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(14)60473-4

Larvicidal activity of *Wrightia tinctoria* R. BR. (Apocynaceae) fruit and leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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PEER REVIEW

Peer reviewer

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Comments

The authors have evaluated the impact of various extracts from *W. tinctoria* on *Culex*. Each of the extracts is different in its potential to kill the larvae. Though this is a preliminary investigation, standard methods have been used and the results are interesting.
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ABSTRACT

Objective: To determine the larvicidal activity of crude aqueous and petroleum ether extracts of *Wrightia tinctoria* fruits and leaves against the filarial vector, *Culex quinquefasciatus*.
Methods: The larvicidal activity was evaluated at concentrations of 0.06%, 0.12%, 0.25%, 0.50% and 1.00%. Larval mortality was observed for 24 and 48 h.
Results: Among the plant parts tested, aqueous fruit extract exhibited highest larvicidal activity followed by aqueous leaf extract with LC_{50} values of 0.17% and 0.09%; 0.21% and 0.11% after 24 and 48 h respectively.
Conclusions: Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

KEYWORDS

Wrightia tinctoria, Crude fruit and leaf extracts, *Culex quinquefasciatus*, Larvicidal activity

1. Introduction

Blood feeding female mosquitoes are responsible for the biting nuisance and also in the transmission of more diseases than any other group of arthropods and play an important role as etiologic agents of vector-borne diseases[1]. Mosquitoes are one of the most significant vectors in medical point of view and they transmit parasites and pathogens which continue to have a devastating impact on human beings[2]. The vector-borne diseases caused by mosquitoes are one of the major health problems in many countries. Several species belonging to genera *Culex*, *Anopheles* and *Aedes*, are vectors for the pathogens of various diseases *viz*., filarial fever, Japanese encephalitis, malaria, dengue and chickungunya. *Culex* mosquitoes play a vital role in transmitting vector-borne diseases like lymphatic filariasis and Japanese encephalitis. *Culex quinquefasciatus* Say (Diptera: Culicidae) (*Cx. quinquefasciatus*) is a predominant house-resting mosquito in many tropical countries[3] and a pantropical pest and urban vector of *Wuchereria bancrofti* which causes filarial fever[4].

Culex mosquitoes breed in polluted waters such as blocked drains, damaged septic tanks, or soak age pools

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Foundation Project: Supported by Council for Scientific and Industrial Research (CSIR), Government of India (Grant No. 9/656(12)/2004-EMR-I).

Article history:
Received 15 Nov 2013
Received in revised form 27 Nov, 2nd revised form 4 Dec, 3rd revised form 13 Dec 2013
Accepted 29 Dec 2013
Available online 28 Jan 2014

close to human habitations and are the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. A primary element in the current global strategy for the control of vector-borne diseases is vector control, with chemical control remaining a main component of integrated vector management. Resistance has been reported to every chemical class of insecticide used in vector control programs[5]. There have been widespread applications of various commercial insecticides since few decades to combat these vector species. However, indiscriminate use of those synthetic chemicals unequivocally produced different adverse consequences on soil, water and air ecosystem, creating undesirable effects like toxicity to non-target organisms, human health and ultimately posing threat to/for global environment[6-8]. In addition, development of resistance in vectors against these widespread chemical insecticides results in rebounding of vectorial capacity[9], thus reducing the effective efficacy of those chemicals. It is established that repeated use of synthetic larvicide results in the development of resistance in mosquitoes, and hence effective ecofriendly phytochemicals are the need of the day. Plant world comprises a store house of biochemicals that could be tapped for use as insecticides and they are the richest source of renewable bioactive phytochemicals[10].

The search for alternative insecticides and control measures posing minimal risk to human health and the environment is of great interest from the preventive medicinal point of view. Several plant extracts and their phytochemical compounds have been reported to have detrimental effects on mosquitoes[11-16]. The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent[17-19]. The use of different parts of locally available plants and their various products in the control of mosquitoes have been well established globally by numerous researchers. The larvicidal properties of indigenous plants have also been documented in many parts of India along with the repellent and anti-juvenile hormones activities[20]. Almost all tropical regions of the world are experiencing the resurgence and reoccurrence of one of the world's most deadly diseases (filarial fever malaria, dengue and chikungunya) of the world and India is no exception. Traditionally, plants and their derivatives were used to kill mosquitoes and other household and agricultural pests. In all probability, plants used to control insects contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects[18,21].

Wrightia tinctoria (W. tinctoria) is a small deciduous tree, native to India[22] found grown abundantly in dry, hilly and rocky areas of Tamil Nadu[23]. Its vernacular name is Pala indigo plant in English, Vetpalai and Thondampalai in Tamil[24], and Dhudi in Hindi[22]. *W. tinctoria* is extensively used in the Indian system of medicine[25], and is commonly called as "jaundice curative tree" in South India since it possesses high medicinal value[26]. In folk medicine, the dried and powdered leaves are used to treat piles, fever, diarrohea and round worms[22]. Besides these, the plant also possesses pharmacological properties *viz*., antidandruff[22], antidiarrhoeal, antihaemorrhagic, antidysenteric[27], antiulcer[28], antibacterial and antifungal properties[29]. Very few reports are present pertaining to the insecticidal property of *W. tinctoria* wherein the leaf extract of this plant possesses larvicidal activity against the larvae of *Aedes aegypti* and *Cx. quinquefasciatus*[11].

Indeed, source reduction is one of the key components in vector control program since the target is exceptionally specific unlike adult control. Innovative vector control strategy like use of phytochemicals as alternative sources of insecticidal/larvicidal agents in the fight against vector-borne diseases has become inevitable. Above and beyond, in recent epoch, around the globe phytochemicals have gained massive attention by various researchers because of their bio-degradable and eco-friendly values[30]. In this context, the purpose of the present investigation is to explore the larvicidal properties of *W. tinctoria* crude fruit and leaf extracts against the filarial vector, *Cx. quinquefasciatus* under laboratory conditions.

2. Materials and methods

2.1. Plant collection and extraction

Mature fresh fruits and leaves of *W. tinctoria* collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered fruits (1 kg) each was macerated with 3 L of distilled water and petroleum ether for a period of 96 h each separately and filtered. The filtrate was then concentrated at reduced temperature on a rotary evaporator. The crude aqueous and petroleum ether fruit extracts thus obtained were lyophilized and a stock solution of 100 000 mg/L prepared by adding adequate volume of acetone was refrigerated at 4 °C until testing for bioassay. Likewise, the same methodology was followed for obtaining the crude aqueous and petroleum ether leaf extracts of the above mentioned plant.

2.2. Larvicidal bioassay

The studies were carried out against laboratory reared vector mosquitoes free of exposure to insecticides and pathogens. Standard WHO[31] protocol with slight modifications was adopted for the study. The tests were conducted in glass beakers. Mosquito immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of F1 generation. From the stock solutions, concentrations of 0.06%, 0.12%, 0.25%, 0.50% and 1.00% were prepared. Twenty healthy larvae were released into each 250 mL glass beaker containing 200 mL of water and test concentration. Mortality was observed for 24 and 48 h after treatment. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Control was prepared by the addition of acetone to water. The larval per cent mortality was calculated and when control mortality ranged from 5%-20% it was corrected using Abbott's[32] formula. SPSS 11.5 version package was used for determination of LC_{50} and LC_{90}[33]. Two way ANOVA followed by Tukey's test was performed to determine the difference in larval mortality between concentrations.

3. Results

The results of *Cx. quinquefasciatus* larval mortality tested against *W. tinctoria* crude aqueous and petroleum ether fruit and leaf extracts are presented in Table 1. The results of the present study revealed that among the plant parts tested, aqueous fruit extract exhibited highest larvicidal activity followed by aqueous leaf extract with LC_{50} values of 0.17% and 0.09%; 0.21% and 0.11% after 24 and 48 h respectively (Table 2). Further, the effect of larval mortality was dependent on the concentration of extracts and the results of present study are comparable with earlier reports of plant extracts tested against *Cx. quinquefasciatus*.

Table 1

Larval mortality of *Cx. quinquefasciatus* against *W. tinctoria* crude extracts.

Plant part	Solvent	Concentration (%)									
		0.06		0.12		0.25		0.50		1.00	
		24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Fruit	Aqueous	2.66 ± 2.31^{b} (13.3)	9.34 ± 1.15^{b} (46.7)	10.00 ± 1.00^{b} (50.0)	17.34 ± 3.06^{c} (86.7)	15.34 ± 1.15^{c} (76.7)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)
	Petroleum ether	0.00 ± 0.00^{a} (0.0)	2.66 ± 3.06^{b} (13.3)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	4.66 ± 2.31^{b} (23.3)	2.66 ± 3.06^{b} (13.3)	6.66 ± 2.31^{b} (33.3)
Leaf	Aqueous	0.00 ± 0.00^{a} (0.0)	2.00 ± 0.00^{b} (20.0)	9.34 ± 1.15^{b} (46.7)	14.66 ± 1.15^{c} (73.3)	12.88 ± 1.15^{d} (64.4)	18.66 ± 1.15^{e} (90.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)
	Petroleum ether	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	2.66 ± 3.06^{b} (13.3)	17.34 ± 1.15^{c} (86.7)	17.34 ± 1.15^{c} (86.7)	20.00 ± 0.00^{e} (100.0)	20.00 ± 0.00^{e} (100.0)
Control		0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)

Values are mean (%) of the three replicates of three trials±standard deviation. Figures in paranthesis denote per cent larval mortality. Different superscript alphabets in column indicate statistical significant difference at $P<0.05$ level by two way ANOVA followed by Tukey's test.

Table 2

Probit analysis of larvicidal efficacy of *W. tinctoria* crude extracts against *Cx. quinquefasciatus*.

Plant part	Solvent	LC_{50} (%)		LC_{90} (%)	
		24h	48h	24h	48h
Fruit	Aqueous	0.17	0.09	0.30	0.24
	Petroleum ether	1.71	1.31	2.61	2.28
Leaf	Aqueous	0.21	0.11	0.32	0.21
	Petroleum ether	0.37	0.37	0.60	0.60

4. Discussion

Vector control is one of the most powerful weapons in the process of managing vector populations so as to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control program due to lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vectors[34]. Plants and plant parts have been provided as a good source of inspiration for novel drug compounds, as plant derived drugs have made large contribution to human health. The use of plant extracts, as well as other alternative forms of medical treatment, is enjoying great popularity in the late 1990s[35]. Different parts of plants contain a complex of chemicals with unique biological activity[36]. Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants[11,15,16] and the activity of crude plant extracts subjected further to partial purification with respective solvent washed fraction is often distributed to the complex mixture of active compounds[37].

Results regarding the larvicidal efficacy of this plant are supported by findings of Singh and Bansal[20], who studied the larvicidal activity of aqueous fruit (LC_{50} 0.05%) and root extract (LC_{50} 1.08%) of *Solanum xanthocarpum* against the larvae of *Anopheles stephensi*. Aliero[38] investigated that aqueous seed, leaf and bark extracts of *Azadirachta indica* showed larvicidal activity with 100%, 98% and 48% mortality respectively after 12 h of treatment against *Anopheles stephensi*. The crude aqueous extracts of *Hemidesmus indicus* root, and leaf of *Gymnema sylvestre* and *Eclipta prostrata* showed 100% mortality at 5% concentration after 48 h of exposure against the larvae of *Cx. quinquefasciatus*[39]. The crude acetonic extracts of *Jatropha curcas, Syzygium cumini, Delonix regia, Limonia acidissima, Millingtonia hortensis, Capparis spinosa* and *Piper cubeba* exhibited larvicidal activity against *Aedes aegypti* with LC_{50} values of 0.85%, 0.86%, 1.07%, 1.24%, 1.61%, 1.77% and 1.83% respectively[40]. Abdullahi *et al*.[41] reported the crude aqueous leaf extract of *Striga hermonthica* and *Mitracarpus scaber* to exhibit more than 80% larval mortality in *Cx. quinquefasciatus* at 1.0% and 0.5% concentration after 24 h. The crude leaf extracts of *Mesua ferra* exhibited larvicidal activity against the third instar larvae of *C. quiqnuefasciatus* with LC_{50} values of 0.10%

and 0.07% for 24 and 48 h respectively[42]. Karthikeyan *et al*.[43] reported 100% in ethyl acetate, ethanol, petroleum ether, 97% mortality in crude aqueous leaf extract of *Vitex negundo* and less than 40% in *Leucas aspera* leaf extracts of the above mentioned solvents against *Cx. quinquefasciatus* at 1.0% concentration. The crude ethyl acetate seed coat extract of *Cassia sophera* showed larvicidal activity with LC_{50} value of 1.13% and 0.47% for 24 and 48 h respectively against *Cx. quinquefasciatus*[44].

Natural product research by using botanicals are progressively going on to identify novel bio-potency compounds to replace environmentally hazardous synthetic pesticides. The indiscriminate application of synthetic pesticides adversely affects the environment and human health. In addition, pesticide resistance has resulted in the resurgence of mosquito-borne diseases[45]. Recent report highlights botanical extracts not only having mosquitocidal properties but also water purification properties[46]. The mass breeding of *Cx. quinquefasciatus* is very common in highly polluted water with rich organic resources. Plant extracts possessing larvicidal and water purification properties are very much useful to maintain the aquatic environment free from pollution and at the same time to control immature mosquitoes in their breeding sites. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also to identify the active ingredient(s) of the extract responsible for larvicidal activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors thank the Director, King Institute of Preventive Medicine and Research for the facilities provided, and Council for Scientific and Industrial Research (CSIR), Government of India for the financial support (Grant No. 9/656(12)/2004-EMR-I).

Comments

Background

This paper describes the larvicidal activity of *W. tinctoria* fruit and leaf extracts against the filarial vector *Cx. quinquefasciatus*. The work is relevant to the Asian countries where the menace due to *Culex* is high. The use of safe plant based products for control has been experimented.

Research frontiers

The paper discusses the use of *W. tinctoria* fruit and leaf extracts. The role of extracts from different solvents have been tested. The results are interesting as the extracts are able to cause the mortality of *Cx. quinquefasciatus* larvae.

Related reports

With regard to the materials and methods part, standard WHO method was adopted. The aqueous fruit extract of *W. tinctoria* exhibited larval mortality and data subjected to statistical analysis were found to be significant and good.

Innovations & breakthroughs

Scientific data on the use of natural pest control agents is encouraging and is eco-friendly. This paper has highlighted the use of a plant which has not been widely explored as a pest control agent.

Applications

The experimental results indicate that the extracts of the plant could be used as an effective source of natural pest control especially of a vector known to cause filariasis.

Peer review

The authors have evaluated the impact of various extracts from *W. tinctoria* on *Culex*. Each of the extracts is different in its potential to kill the larvae. Though this is a preliminary investigation, standard methods have been used and the results are interesting.

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