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Larvicidal Activity of Some *Curcuma* and *Kaempferia* Rhizome Extracts Against Dengue Fever Mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae)

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Investigations were carried out to test the larvicidal activity of extracts of some Zingiberaceae plants species against the larvae of dengue fever mosquito *Aedes aegypti*. Fifteen crude extracts from three *Curcuma* species and two *Kaempferia* species were used for the test. Most of these plants were toxic to the mosquito larvae and indicated promising activity with LC_{50} values of less than 90 µg/mL. Among the five plants, hexane extract of *Curcuma xanthorrhiza* was the most toxic against *Aedes aegypti* with LC_{50} value of 26.4 mg/mL.

Key Words: Larvicidal, Zingiberaceae, *Aedes aegypti*, *Curcuma*, *Kaempferia*.

INTRODUCTION

The plants belonging to Zingiberaceae family are found to be rich source of substances of biological interest. A large numbers of plants from this family are being used in traditional medicine practices. Traditional medicinal practitioner in India and China has long used turmeric as a remedy for hepatitis, sepsis, dyspepsia and a number of other disorders. Recent studies have not only confirmed the scientific grounds of many of these older applications, but have found also several new and useful properties as well, such as anticancer, antiviral and antiinflammatory[1,2].

Several frequently used herbal drugs were derived from the *Curcuma* species of the family Zingiberaceae. The rhizomes and roots of *Curcuma* are commonly used as traditional drugs. The plants have been used for spices, medicines, dyes, foods, perfumes and tonic and as tropical greenhouse ornamentals. *Kaempferia* species are well known for its nice smell and usually used as medicine to treat cold, stomachache and dysentery by the natives. Besides that, the rhizomes are used for coughs and as a masticatory[3]. Phytochemical studies had shown that *Kaempferia* plants are rich in cyclohexane oxide derivatives, chalcone derivatives, cinnamates, terpenoids and flavonoids[4].

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Mosquitoes are widespread in most of the tropical regions and become serious vectors of several diseases like malaria, dengue fever and yellow fever and cause serious health problems to human beings. One of the available methods used for the control of mosquitoes is the use of chemical insecticides. The method is favourable because of their fast action and easy application. However, their repeated and excessive uses have developed the resistance among the insects and poses severe health hazards and environmental problems. All these highlight the need for alternative method to control the mosquito larvae. Globally there have been conscientious efforts to overcome these problems and great emphasis has been placed recently on environment-friendly and economically viable methodologies for pest control[5].

Plants are the richest source of renewable bioactive organic chemicals and have potential to be used as botanical pesticides for eradication of mosquito larvae. Besides inexpensive, in some cases they are equally effective and easily biodegradable. Over the past 50 years, more than 2,000 plant species belonging to different families and genera have been reported to contain toxic principles which are effective against insects. Mosquito larvae of different species display different susceptibilities to some phytochemicals. Here we wish to report on the larvicidal activity of rhizome extracts of there *Curcuma* and two *Kaempferia* species against larvae of *Aedes aegypti*. The biological activities of these Zingiberaceous plants towards the mentioned dengue fever mosquito have never been reported previously.

EXPERIMENTAL

The plant materials were collected from Yogyakarta, Central Java, Indonesia. The plants were identified by Dr. Suwijio Pramono and voucher specimen has been deposited at the Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University. The finely ground air-dried rhizomes (1 kg) of each plant were extracted three times with petroleum ether or *n*-hexane each for 72 h. The extracts were filtered and concentrated under reduced pressure. The residue was re-extracted with chloroform or dichloromethane and methanol or ethanol each for three times. The larvicidal activities of these dried extracts were then tested against larvae of *A. aegypti*.

The bioassay tests on *A. aegypti* were carried out to investigate the larvicidal activity of samples following standard methods recommended by World Health Organization (WHO) with some modification[6]. A stock solution with concentration of 5000 µg/mL was prepared by dissolving 100 mg of crude extract in 20 mL absolute ethanol. A series of 50 mL of stock solutions with concentration of 50, 100 and 150 µg/mL were prepared for range finding. A control solution was prepared using 1.5 mL of absolute ethanol and chlorine-free water. Ten late third instars larvae were introduced into each glass containing dechlorinated water and the extract standard solution was then added to each glass. After 24 h mortality counts were made and the experiments were repeated twice. The values of LC_{50} which account to 50 % mortality of larvae were calculated using the Probit Analysis Program[7].

RESULTS AND DISCUSSION

Several plants samples from genus *Curcuma* and *Kaempferia* were extracted using various solvents of increasing polarity at room temperature. The extracts were screened for its larvicidal activities against the larvae of *A. aegypti*. In this investigation the larvicidal activity of plant extracts from the *C. xanthorrhiza*, *C. heyneana*, *C. mangga*, *K. angustifolia* and *K. rotunda* are reported. Tables-1 shows LC_{50} values of various extracts of plant samples. The hexane extract of *C. xanthorrhiza* indicated high toxicity against larvae of *A. aegypti* with LC_{50} value of 26.4 μg/mL. Besides this, the petroleum ether extract of *C. heyneana* also gave high toxicity with LC_{50} value of 34.9 μg/mL. However the larvicidal activity of these plant extracts were considered not that prominent if compared with two well known botanical insecticides, *Azadirachta indica* and *Asimina triloba*, both are having LC_{50} vales below 20 μg/mL.

TABLE-1
LARVICIDAL (*Aedes aegypti*) ACTIVITY OF CRUDE EXTRACTS OF THE PLANTS

Plant	Extract	LC_{50} (μg/mL)*	Slope ± S.E.**
Curcuma xanthorrhiza	Hexane extract	26.4	3.54 ± 0.93
	Dichloromethane	65.9	4.24 ± 0.99
	Ethyl acetate	52.6	4.13 ± 0.66
	Methanol	68.6	5.34 ± 0.99
Curcuma heyneana	Petroleum ether	34.9	2.60 ± 0.59
	Chloroform	64.1	2.76 ± 0.47
Curcuma mangga	Hexane	185.9	8.07 ± 3.76
	Diclorometh ane	193.2	22.3 ± 3.27
	Ethanol	133.7	6.60 ± 1.21
Kaempferia angustifolia	Petroleum ether	108.3	8.12 ± 2.10
	Chloroform	96.0	5.28 ± 0.84
	Methanol	88.4	4.33 ± 0.64
Kaempferia rotunda	Petroleum ether	110.8	4.62 ± 1.75
	Chloroform	67.8	1.91 ± 1.38
	Methanol	86.5	5.98 ± 1.01
Asimina triloba[14]		20.0	N.A.
Azadirachta indica[15]		1.2-18.0	N.A.

*LC = Lethal concentration (at 95 % CL), **S.E. = Standard error, N.A. = Not available.

Phytochemical studies on *C. xanthorrhiza* and *C. heyneana* have led to the isolation of bioactive sesquiterpenes including α-curcumene, germacrone, zederone, xanthorrhizol, curcumin, oxycurcumenol epoxide, curcumenol and isocurcumenol[8,9]. The mosquitocidal activity of *C. longa* constituents against *A. aegypti* larvae has been reported previously[10]. Labda-8(17),12-dien-15,16-dial (**1**) was the most effective larvicidal compound (Fig. 1) when compared to the activity *ar*-turmerone (**2**) and curcumin (**3**). It gave 100 % mortality at 10 μg/mL when tested on 4th instar *A. aegypti*. This should account for the rather high larvicidal activity of the *Curcuma*

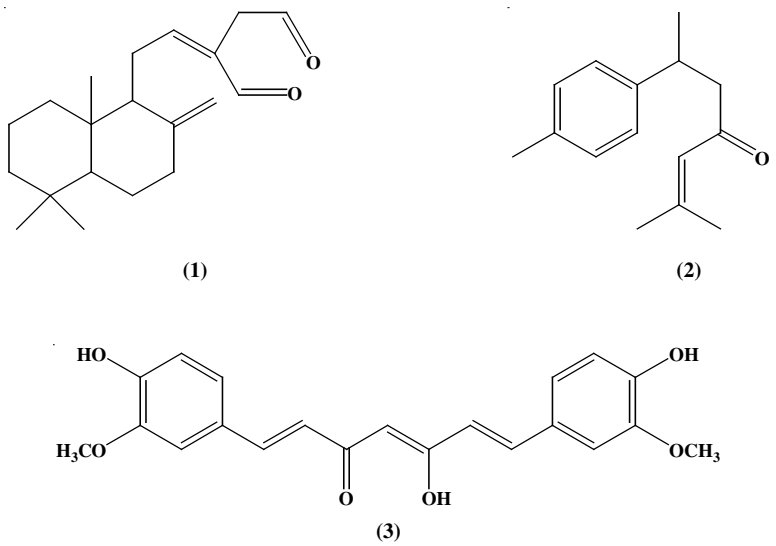

Fig. 1. Structures of larvicidal compounds

plant extracts against larvae of *A. aegypti*. Thus, the hexane extract of *C. xanthorrhiza* and petroleum ether extract of *C. heyneana* can be potential source of natural larvicides against larvae of *A. aegypti*. The crude chloroform, dichloromethane and ethyl acetate extracts of *C. xanthorrhiza*, *C. heyneana*, *K. angustifolia* and *K. rotunda* were moderately toxic with LC_{50} values of between 60 and 90 µg/mL. The methanol extracts of *C. xanthorrhiza*, *K. angustifolia* and *K. rotunda* also indicated moderate larvicidal activity, while petroleum ether extracts of two *Kaempferia* species were least toxic with LC_{50} values more than 100 µg/mL.

Previous studies indicated that the most promising botanical mosquito pesticides originated from plant species of families Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Oocystaceae and Rutaceae[11]. The mosquito-larvicidal activity of Sri Lankan Zingiberaceae species (*Curcuma domestica*, *Curcuma zedoria*, *Languas galanga*, *Elettara repens* and *Zingiber zerumbet*) against late third-instar larvae of *Culex quinquefasciatus*, *Aedes aegypti*, *Aedes albopictus*, *Anopheles culicifacies*, *Anopheles tessellates* and *Anopheles subpictus* were reported[12]. In addition, similar work on tuberous part of *Kaempferia galanga* against third-instar larvae of *Culex pipiens*, *Aedes aegypti* and *Ochlerotatus togoi* was also studied. The larvicidal-active constituents of *K. galanga* were identified as the phenylpropanoids ethyl cinnamate and ethyl *p*-methoxycinnamate, besides monoterpene-3-carene[13].

Out of the five plants tested, non-polar extracts of *C. xanthorrhiza* and *C. heyneana* showed promising bioactivity against larvae of *A. aegypti* with LC_{50} values of less than 35 µg/mL. Two *Kaempferia* species exhibited low activity while extracts of *C. mangga* were non-toxic towards *A. aegypti*. These results complement previous findings that Zingiberaceous species could be utilized as mosquito larvae control agents for protection of humans and domestic animal as well.

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