0.99
2nd	24 h	0.01	0.54	Y= 3.00x + 7.17	0.87	0.08	0.51	Y= 6.67x + 5.73	0.97
	48 h	0.05	0.15	Y= 4.34x + 7.67	0.95	0.07	0.37	Y= 8.01x + 5.59	0.94
	72 h	0.07	0.10	Y= 1.68x + 8.89	0.95	0.06	0.30	Y= 7.33x + 6.27	0.95
3rd	24 h	0.07	0.31	Y= 7.67x + 5.76	0.98	0.05	0.29	Y= 6.34x + 6.49	0.97
	48 h	0.05	0.23	Y= 6.01x + 6.86	0.98	0.04	0.19	Y= 6.00x + 7.06	0.98
	72 h	0.03	0.13	Y= 4.34x + 8.09	0.95	0.04	0.22	Y= 4.67x + 7.79	0.96
4th	24 h	0.09	1.12	Y= 7.33x + 4.67	0.94	0.30	7.03	Y= 6.00x + 3.06	0.89
	48 h	0.08	0.52	Y= 7.34x + 5.40	0.94	0.17	4.55	Y= 6.01x + 3.79	0.94
	72 h	0.05	0.42	Y= 5.33x + 6.73	0.87	0.11	1.39	Y= 6.67x + 4.53	0.98

R= regression coefficient, LC= lethal concentration

Table 3
Result of larval mortality of different concentration of solvent extracts of bud of *P. tuberosa* on third instar of *Cx. quinquefasciatus* (mean±SE) (%).

Solvent extract	Concerntration (mg/L)	*Cx. quinquefasciatus*			*An. stephensi*		
		24 h	48 h	72 h	24 h	48 h	72 h
Ethyl acetate	20	53.3±0.33	60.0±0.58	70.0±0.58	46.7±0.33	66.7±0.33	73.3±0.33
	40	60.0±0.58	70.0±0.58	73.3±0.33	53.3±0.33	56.7±0.33	73.3±0.33
	60	76.7±0.33	83.3±0.33	86.7±0.33	66.7±0.33	76.7±0.33	83.3±0.33
Chloroform: methanol(1:1)	20	63.3±0.33	76.7±0.33	80.0±0.00	56.7±0.33	70.0±0.00	80.0±0.58
	40	83.3±0.33	90.0±0.00	93.3±0.33	60.0±0.58	70.0±0.67	76.7±0.33
	60	86.7±0.33	93.3±0.33	96.7±0.33	73.3±0.33	80.0±0.00	86.7±0.33
Acetone	20	56.7±0.33	63.3±0.33	70.0±0.00	36.7±0.33	43.3±0.33	50.0±0.00
	40	63.3±0.33	66.7±0.67	70.0±0.58	43.3±0.33	53.3±0.33	56.7±0.33
	60	73.3±0.33	76.7±0.33	80.0±0.00	53.3±0.33	60.0±0.00	70.0±0.58

Table 4
Result of biting deterrency activity of bud of *P. tuberosa* [chloroform: methanol (1:1, v/v)] on *Cx. quinquefasciatus* and *An. stephensi*.

Mosquito species	Concentration (%)	Percentage protection (%)	Average Protection time (min)
Cx. quinquefasciatus	1.0	60	2.0 h
	1.5	72	3.0 h
	2.0	80	4.0 h
An. stephensi	1.0	65	2.3 h
	1.5	80	4.0 h
	2.0	90	5.0 h

4. Discussion

Now a day's vector control becomes problematic due to resistance of mosquitoes to conventional synthetic insecticides. Therefore, it is necessary to look for and find a better insecticide or larvicide, which could provide a safer and long-lasting control against all mosquito species. Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources. In addition, the use of medicinal plants for mosquito control is likely to generate local employment, reduce dependence on expensive imported products, and stimulate efforts to enhance public health.

The present investigation revealed that the bud extract of *P. tuberose* possess larvicidal and repellent activity against *Cx. quinquefasciatus* and *An. stephensi*. Crude extract of bud of *P. tuberose* showed effective result against third instar larvae of *Cx. quinquefasciatus* and *An. stephensi*. Among the solvent extracts chloroform: methanol extract (1:1) showed the best result against both the mosquito species followed by ethyl acetate and acetone. The present study also revealed that larvae of *Cx. quinquefasciatus* were more susceptible than *An. stephensi*. But chloroform: methanol extract (1:1) showed more repellent activity against *An. stephensi* than *Cx. quinquefasciatus*. Though several compounds of plant origin have been reported as larvicide[15], there is a wide scope for the discovery of more effective plant products. Govindarajan *et al*[16] reported the larvicidal and repellency efficacy of the leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against late third instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The highest larval mortality was found in benzene extract of *Ervatamia coronaria* against the larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values were 79.08, 89.59, and 96.15 mg/L and 150.47, 166.04, and 174.10 mg/L, respectively. The results of the repellent activity of benzene and ethyl acetate extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* plants at three different concentrations of 1.0, 2.5, and 5.0 mg/cm^2 showed

the effective repellency against these mosquito species. Kalu *et al*[17] studied on the ethanol extract of *Alium sativum* against the filarial vector *Cx. quinquefasciatus*. The LC_{50} values calculated were 144.54, 165.70, 184.18 mg/L against second, third, fourth instar larvae respectively.

Repellents are used as personal protection methods and are best alternative where other control measures are neither practical nor possible. Repellents are inexpensive means for reducing the man and vector contact. Many plant extracts and essential oils manifest repellent activity against different mosquito species[18]. Bream *et al*[19] reported the larvicidal and repellent activity of six plant extracts (using ethanolic and petroleum ether) of the indigenous aquatic plant *Echinochloa stagninum* against *Cx. pipiens*. The most effective plant extracts that evoked 100% repellency or biting deterrence were petroleum ether extracts of leaves, stems and roots at a dose of 5, 5 and 4.3 mg/cm^2. The toxicity values of the tested ethanolic extracts of different plant parts based on LC_{50} values are arranged in a decreasing order as follows: leaves (LC_{50} 80.32 mg/L) > stems (LC_{50} 112.78 mg/L) > roots (LC_{50} 2413.48 mg/L). Prabhu *et al*[20] studied on the repellent activity of *Moringa olifera* against *An. stephensi* where he recorded the 90.41% repellency at 100% concentration and 23.28% repellency was reduced after the treatment of 20% concentration.

Biopesticides from plant origin may contribute effective, inexpensive and safe method for vector control. These new findings may helpful to be applied in integrated control strategies to gain maximum impact on vector control. However, further investigation needed to carry out the identification of active compound which is environmentally acceptable and can be used against wide range of mosquito species.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors acknowledge with thanks the help of Dr. Ambarish Mukherjee, Professor of Botany, The University of Burdwan, for identification of the plant. The financial support provided by the DST, New Delhi, India (D.O. NO.SR/SO/HS/84/2007 dated 08/02/08) is thankfully acknowledged.

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