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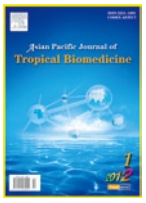

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Laboratory evaluation of six crude plant extracts as repellents against larval *Leptotrombidium deliense* (Acari: Trombiculidae)

Azima Laili Hanifah*, Ho Tze Ming, Vishalani Vishnu Narainasamy, Ahmad Taufik Yusoff

Acarology Unit, Infectious Disease Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia

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ABSTRACT

Objective: To examine the repellency of crude plant extracts of onion (*Allium cepa var. aggregatum* (*A. cepa*)), garlic (*Allium sativum* (*A. sativum*)), clove (*Syzygium aromaticum* (*S. aromaticum*)), cinnamon (*Cinnamomum zeylanicum* (*C. zeylanicum*)), ginger (*Zingiber officinale* (*Z. officinale*)) and pandanus (*Pandanus amaryllifolious* (*P. amaryllifolious*)) on larval *Leptotrombidium deliense* (*L. deliense*). **Methods:** The repellency assay used is a modification of the technique developed by Azima. Extracts at different concentrations (10%, 5%, 2.5%, 1%, 0.1%, and 0.01%) were evaluated. **Results:** All extracts except that from ginger had similar repellencies ranging between 80%-96% at 10% concentration. At the lowest concentration (0.01%), onion extract gave the highest repellency (30%) followed by clove and cinnamon (23%). **Conclusion:** Generally, the plant extracts showed various degrees of repellency against the larval mites and the repellency increased with increasing concentrations of the extracts.

1. Introduction

Scrub typhus, caused by antigenically disparate isolates of *O. tsutsugamushi*, is a widely distributed mite-borne human disease in the Asia Pacific region[1]. It is distributed throughout the Asia Pacific rim, being endemic in Korea, China, Taiwan, Japan, Pakistan, India, Thailand, Malaysia, and northern portions of Australia[2]. All known vectors of this disease belonged to the genus and subgenus *Leptotrombidium*. These *Leptotrombidium* previously had been found in areas with mixed secondary forest and mangrove swamp and lalang fringing[3]. Scrub typhus encountered in grassy fields along the banks of rivers in neglected or abandoned rice fields, gardens and plantation in over grown man-made clearings, in forests or jungles and in the junctional shrubby fringe between field and forest. The use of repellent can be applied to avoid the bites of this mite. Repellents are substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin (or a surface in general)[4]. Plants have historically been valuable sources of agents for the control of insects[5]. Numerous plant-derived substances have demonstrated physiological and behavioral activity against insect pests, and they can provide new sources for the development of natural pesticides[6]. Products with botanical origin have shown a wide range of biological activities including toxicity, repellence, antifeedant, and growth regulatory properties[7]. In recent years, several essential plant oils had been found to have repellent properties. Such plants included citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint[8,9]. Aromatic essential oils from 13 plants species had been tested and four were effective as repellents against *Leptotrombidium* imphalum chiggers; *Syzygium aromaticum* (*S. aromaticum*)(clove) oil exhibited 100% repellency at 5% concentration (dilution with absolute ethanol), whereas *Melaleuca alternifolia* (*M. alternifolia*) (tea tree) oil exhibited 100% repellency at 40% concentration, undiluted oils of *Zingiber cassamunar* (*Z. cassamunar*)(plai) and *Eucalyptus globules* (*E. globules*)(blue gum) exhibited 100% repellency[10]. The purpose of this study was to evaluate the repellency of six plant extracts against larval *Leptotrombidium deliense* (*L. deliense*).

2. Materials and methods

2.1. Chiggers

Unfed, uninfected and 20-30 days old *L. deliense* larvae used in the study were obtained from laboratory colonies maintained at Acarology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia, since 1980s. These colonies were maintained at room temperature and had not been exposed to any pesticide or repellent prior to the study.

*Corresponding author: Azima Laili Hanifah Acarology Unit, Institute for Medical Research, Jalan Pahang, 50588, Kuala Lumpur, Malaysia.
Tel: +603 2616 2445
Fax: +603 2693 5928
E-mail: azima@imr.gov.my

2.2. Repellents

Six crude plant extracts were evaluated. These were onion (*Allium cepa* var. aggregatum (*A. cepa*)), garlic (*Allium sativum* (*A. sativum*)), clove (*S. aromaticum*), Cinnamon (*Cinnamomum zeylanicum* (*C. zeylanicum*)), ginger (*Zingiber officinale* (*Z. officinale*)) and pandanus (*Pandanus amaryllifolious* (*P. amaryllifolious*)). The rhizomes and leaves (pandanus only) were crushed using a heavy duty blender and extracts squeezed out from the blended materials through several layers of cotton gauze. Extracts were then centrifuged for ten minutes at 5 000 rpm and the supernatants used in bioassays at several concentration (10%, 5%, 2.5%, 1%, 0.1%, and 0.01%) were diluted with double distil water.

2.3. Bioassay

The bioassay used to evaluate repellency was that used by Azima *et al*[11]. Commercial cotton buds with plastic shafts were used to hold the test repellent. The shafts were cut into 2.5 cm length from the base of the cotton buds. Approximately 100 μL of test repellent were dropped on each cotton bud. Untreated cotton buds were used as controls. Each shaft was then embedded 0.5 cm into a round piece of plasticine placed in the middle of a 9 cm diameter petri dish. The dish was filled with water up to the base of the plastic shaft just covering the plasticine. This was to prevent the chiggers from escaping. A single chigger was placed at the bottom of the plastic shaft just above the water level and observed for 5 min. A chigger that climbed up to the top of cotton bud was considered as not repelled by the test repellent and a chigger that did not reach the base of the cotton bud was considered repelled. A total of 30 chiggers were tested for each type of repellent.

2.4. Statistical analysis

Results were analyzed by One-Way Anova at 95% confidence level.

3. Results

The larvae of *L. deliense* exhibited different sensitivities to the plants extracts. The repellency rates are shown in Table 1. The repellencies ranged from 6% for lowest concentration up to 96% for the highest concentration. Onion (*A. cepa* var. aggregatum), clove (*S. aromaticum*) and cinnamon (*C. zeylanicum*) showed the highest repellency rate compared to the other plants extracts.At the highest concentration test (10%), clove (*S. aromaticum*) apparently had the highest repellency rate compared to the other plant extracts. There was a significant difference in repellency rate between concentrations for each extract ($P<0.01$) with repellency increasing with increasing concentration. When the different extracts are compared at each concentration, there was significant differences in repellency ($P<0.05$) but not at 0.01% concentration ($P=0.275$).

Table 1
Repellency of plant extracts against *L. deliense*.

Type of plants extracts	Number of chiggers tested	Repellency (%) rate (Mean + SD) Concentrations of plant extracts							Mean±SD	*P* value
		Control	10.0%	5.0%	2.5%	1.0%	0.1%	0.01%		
Onion	30	0	93	86	73	56	53	30	65.17±23.40	0.000
Clove	30	0	96	83	80	60	36	23	63.00±28.69	0.000
Cinnamon	30	0	93	90	76	60	56	23	66.33±26.23	0.000
Pandanus	30	0	83	76	70	63	56	16	60.67±23.85	0.000
Garlic	30	0	80	76	60	56	20	6	49.67±30.16	0.000
Ginger	30	0	50	46	33	26	23	10	31.33±14.96	0.007
P value	0.000	0.001	0.001	0.041	0.006	0.275				

4. Discussion

Unfed larvae naturally climb up surrounding vegetation to await a passing host. The test procedure in this study is based on this behavior. It is very effective, inexpensive and easy to conduct. The time required per test is less than 5 min from the time of dropping a repellent on the cotton bud to the time of recording whether a chigger is repelled. Larvae on the plastic shaft are easily seen with a magnifying glass.

Repellents made from plant extracts would be safer for humans than ones based on chemicals. In addition, they could be better for the environment and more economical. Nowadays, many researchers tend to return to investigations involving plant extracts for natural control of parasites[12] and pests[7] of importance in agricultural and veterinary industries. Aromatic, arthropod-repellent plants are traditionally used to protect people from blood feeding ectoparasites[13] such as ticks, bird-nest mites, lice, fleas, hippoboscid flies, blackflies, *etc*. Among the six plants extracts tested in this study, ginger extract was the least effective in repelling *L. deliense*. The low repellency of ginger extract has been reported too by Yuwadee *et al*[14]; they found 10% concentration of ginger (*Z. officinale*) gave zero protection from *Aedes aegypti* (*Ae. aegypti*) compared with clove (*S. aromaticum*) extract that gave about 30 min′ protection against the same species of mosquito. In the other hand, previous ethno botanical considerations suggested ginger, *Z. officinale* found in Southeast Nigeria, have the ability to control stored grain insect pests[15]. To support this finding, Ukeh[16,17] done a laboratory evaluation and confirmed that *Z. officinale* rhizomes were directly repellent to adult maize weevil, *Sitophilus zeamais* (*S. zeamais*). Nieves[18] conducted a study on essential oil of *C. zeylanicum* under laboratory condition and found that this essential oil provided 95% protection against bite of Lutzomyia migonei or sand fly for 3 h. Eamsobhana *et al*[11] demonstrated that several aromatic, essential oils of plants may be useful as chigger repellents for the prevention of scrub typhus; *S. aromaticum* (clove; syn. *Eugenia aromaticum*, *E. caryophyllata*) oil repelled chiggers at relatively low concentration.

Garlic extract at 0.1% and 0.01% concentration in this study, gave the lowest repellency rates. However Mohinder[9] reported that, aqueous garlic extract have toxic effect on the hatching eggs and emergence of larvae of *A. aegypti*. Another study by Nicodemo[19]

found garlic extract was effective as a repellent against *Apis mellifera* honeybees from confined beef cattle feeder for six hours. Sterjernberg[20] suggested garlic extract may be considered as a tick repellent for individual and population at high risk for tick bites. Denloye[21] was carried out a study of powder, extracts and essential oil of garlic (*A. stivum*) against *Callosobruchus maculatus* and result showed that the aqueous extracts were more toxic to *C. maculatus* compared to the other types of extracts

Onion extract produced high repellency rate in this study. The juice of onion extract was used as a moth repellent and can be rubbed onto skin to repel insect (www.naturalmedicinalherbs.net). Other has reported that, the gasses emitted by the onion, when sliced, have a strong odor and are known irritants effective on repelling insects (www.lifehackery.com). Packer reported that onion mixed with garlic, pepper and one quart of water are environmentally friendly spray to keep insects off the plants (www.wisebread.com). Besides being an effective repellent, Cinnamon oil had also been reported to be effective in controlling mites. Saad *et al*[22] found that on the basis of LC_{50} values, the active compound of cinnamon oil, cinnamaldehyde was most toxic against a dust mite Dermatophagoides pteronyssinus.

The repellency of pandanus extract is not just restricted to chiggers; Ahmad[23] reported pandananus extract was able to repel Americana cockroaches (*Periplaneta americana*).

Conflict of interest statement

We declare that we have no conflict of interest.

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