Parasite Epidemiology and Control 1 (2016) 78–84

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

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

Bioefficacy of ecbolin A and ecbolin B isolated from *Ecbolium viride* (Forsk.) Alston on dengue vector *Aedes aegypti* L. (Diptera: Culicidae)

Appadurai Daniel Reegan [a,b], Munusamy Rajiv Gandhi [a], Govindan Sivaraman [a], Kalaimaran Francina Cecilia [c], Ramalingam Ravindhran [c], Kedike Balakrishna [a], Michael Gabriel Paulraj [a], Savarimuthu Ignacimuthu [a,d]

[a] Division of vector control, Entomology Research Institute, Loyola College, Chennai 600 034, Tamil Nadu, India
[b] National Vector Borne Disease Control Programme, ROH&FW, Govt. of India, Besant Nagar, Chennai 600 090, Tamil Nadu, India
[c] Department of Plant Biology and Biotechnology, Loyola College, Chennai 600 034, India
[d] Visiting Professor Programme, Deanship of Research, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form 15 March 2016
Accepted 15 March 2016
Available online 6 April 2016

Keywords:
Ecbolin A
Ecbolin B
Ecbolium viride
Bioassay
Aedes aegypti

ABSTRACT

Ecbolin A and ecbolin B were isolated from ethyl acetate extract of *Ecbolium viride* (Forsk.) Alston root and evaluated for larvicidal and growth disturbance activities against *Aedes aegypti* L. (Diptera: Culicidae). For larvicidal activity, the third instar larvae of *A. aegypti* were exposed to different concentrations viz., 1.0, 2.5, 5.0 and 10 ppm for each compound. Among the two compounds screened, ecbolin B recorded highest larvicidal activity with LC_{50} and LC_{90} values of 0.70 and 1.42 ppm, respectively. In control, the larval behaviour was normal. The active compound ecbolin B was tested for growth disruption activity at sub lethal concentrations viz., 0.5, 1.0 ppm and observed for malformation like larval gut elongation, larval longevity, intermediates, malformed adults, failed adult emergence and compared with methoprene. The results showed significant level of larva–pupa intermediates, pupa–adult intermediates, malformed adult emergence and less adult formation against *A. aegypti*. The histopathological results revealed a severe damage on the midgut epithelial columnar cells (CC) and cuboidal cells (CU) in ecbolin B treated larvae of *A. aegypti*. Similarly peritrophic membrane (pM) was also observed to be damaged in the treated larvae. The present results suggest that, ecbolin B could be used as a larvicidal agent against dengue vector *A. aegypti*.

1. Introduction

Mosquitoes are arthropod vectors responsible for transmitting various pathogens and mosquitoes are called as 'Public Enemy Number One' (WHO, 1996a). *Aedes aegypti* L. is the primary vector, which is involved in the transmission of arboviruses responsible for major diseases like dengue, dengue hemorrhagic fever, chikungunya and zika (Harrington et al., 2005; Kannathasan et al., 2011). Dengue fever is endemic to many tropical countries including India (Ahmed and Akram, 2005; Valenca et al., 2013).

Mosquito borne diseases are major threat to human health. Currently, biological and chemical compounds like *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (BS), pyrethroids, pyriproxyfen, permethrin, diflubenzuran and methoprene are used as larvicidal and growth regulating products in integrated vector management (IVM) against *A. aegypti* (Bellini et al.,

E-mail address: eriloyola@hotmail.com (S. Ignacimuthu).

http://dx.doi.org/10.1016/j.parepi.2016.03.004

2014). Botanical compounds are also a good choice for IVM based control of mosquito larvae (Sutthanont et al., 2010; Madhu et al., 2010; Bayen, 2012; Muthu et al., 2012; Costa et al., 2012). Literature reveals many reports on phytocompounds against vector mosquitoes (Pelah et al., 2002; Jang et al., 2005; Chapagain et al., 2008; Perumalsamy et al., 2009; Han et al., 2013; da Silva Gois et al., 2013). Many authors proved that, phytocompounds primarily affect the biosynthesis or the mechanisms of ecdysone, showed disturbing effects on mosquito larval growth and moulting (Reegan et al., 2014; Sakthivadivel and Thilagavathy, 2003; da Silva et al., 2013; Corzo et al., 2012).

Ecbolium viride (Forsk.) Alston (Acanthaceae) is a perennial woody under shrub (also known as Green Shrimp) found in the plains of India and also in Arabia, Malaysia, Sri Lanka and Tropical Africa (Rastogi, 1979; Cecilia et al., 2014). This plant is widely used in Indian traditional medicinal system such as Siddha, Ayurveda, Unani and Folk (Nair et al., 1985; Khare, 2007). In folk medicine, aqueous extract of dried roots of the plant is used for menorrhagia (Datta and Maiti, 1968; Kirtikar and Basu, 1987). The roots of *E. viride* used for the treatment of jaundice (Nair et al., 2007) and rheumatism (Shanmugam et al., 2009); while the roots and leaves together are used against tumour (Yusuf et al., 2009). Further, the extracts obtained from the root of *E. viride* showed a number of pharmacological activities viz., antioxidant (Babu et al., 2011), anti-inflammatory (Lalitha and Sethuraman, 2010), anti-hepatotoxicity (Priyadharshni et al., 2011; Pandey, 2011), antiplasmodial, antitrypanosomal and antimalarial activity (Abdel-Sattar et al., 2009).

In our earlier study, we have reported the larvicidal and pupicidal activities of ecbolin A and ecbolin B isolated from the ethyl acetate extract of *E. viride* root against *Culex quinquefasciatus* (Cecilia et al., 2014). In the present study, the isolated compounds, ecbolin A and ecbolin B were assessed for their effects on *A. aegypti*.

2. Materials and methods

2.1. Plant collection, extraction and isolation of ecbolin A and ecbolin B

Roots of *E. viride* (Fig. 1) were collected from Srirangam, Trichy, Tamil Nadu, India. The crude ethyl acetate extract (40 g) of *E. viride* root was subjected to column chromatography over silica gel (200 g- Qualigens 100–200 mesh) and eluted with n-hexane followed by combinations of n-hexane: ethyl acetate (95:5 to 0:100) and ethyl acetate: methanol (95:5 to 0:100). The eluted fractions were combined based on the TLC results and finally a total of twelve fractions were obtained. Based on the activity results, the fraction 6 and fraction 7 were selected for further crystallization and identified as ecbolin B and ecbolin A, respectively. A detailed procedure on processing plant material, isolation of ecbolin A and ecbolin B (Figs. 2 and 3) and structural elucidation have been described in our earlier reports (Cecilia et al., 2012a,b; 2014).

2.2. Insect rearing

Larvae of *A. aegypti* were obtained from the stock culture maintained at the Entomology Research Institute laboratory, which were free of exposure to pathogens, insecticides or repellents. Rearing conditions were 27 ± 2 °C temperature, 75–85% relative humidity and a photoperiod of 14 ± 0.5 h.

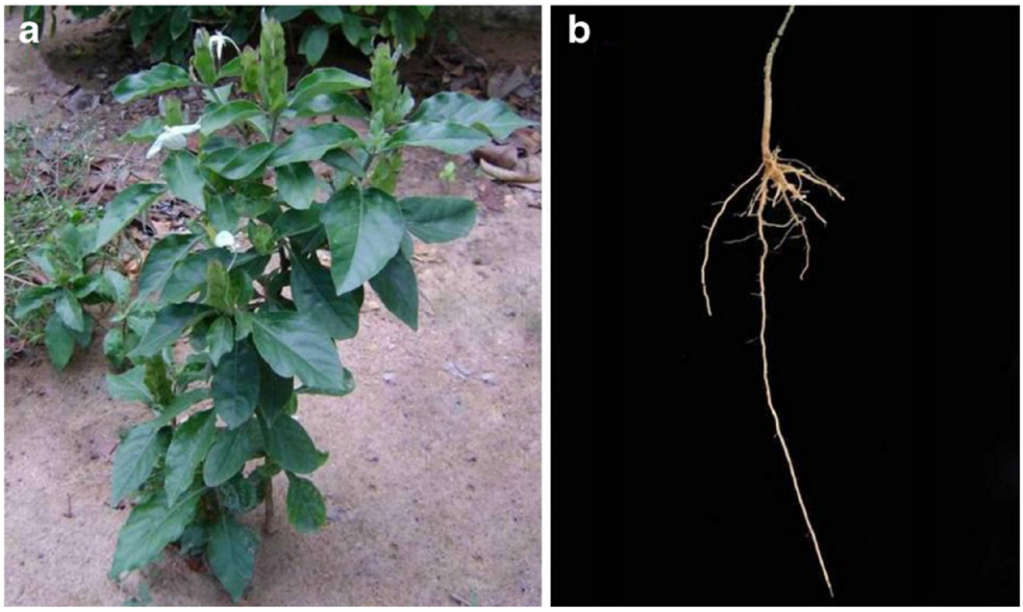

Fig. 1. *Ecbolium viride* whole plant (a) and its root (b). Cecilia et al. (2014).