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REPELLENCY OF AROMATIC MEDICINAL PLANT EXTRACTS AND A STEAM DISTILLATE TO *AEDES AEGYPTI*

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ABSTRACT. The repellent activity of methanol extracts from 23 aromatic medicinal plant species and a steam distillate against female blood-starved *Aedes aegypti* was examined in the laboratory by skin test and compared with that of *N,N*-diethyl-*m*-toluamide (deet). Responses varied according to plant species. At a dose of 0.1 mg/cm^2, the repellency of extracts of *Cinnamomum cassia* bark (91%), *Nardostachys chinensis* rhizome (81%), *Paeonia suffruticosa* root bark (80%), and *Cinnamomum camphora* steam distillate (94%) was comparable to deet (82%). The duration of the effectiveness for extracts from *C. cassia* bark and *N. chinensis* rhizome was comparable to deet and lasted for ~1 h. Relatively short duration of repellency was observed in *P. suffruticosa* root bark extract and *C. camphora* steam distillate. The plants described merit further study as potential mosquito repellent agents.

KEY WORDS Natural repellent, mosquito, *Aedes aegypti,* aromatic plant, deet, skin test

INTRODUCTION

Mosquito repellents could be one of the most effective tools for protecting humans from mosquito attack and from mosquito-borne diseases, such as dengue hemorrhagic fever, malaria, encephalitis, and filariasis (Curtis et al. 1990). Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations (Croft and Brown 1975). It has also resulted in the development of resistance (Brown 1986), had undesirable effects on nontarget organisms, and fostered environmental and human health concerns (Hayes and Laws 1991). The most commonly used mosquito repellent is *N,N*-diethyl-*m*-toluamide (deet), which is still most effective. However, this compound has an unpleasant odor, can damage plastics and synthetic rubber, and has high skin penetration characteristics (Qiu et al. 1998). These problems indicate a need for new and improved repellents and strategies for protection from mosquito attack.

Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals (Wink 1993) and typically are free from harmful effects (Isman 1995). Because of this, much interest has focused on plant extracts, or plant essential oils, as potential mosquito repellent agents. The effectiveness and duration of repellency depend on the type of repellent (active ingredient and formulation), mode of application, local conditions, attractiveness of individual people to insects, loss of repellent with removal by perspiration and abrasion, sensitivity of the insects to repellents, and biting density (Rozendaal 1997).

This paper describes a laboratory study that was made to assess the potential of plant extracts for use as commercial mosquito repellents. Repellent activity of methanol extracts from 23 aromatic medicinal plant species and a steam distillate were assessed against female blood-starved *Aedes aegypti* (L.) and compared with that of deet.

MATERIALS AND METHODS

Insects: The Ae. aegypti used in this study were from cultures maintained in the laboratory for 7 years without exposure to insecticide. Adult mosquitoes were reared on a 10% sucrose solution and blood-fed on live mice. Larvae were reared in plastic trays (24 × 35 × 5 cm) containing 2,000 ml of water supplied with 0.5 g of sterilized diet (40-mesh chick chow powder and yeast, 4:1 by weight). They were held at 28 ± 2°C and 75 ± 5% relative humidity (RH) and a 16:8 h photoperiod.

Plants and sample preparation: A total of 24 aromatic medicinal plant species were selected (Namba 1993). The parts of each plant that have been used in Chinese medicine (Namba 1993) were purchased from Boeun medicinal herb shop, Kyungdong market, Seoul, and used in extractions (Table 1). With the exception of *Chaenomeles sinensis* (Thouin) Koehn. and *Cinnamomum camphora* Presl, the plants were dried in an oven at 40°C for 2 days and finely powdered with a blender. Each sample (50 g) was extracted twice with 300 ml of methanol at room temperature for 2 days and filtered. Slices (200 g) of the fresh *Chaenomeles* fruits were ground in a blender, extracted twice each with 900 ml of methanol at room temperature for 1 day, and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40°C. The yield of each methanolic extraction is given in Table 1. *Cinnamomum camphora* was purchased as a steam distillate.

Bioassay: The method of Frances et al. (1996)

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Table 1. Aromatic medicinal plants tested.

Family	Species	Tissue used[1]	Yield (%)[2]
Apiaceae	*Angelica dahurica*	Ro	17.7
Araceae	*Acorus calamus* var. *angustatus*	Rh	10.1
	Acorus gramineus	Rh	9.5
Compositae	*Artemisia princeps* var. *orientalis*	Wp	6.6
	Inula helenium	Ro	16.3
Dioscoreaceae	*Dioscorea batatas*	Rh	2.4
Fabaceae	*Gleditsia horrida*	Fr	17.3
	Glycyrrhiza glabra	Ro	21.9
Labiatae	*Agastache rugosa*	Wp	9.5
	Schizonepeta tenuifolia	Wp	8.1
Lauraceae	*Cinnamomum camphora*[3]	—	—
	Cinnamomum cassia	Ba	5.1
Magnoliaceae	*Magnolia obovata*	Ba	5.8
Myrtaceae	*Eugenia caryophyllata*	Fb	37.8
Paeoniaceae	*Paeonia suffruticosa*	Rb	18.6
Piperaceae	*Piper nigrum*	Fr	10.1
Polygonaceae	*Rheum coreanum*	Rh	41.6
Prumulaceae	*Lysimachia davurica*	Wp	9.0
Rosaceae	*Chaenomeles sinensis*	Fr	60.8
Rutaceae	*Evodia rutaecarpa*	Fr	9.5
Solanaceae	*Solanum melongena*	Fr	47.7
Stemonaceae	*Stemona japonica*	Ro	15.2
Thymelaeaceae	*Aquilaria agallocha*	Hw	6.6
Valerianaceae	*Nardostachys chinensis*	Rh	12.9

[1] Ba, bark; Fb, flower bud; Fr, fruit; Hw, heart wood; Rb, root bark; Rh, rhizome; Ro, root; and Wp, whole plant.
[2] (Weight of crude methanol extract/weight of dried test material) × 100; except *Chaenomeles sinensis*, (weight of crude methanol extract/weight of fresh fruits) × 100.
[3] Steam distillate.

with a slight modification was used to determine the repellent activity of test samples against female blood-starved *Ae. aegypti.* Every bioassay was conducted from 1200 to 1700 h. In a preliminary test, 0.1 mg of each plant extract was solubilized in 20 μl of ethanol by sonication for 10 sec and provided an appropriate amount for repellent bioassays. Ethanol (20 μl) was applied directly to exposed skin through a 5-cm-diameter hole on the back of a rubber glove and dried for 3 min. Because biting density plays an important role in studies of repellency (Rozendaal 1997), skin was exposed for 5 min in a screen wire cage (30 × 30 × 30 cm) containing 300 blood-starved females (6–8 days old). Immediately after the control exposure, the hand was removed from the cage and a dose of 0.1 mg/cm^2 of each test plant material and deet (Sigma, St. Louis, MO) in 20 μl of ethanol were applied evenly over the skin surface. After air drying for 3 min, the treated hand was exposed to mosquitoes in the same test cage for 5 min at 30-min intervals. The number of test mosquitoes biting on the skin was recorded. Experiments were conducted at 28 ± 2°C and 75 ± 5% RH. Each assay was replicated 3 times.

Repellency was calculated according to the formula from Schreck et al. (1977): % repellency = [(Ta − Tb)/Ta] × 100, where Ta is the number of mosquitoes in the control and Tb is the number of mosquitoes in the treated group.

Statistical analyses: Percent repellency was determined and transformed to arcsine square root values for ANOVA. Treatment means were compared and separated by the Scheffe test at $P = 0.05$ (SAS Institute 1990). Means ± SE of untransformed data are reported.

RESULTS AND DISCUSSION

The repellent activity of methanol extracts from 23 aromatic medicinal plant species and a steam distillate against starved *Ae. aegypti* females varied according to plant species (Table 2). At a dose of 0.1 mg/cm^2, potent repellency against mosquito adults was obtained with the extracts of *Cinnamomum cassia* Blume bark (91%), *Nardostachys chinensis* Batalin rhizome (81%), *Paeonia suffruticosa* Andrews root bark (80%), and *C. camphora* steam distillate (94%). Repellency in each case was comparable to that of deet (82%). *Eugenia caryophyllata* Thunb. extract provided 75% repellency. The other 19 plant extracts exhibited <70% repellency.

We know that plant-derived insect repellent agents are selective, have no or little harmful effect on nontarget organisms or the environment, and can be applied to human skin and clothing in the same way as conventional repellents (Curtis et al. 1990, Isman 1995, Rozendaal 1997). Furthermore, many plant extracts and essential oils manifest repellent activity against different mosquito species (Curtis et al. 1990, Sukumar et al. 1991, Rozendaal 1997). Sukumar et al. (1991) noted that the most promis-

Table 2. Repellent activities of aromatic medicinal plants against female *Aedes aegypti* skin test.

Plant species[1]	% repellency[2]
Acorus calamus var. *angustatus*	56 ± 9.6 CDEF
Acorus gramineus	44 ± 1.8 DEFG
Agatache rugosa	54 ± 1.6 CDEF
Angelica dahurica	50 ± 1.5 CDEFG
Aquilaria agallocha	62 ± 2.2 BCDEF
Artemisia princeps var. *orientalis*	60 ± 3.6 BCDEF
Chaenomeles sinensis	49 ± 2.5 CDEFG
Cinnamomun camphora	94 ± 1.8 A
Cinnamomum cassia	91 ± 3.9 AB
Dioscorea batatas	39 ± 1.6 EFG
Eugenia caryophyllata	75 ± 3.3 ABCDE
Evodia rutaecarpa	32 ± 3.2 FG
Gleditsia horrida	57 ± 3.6 CDEF
Glycyrrhiza glabra	28 ± 4.5 FG
Inula helenium	50 ± 3.7 CDEFG
Lysimachia davurica	50 ± 2.7 CDEFG
Magnolia obovata	59 ± 2.3 CDEF
Nardostachys chinensis	81 ± 5.8 ABCD
Paeonia suffruticosa	80 ± 4.3 ABCD
Piper nigrum	52 ± 3.9 CDEFG
Rheum coreanum	51 ± 1.9 CDEFG
Schizonepeta tenuifolia	53 ± 4.0 CDEF
Solanum melongena	15 ± 3.2 G
Stemona japonica	61 ± 1.3 BCDEF
Deet	82 ± 3.7 ABC

[1] Exposed to 0.1 mg/cm² for 15 min.

[2] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe test; SAS Institute 1990). Repellency was transformed to arcsine square root values before ANOVA. Means ± SE of untransformed data are reported.

ing botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae, and Rutaceae. In this study, repellency against female *Ae. aegypti* comparable to deet was observed for extracts from plants in the Lauraceae, Myrtaceae, Paeoniaceae, and Valerianaceae families. Repellent activity of the extracts of *C. cassia* bark, *E. caryophyllata* flower bud, *N. chinensis* rhizome, and *P. suffruticosa* root bark, as well as the steam distillate of *C. camphora,* against female *Ae. aegypti* was comparable to that of deet.

The repellent activity of extracts from *C. cassia* bark, *N. chinensis* rhizome, *P. suffruticosa* root bark, and *C. camphora* steam distillate against female *Ae. aegypti* at 0.1 mg/cm² was comparable to that of deet (Fig. 1). Their efficacy lasted for 1 h. Relatively short duration of repellency (30 min) was observed in *P. suffruticosa* extract and *C. camphora* steam distillate.

Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols, and aldehydes are repellent to mosquitoes for periods ranging from 15 min to 10 h (Rozendaal 1997). Furthermore, various formulations for controlled release have been developed to increase the protection period provided by repellents (Gupta and Rutledge 1989). Sharma and Ansari (1994), for example, reported that a 1% neem oil–kerosene mixture could provide economical personal protection from mosquito bites. *Lantana camara* L. flower extract in coconut oil provided 94.5% protection from *Aedes albopictus* (Skuse) and *Ae. aegypti* without adverse effects on the human volunteers for a 3-month period after the application (Dua et al. 1996).

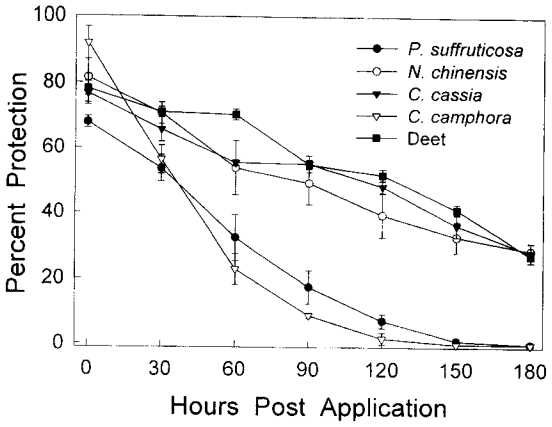

Fig. 1. Duration of protection of methanol extracts from 3 plant species, a steam distillate of *C. camphora,* and deet against female *Ae. aegypti* when dosed at a rate of 0.1 mg/cm². Bar represents standard error.

Results of this study indicate that some plant extracts could be useful for protecting human and domestic animals from mosquito attack, provided a slow-release effect for the repellents can be developed. For practical use of these plants as novel mosquito repellents to proceed, however, further research on their safety in human health, as well as formulations that improve repellent potency and stability, is necessary.

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