Efficacy of Plant Essential Oils for the Repellents Against Chiggers (*Leptotrombidium Imphalum*) Vector of Scrub Typhus

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Scrub typhus caused by the Orientia tsutsugamushi. Rodents, particularly rats, serve as principal reservoir hosts. Infection in man is transmitted by the, chigger bite. Repellents provide an effective agent of protecting individuals from chigger. In the present study 6 plant essential oils were tested for evaluation of their repellent activity against the chigger, Leptotrombidium imphalum. The results showed that Clove oil was significantly more effective than others with ED_{50} and EC_{50} of 0.420 mg and 2.3%, followed by Zingiber oil (8.458 mg and 42.3%), Vetiver oil (19.582 mg and 97.9%), Turmeric oil (24.343 mg and 121.7%), Orange oil (27.310 mg and 136.6%) and Boesenbergia oil (30.486 mg and 152.4%). These results suggested that Clove oil was the most efficient repellent against chigger which is the vector for scrub typhus.

Keywords: *Scrub typhus, Chigger, Repellent, Essential oil*

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Scrub typhus is an important febrile disease endemic in the Asia-Pacific region such as Korea, Japan, Southeast Asia, and Australia. More than a billion people are at risk of infection and about one million cases occur annually[1].

Scrub typhus is caused by the bacterium *Orientia* (formerly named *Rickettsia*) *tsutsugamushi*[2]. *O. tsutsugamushi* is a gram negative, intracellular, small rod-sharped bacterium (coccobacillus). To persist in nature, the bacterium parasitizes in trombiculid mites (Acari: Trombiculidae) and small mammals. Small mammals serve as reservoir host in its natural life cycle, and larval stage of mites, so called chiggers, act as vector[1]. The bacterium can develop in mite transovarially (from adult female to eggs) as well as transstadially (from egg to larva to adult). Humans and rodents get infected by the bite of chigger harboring *O. tsutsugamushi*. After the incubation period of 7 to 14 days, patients usually develop high fever, chill, headache, rash and may be eschar lesion. Typically, the disease responds well to doxycycline and chloramphenicol antibiotic treatment. However, delayed response to usual antibiotic therapy of scrub typhus patients was reported in Chiangrai Province, in Northern Thailand. The present report suggested that antibiotic-resistant strains of *O. tsutsugamushi* may exist[3].

Repellents are used as an effective method for protection of individuals from biting arthropods[4]. The discovery of DEET (N,N-diethyl-3-methyl-benzamide) in 1954[5] was probably the single most important event in the evolution of arthropod repellents[4]. Unfortunately, DEET is not effective against all arthropods[6]. Therefore many investigators have been seeking more effective new repellents and repellent formulations[7]. There were at least five crude-extracts of plants having larvicidal actions[8]. Thus, the essential oils from these plants were investigated for their repellent effect against chigger vector of scrub typhus.

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Material and Method

Arthropod Species

Chiggers were reared in the laboratory at the

Department of Parasitology, Faculty of Medicine Siriraj Hospital. Larval mite (*Leptotrombidium imphalum* Vercammen-Grandjean and Langston) were used in the present study. The mites were maintained in plastic containers (4.0 cm high) with a plaster of Paris and charcoal (9:1 ratio) substrate (1.5 cm deep). Nymphs and adults were fed collembola (*Sinetta curciseta* Brook) eggs, whereas larvae (chiggers) were fed on 4-wk old mice using the method of Nadchatram (1968)[9]. All mite stages were held in a biosafety cabinet at 27°C ± 3°C, 50%-60% RH and natural light.

Plant extraction

Six essential oils *i.e.* 1) clove oil (*Syzygium aromaticcum* (L) Merr & Perry), 2) zingiber oil (*Zingiber cassumunar* Roxb), 3) turmeric oil (*Curcuma longa* L), 4) vetiver oil (*Vetiveria zizanioides* (L) Nash), 5) boesenber oil (*Boesenbergia pundurata* (R0xb) Schitr) and 6) orange oil (*Citrus aurantium* Amara) were purchased from a market used to evaluate the efficiency of repellent property against chiggers. All of the plant oils were diluted (v:v) with ethanol to prepare 20%, 50%, 70% and 100% concentrations. Ethanol solvent was used as a control.

In vitro test procedure

The procedure is based on the propensity for chiggers to climb upwards. A plastic Q-tip (Fancy Inc., Bangkok, Thailand) was used as the substrate for the repellent essential oil. Q-tip was longitudinal-half cut with 1cm long cotton bud and 2 cm long shaft. 20 µl of each essential oil was dropped on the cotton bud and allowed to air-dry for 1 h. The Q-tip was embedded into the charcoal substrate with 0.5 cm depth at the center of the vial. A perimeter line around the vial was marked at 1 cm below the top rim of the vial (Fig. 1). The area above the line, either on the wall of the vial or on the Q-tip, was considered as the repellent zone. A single 2-3 wk-old chigger was released into the plastic vial and observed for 5 min. The number of chiggers which moved into the repellent zone for at least 5 sec was recorded. Each test was replicated 10 times using a new chigger each time.

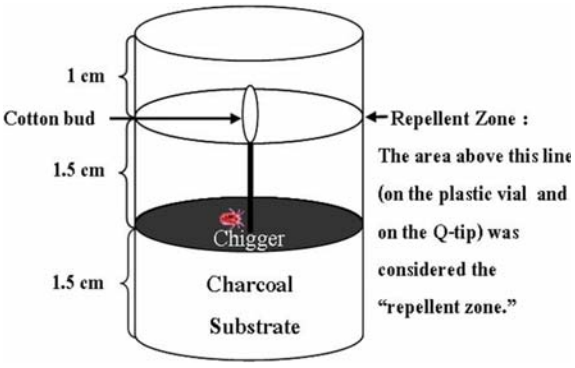

Fig. 1 A tool for repellent activity of essential oils against the chigger tested

Statistical analysis

The number of chiggers which moved into the repellent zone from each essential oil was recorded. The dose of each essential oil was calculated from the percent concentration and volume used. The median effective dose to repel 50% (ED_{50}) and the median effective concentration to repel 50% (EC_{50}) of the test chigger were then calculated by the probit analysis of SPSS for Windows Advanced Statistics. The linear regression equation was also determined to predict the probability of a response to each of the concentrations and doses.

Results

The data demonstrated that there are significant differences in the efficacy of the various test essential oils against the *L. imphalum* chigger. Clove oil was clearly more effective than most of the others followed by zingiber oil, whereas the rest essential oils were not at all protective.

With regard to ED_{50} values, clove oil was significantly more effective than others with ED_{50} of 0.420 mg followed by zingiber oil (8.453 mg), vetiver oil (19.582 mg), turmeric oil (24.343 mg), orange oil (27.310 mg) and boesenber oil (30.486 mg) (Table 1).

Concerning EC_{50} values, Clove oil was significantly more effective than others with EC_{50} of 2.282% followed by Zingiber oil (42.289%), Vetiver oil (97.908%), Turmeric oil (121.716%), Orange oil (136.550%) and Boesenber oil (152.432%) (Table 2).

Discussion

Repellents are an inexpensive and practical means of providing against nuisance and disease vector arthropods[4]. Although DEET is currently the most widely used repellent, it has certain drawbacks. DEET is incompletely effective against arthropods[10]. There are concerns about its potential toxicity and safety despite a long history of safe application[11]. Hence, health risks may take place when used at high concentrations[12]. There were at least five plant extracts having larvicidal action[8]. Consequenctly, the study of essential oils from these plants on repellent activity

Table 1. ED_{50} of repellent essential oils against the chigger

Plants extract	ED_{50} (mg)	Formula
Clove oil	0.420	Y = -1.472 + 3.504 x X
Zingiber oil	8.458	Y = -0.964 + 0.114 x X
Turmeric oil	24.343	Y = -0.917 + 0.380 x X
Vetiver oil	19.582	Y = -1.044 + 0.053 x X
Boesenbergia oil	30.486	Y = -1.641 + 0.053 x X
Orange oil	27.310	Y = -1.095 + 0.040 x X

ED_{50}: median effective dose

Table 2. EC_{50} of repellent essential oils against the chigger

Plants extract	EC_{50} (%)	Formula
Clove oil	2.282	Y = -2.317 + 1.015 x X
Zingiber oil	42.289	Y = -0.964 + 0.023 x X
Turmeric oil	121.716	Y = -0.917 + 0.008 x X
Vetiver oil	97.908	Y = -1.044 + 0.011 x X
Boesenbergia oil	152.432	Y = -1.614 + 0.011 x X
Orange oil	136.550	Y = -1.095 + 0.008 x X

EC_{50}: median effective concentration

against chiggers, is nowadays of great importance to avoid contact with scrub typhus vectors.

In the present study the clove oil has the highest repellent activity against chigger followed by that of zingiber oil. Lerdthusnee et al, (2003)[13] determined the efficacy of 11 different chemicals against chiggers and three of them, *i.e.* had DM-159-2 (N, N-diethyl-3-(trifluoromethyl) benzamide), DEPA (N, N-diethy phenyl aeeteamide) and DM-36-2 (2-cyclohexyl-N, N-diethylacetamide), had ED_{50} values of 3.47 mg, 70.25 mg and not determined respectively. It was found that these chemicals had less efficacy than the authors' clove oil with ED_{50} value of 0.420 mg. Akkagraisee et al, (2006)[14] also investigated the repellent property of 9 essential oils against chiggers and found that the most active essential oil was from the *Paeperia* sp. which gave the ED_{50} value of 1.03 mg. In comparison the authors' findings to those of Lerdthusnee et al, (2003)[13] and Akkagraisee et al, (2006)[14], it can be concluded that clove oil had better activity (ED_{50} = 0.420 mg and EC_{50} = 2.282%) than some pure chemicals and many essential oils against chigger. This means that clove oil had repellent activity against vector for scrub typhus. Thus, it probably use clove essential oil as repellent substance instead of chemicals such as DEET which can cause the allergy to users. Finally, clove oil might be a great potential to be the alternative way in chigger protection.

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Potential conflicts of interest

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การศึกษาประสิทธิภาพของน้ำมันหอมระเหยจากพืชสมุนไพรต่อการป้องกันตัวไรอ่อน (Leptotrombidium imphalum) พาหะนำโรคสครับไทฟัส

วุฒิกรณ์ รอดความทุกข์, อัญชนา ปราศาสตร์วิทย์, ชัยวัฒน์ จาติเสถียร, อารยา จาติเสถียร, จริยาณาฏ เกวี , ประภาทิพย์ เอี่ยมโสภนา

โรคสครับไทฟัสมีสาเหตุมาจากการติดเชื้อ Orientia tsutsugamushi มีตัวไรอ่อน (Chigger) สายพันธุ์ Leptotrombidium spp. เป็นพาหะนำโรค มีสัตว์ฟันแทะจำพวก หนู เป็นแหล่งรังโรคมนุษย์ติดเชื้อก่อโรคสครับไทฟัสได้จากการถูกตัวไรอ่อนที่มีเชื้อกัด การป้องกันโรคสครับไทฟัสทำได้โดยการใช้สารป้องกันแมลงป้องกันตัวไม่ให้ตัวไรอ่อนกัดขณะเข้าไปในพื้นที่เสี่ยง เช่น บริเวณพื้นที่ป่า การศึกษาในครั้งนี้ เป็นการศึกษาถึงคุณสมบัติของน้ำมันหอมระเหยจากพืชสมุนไพร จำนวน 6 ชนิด เพื่อประเมินฤทธิ์ในการป้องกันตัวไรอ่อน (chigger) โดยใช้ทดสอบกับตัวไรอ่อนสายพันธุ์ Leptotrombidium imphalum ในห้องปฏิบัติการ ผลการทดลองพบว่า น้ำมันหอมระเหยจากการพลู (Clove oil) มีประสิทธิภาพดีกว่าน้ำมันหอมระเหยจากพืชชนิดอื่น ๆ อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบโดยใช้ค่า ED_{50} และ EC_{50} โดยพบว่า น้ำมันหอมระเหยจากกานพลู มีค่า ED_{50} และ EC_{50} เท่ากับ 0.420 มก. และ ร้อยละ 2.3 ตามลำดับ ตามด้วยน้ำมันหอมระเหยจาก ไพร (Zingiber oil) 8.458 มก. และ ร้อยละ 42.3, หญ้าแฝก (Vetiver oil) 19.582 มก. และ ร้อยละ 97.9, ขมิ้น (Turmeric oil) 24.343 มก. และร้อยละ 121.7, ส้ม (Orange oil) 27.310 มก. และ ร้อยละ 136.6 และ กระชาย (Boesenbergia oil) 30.486 มก. และ ร้อยละ 152.4 จากผลการทดลองยืนยันว่าน้ำมันหอมระเหยจากการพลูมีประสิทธิภาพสูงที่สุดในการใช้ป้องกัน chigger ซึ่งเป็นพาหะนำโรคสครับไทฟัส