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SHORT COMMUNICATION

Antimalarial efficacy of dynamic compound of plumbagin chemical constituent from *Plumbago zeylanica* Linn (Plumbaginaceae) against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae)

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Abstract In the present investigation, the effective root compound of plumbagin of *Plumbago zeylanica* (Plumbaginaceae) was evaluated for chemical constituent and antimalarial effect against the fourth instar larvae of *Anopheles stephensi* Liston (Diptera). In the chromatographic analyses of root compound with Rf value of 0.788 and NMR analyses also revealed that the effective compound contain naphthoquinone plumbagin were identified as the major chemical constituent. Larval mortality was observed after 3 h of exposure period. The plumbagin compound showed remarkable larvicidal activity against *A. stephensi* (LC_{50} 32.65 and LC_{90}72.27 ppm). Histopathological effects of compound was observed in the treated larvae. Based on the results, the plumbagin compound of *P. zeylanica* can be considered as a new source of natural larvicide for the control of malarial vector.

Keywords Mosquito vector · Medicinal plant · NMR · Larvicide · Histopathology

Introduction

The vector-borne diseases caused by mosquitoes are one of the major health problems in tropical and subtropical countries. Malaria is a deadly disease resulted in 207 million cases and about 627,000 deaths in 2012 (WHO 2013). In India, *Anopheles stephensi* is responsible for malaria transmission in urban areas (Senthilkumar et al. 2009). Management of disease vector by means of synthetic chemicals has facing a threat due to the emergence of resistance, effect on environmental pollution and nontarget organisms. This has necessitated requiring for search and development of environmental secure, biodegradable and indigenous method for vector control (Amer and Mehlhorn 2006a). Hence, the search for such effective compound of plant-based insecticides/larvicides (particularly root compound) will be an inheritance to overcome the resistance problem. Furthermore, the resistance by vectors against plant-derived insecticides has not been reported thus far (Kannathasan et al. 2011). A quantity of reports establish the mosquito larvicidal potential of the plant extracts and the essential oils obtained from the different parts of the variety of plants (Amer and Mehlhorn 2006b; Amer and Mehlhorn 2006c; Kalaivani et al. 2012; Thanigaivel et al. 2012; Senthil-Nathan 2007, 2013), though the insecticidal effects of plant chemicals differ not only according to plant species, mosquito species and plant parts, but also to extraction methods. Several studies have focused on insecticidal, larvicidal and repellent properties of natural products for controlling *Anopheles* mosquito, but have reported varied results (Senthil-Nathan et al. 2005, 2006). Previous investigations have indicated that various *Plumbago* plant crude extracts show the highest larvicidal effect on *Anopheles gambiae* (Maniafu et al. 2009).

Plumbago zeylinica Linn. is an age-old Rasayan herb in traditional Ayurved and commonly known as white chitraka, belongs to Plumbaginaceae family. It is distributed as a weed throughout the tropical and subtropical countries of the world (Singh et al. 2011). In the root part of *P. zeylanica*, it is used for

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treatment of different ailments such as parasitic diseases, fever or malaria (Olagunju et al. 2006; Jeyachandran et al. 2009; Jiangsu New Medical College 1979; Dai et al. 2004) and possible therapeutic properties along with enormous antioxidant activity (Tilak et al. 2004). Some active compounds previously identified and isolated from the root of *P. zeylanica* include five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone) (Lin et al. 2003). Moreover, these studies were focused only on antimicrobial, antioxidant and cytotoxic activities. To the best of our data, the effective compound of root of *P. zeylanica* has not been studied for antimalarial activity. So, the present study was made to analyse the chemical constituents and to study the antimalarial activity of *P. zeylanica* root compound identified through NMR and histolopathological changes against the fourth instar larvae of *A. stephensi*.

Materials and methods

Plant extraction and chromatographic analysis of root

Methanolic extract of roots of *P. zeylanica* (Fig. 1) were collected from plants of natural forest of Kalakadu Mundanthurai Tiger Reserve (KMTR) Forest, Kadayam. The plant roots were washed thoroughly in 500 ml of distilled water and surface sterile with the mercuric chloride. Fresh roots were air-dried in shade at room temperature, and the materials were powdered. The finely

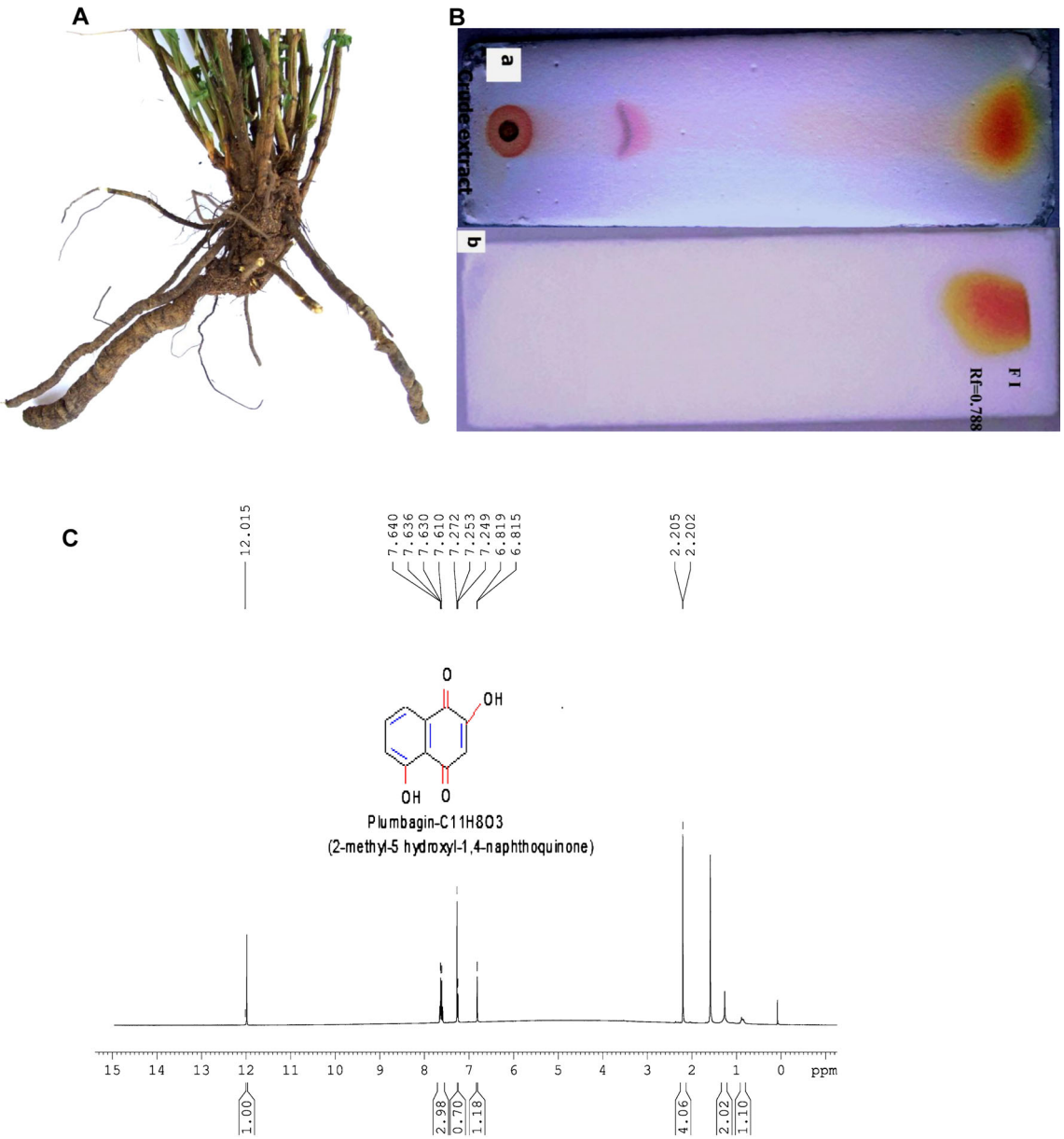

Fig. 1 **a** Morphology of *P. zeylanica* root. **b** Methanolic crude extract of *P. zeylanica* root and isolated *P. zeylanica* root fraction I (plumbagin), silica-gel fluorescence TLC, toluene/ethyl acetate (3:1) mobile phase. (**c**) ^{1}H NMR spectra of fraction I of *P. zeylanica* and the chemical structure of plumbagin

ground (100 g) roots were loaded with 400 ml of methanol for 48 h using Soxhlet equipment until exhaustion (Vogel 1978). The *P. zeylanica* with methanol solvent yielded 80 g of crude residue and stored at 4 °C until further use. The residue (5.0 g) was chromatographed on a 160-g silica-gel column of a mesh size of 60–120, and elution was commenced with gradient toluene and ethyl acetate mixture (6:1). Polarity of the solvent was increased in a gradient of 6:1, 3:1, 1:1, 0:1, and finally, the column was washed with 50 ml of water and the 50 ml of fractions 1–4 (a), 5–9 (b) were collected. Two hundred milliliters of eluents were used. Further, thin layer chromatography (TLC) was also monitored and scraped the required band diluted with 20 ml of methanol. This product was centrifuged at 10,000 rpm for 20 min, and supernatant was used as the experiment. Fraction (b) was known as fraction I, carried out for further process.

NMR analysis of fraction I

^{1}H NMR spectra of methanol extract fraction I of *P. zeylanica* was recorded on a NMR-400 MHz and chemical shifts were recorded as δ values. The result graph was compared with the reference chart and possible functional group present in the plant were determined (Silverstein et al. 2005).

Larvicidal assay and histopathological analysis

The eggs of *A. stephensi* were received from the Indian Council of Medical research-Government of India, Madurai. The larvae were fed with Brewer's yeast/dog biscuit (1:3). The larvicidal activity was observed as per the standard procedures

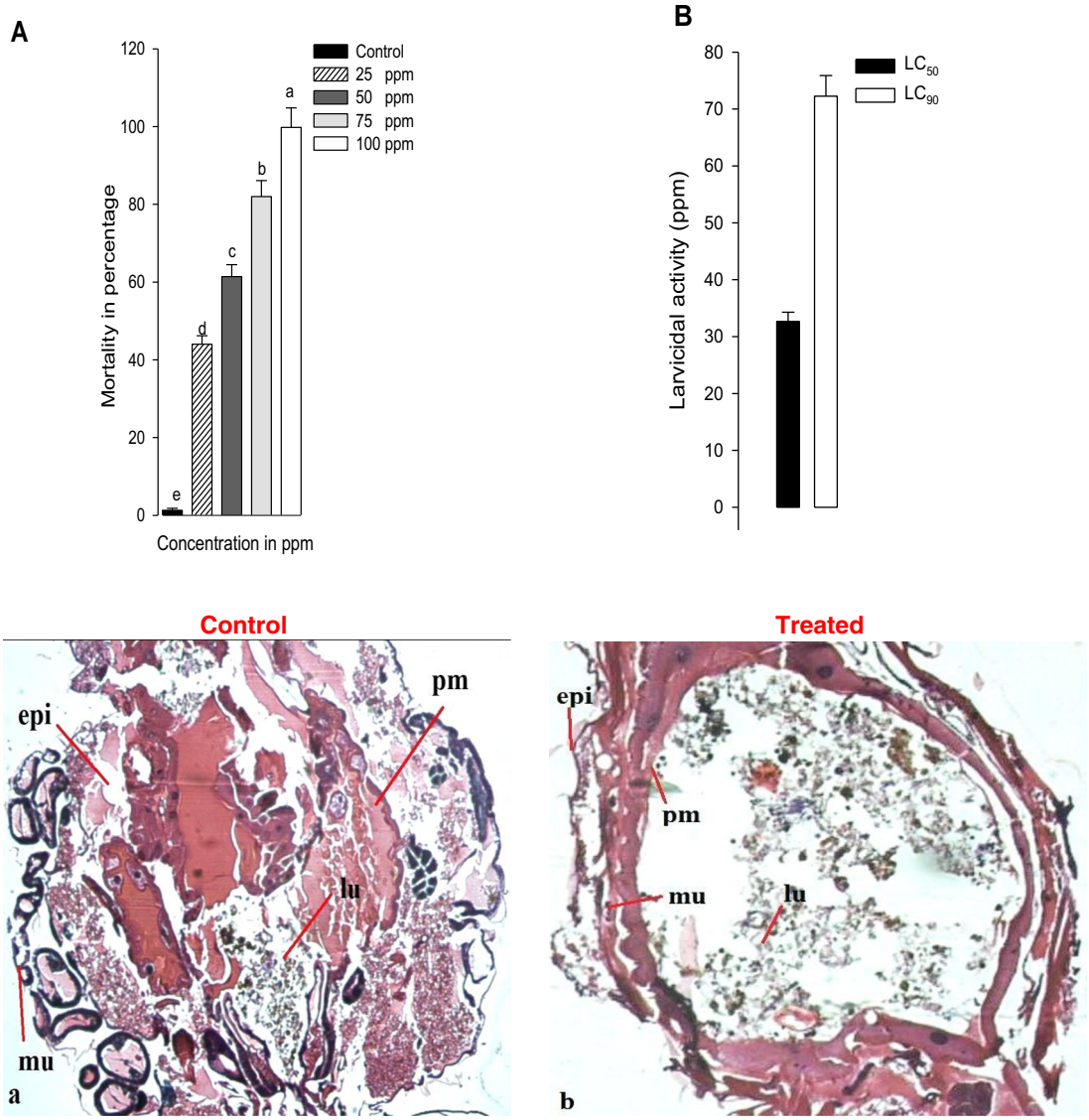

Fig. 2 **a** Percentage mortality of the fraction I of *P. zeylanica* against fourth larval instar and lethal concentrations (LC50 and LC90) of *A. stephensi*. Mean (±SEM) followed by the *same letter* in each larval instar in *bars* indicate no significant difference ($p<0.05$) in Tukey's test. **b** Longitudinal section of the midgut of third instar larvae of *A. stephensi* treated with *P. zeylanica* 0.1 %. *lu* gut lumen, *mu* muscles, *pm* peritrophic matrix, *epi* vacuolated gut epithelium. The *scale bar* (*line*) is 50 μm. Magnification is×40

recommended by the World Health Organization (1981). The methanolic compound (plumbagin) was prepared into different concentrations viz. 25, 50, 75 and 100 ppm with distilled water. Twenty larvae (in a 100-ml beaker) of early fourth instar stage were used for larvicidal assay, and five replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. The larval mortality was calculated after 3 h of the exposure period. The lethal concentrations, LC_{50} and LC_{90} and their 95 % confidence limit was calculated by profit analysis (SPSS, version 11.5). Treated larva was fixed, dehydrated and mounted in paraffin blocks. Eight-micrometer thick sections of larval tissue blocks were cut, mounted on glass slides and stained with haematoxylin and eosin for histopathological examination using bright field light microscope.

Results and discussion

The Rf value was calculated on root of *P. zeylanica* compound (0.788) (Fig.1b). The NMR analyses were presented in Fig.1c. Chemical constituents were identified from the compound and the major constituent signals for a broad singlet value of hydroxyl group at δ 12.015. Three and two mutually coupled aromatic protons appeared at δ 7.640, δ 7.272, δ 6.819 and δ 2.205, δ 2.202 naphthoquinone plumbagin and compared to literature values (Arunachalam 2010). The splitting pattern and coupling constants revealed the presence of a trisubstituted benzene ring. The isolated compound was subjected that determined the number of hydrogen atoms present and the structural name of isolated compound of plumbagin is 2-methyl-5-hydroxyl-1, 4-naphthoquinone ($C_{11}H_8O_3$). When compared to control (Fig. 2c(a)), larvae treated with *P. zeylanica* compound suffered various histological changes (Fig. 2c(b)). The midgut epithelium was smashed and cells were vacuolated but remained enclosing the nuclei. Muscles appeared slightly damaged and disorganized. These results shown that the midgut was most affected tissues in larvae treated with *P. zeylanica* effective compound. The bioactive compound showed 100 % larval mortality, ($F_{4,20}$ =74.75, p<0.001) for the fourth instar of *A. stephensi* at 100 ppm (Fig. 2a). The larvicidal activity of the compound may be due to the presence of the major chemical constituents such as naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone). The effective compound from the root of *P. zeylanica* had remarkable larvicidal activity (Fig. 2b) against *A. stephensi* (LC_{50} 32.65 and LC_{90} 72.27 ppm). Patil et al. (2010) showed larvicidal activity of crude methanol, dichloromethane and chloroform extracts of the leaves and roots of six Indian plants *Aegle marmelos*, *Balanites aegyptiaca*, *Calvatia gigantica*, *Murraya koenigii*, *Nyctanthes arbor-tristis* and *P. zeylanica* were tested against the early fourth instar larvae of *Aedes aegypti* and *A. stephensi*.

In recent years, the mosquito control programme using artificial chemicals have unsuccessful because of the ever-increasing insecticide resistance (WHO 1992). However, control of vector mosquito larvae normally depends on continued application of organophosphates and insect growth regulators (Yang et al. 2002), and these artificial chemical agents have been positive so far, because of their quick action and simple application. So, use of natural products, particularly root compound will be a respond to the questions regarding the insecticide resistance. In that way, the bioactive compound of *P. zeylanica* can be considered as a new source of antimalarial effect for the control of *A. stephensi*.

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