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Original Research Article

Insecticidal activities of sweet marjoram (*Origanum majorana* L.) against *Pediculus humanuscapitis (Anoplura: Pediculidae)*

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K e y w o r d s

Sweet marjoram, insecticidal activity, *Pediculus humanuscapitis (Anoplura: Pediculidae)*

A B S T R A C T

The objective of the present study that has been carried out in the Facultyof Pharmacy, October 6 University, was to investigate insecticidal activity of a number of sweet marjoram extracts against *Pediculus humans capitis*. Four sweet marjoram (*Origanummajorana*L.) fractions obtained by steam distillation, Soxhlet n-hexane extraction, extraction with aqueous ethanol, and with ethanolic ammonia solution were evaluated invitrofor activity against head lice (*Pediculushumanuscapitis).*The contact insecticidal activities ofmarjoram oilsat a dose of 0.25 mg/cm2, against adult*P. humanuscapitis*, were compared with those of thecommonly used insecticides -phenothrin and pyrethrum. Significant differences were observedin the contact toxicity to female head lice. Onthe basis of LT50 values, marjoram oils were more toxic than either &-phenothrin or pyrethrum.Itwas 2.0-fold more toxic than &phenothrin. No mortality was observed for solvent-treated lice over the observationalinterval of the contact bioassay.It was also toxic at 0.0625 mg/cm.In fumigation tests with adult*P. humanuscapitis* at 0.25 mg/cm2, *marjoram* oils were more effective in closed containers than in open ones, indicating that the effect of these oils was largely a result of action in the vapor phase. Neither &-phenothrin nor pyrethrum exhibited fumigant toxicity. These data warrant further study on identifying thecomponents of the extracts with the highest activities.

Introduction

Head lice (*Pediculus capitis*) are an ectoparasite, confined to the scalp and hair of humans. Infestations are prevalent worldwide and especially common among schoolchildren in both developed and developing countries. *P.humanuscapitis* infections cause skin irritation, pruritus, and sleep loss, as well as occasional secondary bacterial infection from scratching (GratzNG ;1997). Although the symptoms are relatively mild, infestation by *P.humanuscapitis* has resulted in various social, mental, and economic problems. The control of human head lice worldwide

depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphoru (malathion), carbamate(carbaryl), pyrethrin,pyrethroid (permethrin and 6-phenothrin),and avermectin insecticides. Topical insecticides based on insecticidal chemicals are still the mainstay of head lice treatment (Heukelbach J, et al.;2008).Growing patterns of insecticidal drug resistance laid the foundation for research in exploring novel anti-insect agents from medicinal plants particularly with fumigant action for ease of application. There are several well documented studies showing that plant extracts could be used as medicinal products against a broad spectrum of ectoparasites of humans and house animals (Semmler Met al. 2009 and Burgess IF.,2009).However, most of the products are derived from fixed oils rather than essential oils. For example, a product containing a neem seed extract was highly effective in *in-vivo* and *in-vitro* tests against head lice (Heukelbach Jet al.2006b and Abdel-Ghaffar F, Semmler M;2007, Heukelbach J, et al.2008). Recently, Burgess et al. ;2010) showed in a clinical trial the superiority of a spray containing coconut, ylangylang and anise oils over a permethrin lotion against head lice.Essential oils from various condiment plants possess multiple biological activities; including antibacterial, antifungal and antioxidant actions (Baratta M T, et al.;1998,Daferera D J et al.2000 andZheng Z L et al.;2009). It is odorous components and secondary metabolites that can be extracted from plant tissues through steam distillation. Essential oils and their volatile constituents are widely used in the prevention and treatment of human illnesses. They are also documented for exhibition of acute toxicity, anti-feeding and oviposition deterrents against a wide variety of insect-pests. Risk lower level of the volatile essential oils to the environment and their minimal residual activity against predator, parasitoid and pollinator insect populations, making essential-oil-based pesticides compatible with integrated pest management programs (Heukelbach J, et al.;2010 in press.**,** Burgess, I F;2004, and Abdel-Ghaffar F,Semmler M ;2007). Marjoram (*Origanum majorana*L., Family: Lamiaceae) is a common spicy medicinal herb, used as a home remedy for the treatment of different ailments. It is well recognized with its popular name "Marjoram". It is also used worldwide in food for better flavor, both in dry form and as fresh vegetable. It is a rich source of polyphenols which are known natural antioxidants. The emulsified oil of marjoram was reported to possess strong antiparasitic activity (1218).Marjoram was initially used by Hippocrates as an antiseptic agent. Itis a well-liked home remedy for chest infection, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, and stomach disorders (Abdel-Ghaffar F, Semmler M'; 2007, Abdel-Ghaffar F, et al.; 2009 and Isman, M 1999). Some sweet marjoram fractions could act as insecticides. For example, the essential oil from sweet marjoram has shown promise as a potential agent for the control of head lice (Yang Y C, et al.; 2009) and German cockroach (Jang Y S, et al.;2005),whereas methanol extract is highly toxic to larvae of the known crop pest *Spodoptera littoralis*(Pavela R; 2004).The present work aimed to evaluate the toxicity effect of sweet marjoram isolates obtained with various extraction methods against the head lice (Adults *P. humanus*).

Materials and Methods

Essential oil:

Sweet marjoram *(Majorana hortensis)* essential oil (EO) was purchased from El-captain Company,(CAP. PHARM Egypt), for extracting natural oils, herbs and

cosmetics, Cairo, Egypt. The experimental material consisted of the fresh sweet marjoram leaves collected at the beginning of flowering time (mid-July) from the garden-plot 2013and dry. A) Volatile fraction was prepared by heating 30 g of ground fresh sweet marjoram leaves with 400 ml of distilled water. The 200 ml of distillate was collected and extracted thrice with methylene chloride (3 × 40 ml). The extracts were combined and dried with anhydrous MgSO4, and the solvent was then removed in a rotary evaporator at +40C(bath temperature) and 470–490 hPa pressure. It was obtained 57 mg of the oily residue. B) The preparation of the n-hexane extract was performed in a Soxhlet apparatus with ground fresh sweet marjoram leaves (30 g) and 400 ml of n-hexane. After 4 h the crude extract was evaporated at 40°C and 230 hPa pressure to give 320 mg of light green oily residue.

C) The aqueous ethanol extract was prepared by stirring 20 g of the dry sweet marjoram herb with 500 ml of aqueous ethanol (70%) in a water bath for 10 h at 50°C.Next, the pulp was filtered through filter paper, and the solvent from filtrate was removed to dryness in a rotary evaporator at +50ºC. To remove the residual water it was evaporated twice to dryness with ethanol-toluene mixture (1:3,v/v, 2 × 100 ml) to yield8.7 g of the light brown powder. D) The ethanolic ammonia extract was processed in the same way as the aqueous ethanol extract with the exception: a mixture of aqueous ethanol (70%) and 25% aqueousammonia (95:5, v/v) was used instead of 70% aqueous ethanol. It was received9.3 g of the light brown powder. Both (aqueous ethanol and ethanolic ammonia extracts)were also assayed for the phenolic compounds content by Folin-Ciocalteu method using gallic acid (Sigma-Aldrich) (GA) as a standard(Singleton and Rossi1965). The content of phenolic compounds in the aqueous ethanol extract responds170 mg GA/g of the plant extract and in the ethanolic ammonia extract 180 mg GA/g of plant extract. Sweet marjoram isolates were evaluated *in vitro* for activity against the lice.

Collection of head lice

Adult's*P. humanuscapitis* were collected from children between the age group of 8-12 by combing through sections of the scalp using a clean comb(Figure 1). After combing, the lice were carefully removed from the teeth of the comb into plastic boxes (Figure 2). Head lice were reared in petri dishes with 0.01- and 1.0-mm mesh screens attached over the central holes (4 cm in diameter) on the lid and bottom sides, respectively, and containing a few strands of human hair (figure 3). To feed head lice with blood meals, the petri dish were placed on the bare lower legof volunteers and maintained therefor 16 h every day according to the method of Leeet al. 2000). All the subjects had not been treated with any anti-lice products for the preceding 3 months. Eggs were held at 32 +_ 1C and 60 +__ 5%RH in darkness. Under these conditions, longevity of eggs and adults was 6.3 and 7.3 day, respectively, and a head louse produces five to six eggs a day.A filter paper contact bioassay was used to evaluate the toxicity of the essential oils and insecticides to adult *P. humanuscapitis*. In a preliminary experiment with marjoram oils as well as 6-phenothrinand pyrethrum, 0.25 mg/cm2 was an appropriatestarting dose for a primary screening. If an essential oilgave similar or better activity than either 8-phenothrinor pyrethrum, further bioassays were conducted. Amounts (0.0625, 0.125, and 0.25 mg/cm9) of the essential oil were applied to filter papers (Whatman No. 2, 4.5 cm in diameter) in 80 p, 1 of acetone. Control filter papers received 80/xl of acetone. After drying ina fume hood for 2

min, each filter paper was placed onthe bottom of a petri dish (5 cm in diameter, 1.2 cm inheight). Batches of 20 *P. humanuscapitis* females (7-9d old), given a human blood meal 4 h before the bioassay, were placed on each petri dish, containing afew strands of human hair, and the dish covered with a lid. In a separate experiment, vapor phase toxicity ofthetest oils against female *P. humanuscapitis* was investigated according to the method of Yang et al. 2003). Briefly, batches of 20 females (7-9 d old) were placedon the bottom of a petri dish (5 cm in diameter, 1.2 cmin height). The petri dish was then covered using a lid with a fine wire sieve (4.7 cm in diameter) attached over a central hole (4.5 cm in diameter). Each filterpaper (4.25 cm in diameter), treated with 0.25 mg/cm2of each essential oil dissolved in 80/xl of acetone, was placed over the wire sieve. This prevented direct contact of female lice with the test oil. Each petri dish was then either covered with another lid (method A) to investigate the potential vapor phase toxicity of the test oils or left uncovered (method B). Control filte rpapers received 80/xl of acetone. Treated and control (solvent only) females were held at 31 IC and 65 5% RH in darkness. Mortalities were determined every 5 min for 5 h. Females were considered dead if they exhibited lethargic response or no movement. In fact, all the individuals scored as dead never recovered. All treatments were replicated three times. i-Phenothrin and pyrethrum served as standards for comparison in toxicity tests. The LT50 values were calculated by probitanalysis (SAS Institute. 1996).

Anti-lice activity

Sweet marjoram isolates and water extracts were tested for pediculocidal activity by filter paper diffusion method (Picollo MI, et al., 2000). All the extracts were dissolved in distilled water to obtain 3 different concentrations (5%, 10%, and 20%). After careful selection under a dissecting microscope, the adults and nymphs were identified and separated. All the test organisms in a ratio of 3.6/1.4 (adult/nymph) were divided into 16 groups (5 lice each) and were placed on a filter paper at the bottom of petri dish and kept open. A 0.5 ml of each test samples was poured on the test organisms and allowed to spread as a thin layer of 4 cm^2. Group 1 was treated with 0.5 ml distilled water and served as control. Group 2 to group 13 received 0.5 ml of various concentrations of aqueous, petroleum ether, chloroform, and methanol extracts respectively. Group 14 to group 16 were treated with 0.5 ml of 5%, 10%, and 20% of benzyl benzoate 25% (w/v) (RidPed). All the Petri dishes were set aside for 1 hr in a dark chamber at 26 ± 0.5℃ and 70 ± 1% humidity (Carpinella MC, et al.;2007). At the end of 1 hr, the dishes were taken out and applied 0.5 ml of distilled water and further placed in the chamber under the condition mentioned above. After 18 hr, the dishes were observed under a dissecting microscope for any possible movement of lice and absence of any movement was considered dead (Meinking TL,et al.;1986). All the treatment was triplicate.

Ovicidal effects

The ovicidal activity was tested by placing 5 brownish oval eggs with an unbroken operculum on the filter paper (Whatmann No. 1; 6 cm diameter) placed in the bottom of each petri dish. Then, 0.5 ml of each test solution and control were applied on the nits. All the dishes were then incubated in a dark chamber at 26 ± 0.5℃ for 14 days. To maintain the moisture, 0.1 ml of distilled water was added at 48 hr. interval. Hatching of eggs was monitored under a microscope and the percentage of emergence, i.e.,

partially hatched nits, was observed, and the findings were recorded -CarpinellaMCetal.; 2007).Each treatment was replicated 3 times.

Results and Discussion

The contact insecticidal activities of marjoram oilsat a dose of 0.25 mg/cm2, against adult *P.humanuscapitis,* were compared with those of the commonly used insecticides - phenothrin and pyrethrum (Table 1). Significant differences were observedin the contact toxicity to female head lice. Onthe basis of LT50 values, marjoram oils were more toxic than either &-phenothrin or pyrethrum. Marjoram oils were 2.0-fold more toxic than &-phenothrin. No mortality was observed for solvent-treated lice over the observational interval of the contact bioassay. Relative toxicity, LT50 value of &-phenothrin/LT5o value of each test material. Marjoram was highly effective and more toxic than either& -phenothrin or pyrethrum. Marjoram oil was also toxic at 0.0625 mg/cm (Table 1)

The vapor phase toxicities of the test Marjoram oil and insecticides against adult *P. capitis* were investigated using a fumigant bioassay in two formats (Table 2). The response of adult P. humanuscapitis tomarjoram oil varied between exposures in a closed container(method A) versus exposures in an open container(method B) over a 5-h exposure. Marjoram oil was50-fold more active in the fumigant assay (closed container) than when using method B (open container).No mortality was observed within the 5-h exposure interval to either-phenothrin or pyrethrum in the closed or open containers, suggesting little or no fumigant action of these insecticides.

Our study indicates that essential oils from local plants of marjoram were highly effective in the vapor phase against head lice. However, *in vitro* efficacy tests of botanical extracts are only the first step of research and much work is needed before they could be used in a commercial product. The incorporation of excipients (alcohols, etc.) that increase the stability of essential oils is of a great concern since essential oils are highly volatile, and the effectiveness of the product could decay in hours if the formulation is incorrect. For instance, active ingredients that were effective *in vitro* could show low or no activity against lice when incorporated into a liquid formulation because certain adjuvants or excipients could affect the insecticidal activity once they are incorporated into a formulation. Once the vehicle base and the excipients are selected, a battery of tests for acute and chronic toxicity (e.g., burning sensation, skin irritation, etc.) is needed. A final step should consider the *in vivo* efficacy of the product (i.e. in clinical trials).Plant essential oils have potential as natural productsfor *P. humanuscapitis* control because some of them are selective, have little or no harmful effects on non-target organisms, and can be applied to humans inthe same way as other conventional insecticides(Hadfield-Law, L ;2000,Morsy, T A et al.; 20008and Mumcuoglu, K Y, etal.; 2002).Many essential oils are known to possess ovicidal, repellent, antifeeding, and insecticidal activities against various insect species(Saxena, BP; 1989 and Isman, M B.1999). For example, neem, *Azadirachta indica* A. Juss,oil is found to have a variety of biological activities, including insecticidal activity against nearly 200 species of insects without any adverse effects on most non target organisms (Saxena, BP; 1989). Additionally, some plant extracts or phytochemicals can be highly effective against insecticide-resistant insect pests(Lindquist, R K, et al.; 1990). The most effective botanical oils would be

those offering a broad spectrum of activity against various life stages of the pest. The control agent should reduce the insect population at all stages, and it should decrease the incidence of the pest(Lamiri, Aet al.; 2001). The present investigation showed that marjoram essential oil exhibited strong contact toxicity and moderate fumigant activity against test stages. Furthermore, adult males and females showed high susceptibility to the fumigation. The results of the current study demonstrated that the pupa was the most tolerant stage and that the adult was the most sensitive one. It is well known that for fumigants, the active stages(adults and non-diapausing larvae) of insects are more susceptible than the sedentary stages (eggs and pupae), owing to differences in their respiratory rates (Rajendran, S, and Sriranjini, V; 2008).In the ovicidal bioassay, the test oil exhibited weak fumigant toxicity and strong contactactivity against egg hatchability. Hence, in the fumigation bioassay the verylow vulnerability of the eggs to vapours at the beginning of embryogenesis results from the fact thatthe permeability of the egg's external surface is lowerat the start of embryogenesis. This relatively impermeable surface opposes the diffusion ofvapours into the young eggs (Maciel, MV, et al.;2010). A second explanation offered by Emekci*et al.,* 2002)was that because respiration rates are much lower at the egg stage than at the active stages, the lower rateof air exchange results in less monoterpenoidm diffusion into the egg. In the present study, essential oil of marjoram showed insignificant oviposition deterrentability. Non-oviposition deterrent toxicity of the insecticide is perhaps because of the absence of corresponding organs or tissues in relation to behavior of oviposition(Hu, QB. et al.;2009). Another plausible suggestion is that low doses were not effective. High doses could not be used to test the oviposition repellency because, as previously described, the adult stage showed high susceptibility to high doses. The contact and fumigation bioassays usedagainst immature stages resulted in some malformed adults. The toxicity of the monoterpenoids has all the characteristics of juvenile hormone activity. The occurrence of deformed adults could be explained by assuming a direct effect on the insect hormonal system similar to that of the insect growth regulators(Schwarz, M, et al.;1970).The deformations induced by essential oils in other pests have been described byde Mendonc *et al.,* 2005;Shekari*et al.,* (2008).Oils from marjoram, have exhibited significant pediculocidal activity in filter paper bioassays (Yang YC, et al. ;2003 and Meinking TL, Taplin D;1996).Penetration of extracts into the alimentary tract of lice could be ignored since all the extracts was applied on lice placed on the filter paper which also subsequently avoided immense dissemination of active constituents into the cuticle when the compound is directly applied to the insect skin(Burkhart CN, Burkhart CG;2001). Additionally, the lice was not exposed in an enclosed environment with the petri dish kept open which limits the possibility of volatile agents getting absorbed through the spiracles. For synthetic pediculocidal agents, the residue which remains in the head even after rinsing with water gives an enhanced control against lice but also noted for the development of resistance for lice (Mumcuoglu KY;1999).Natural extracts from medicinal plants has been noticed for its safe and effective use, and appearance of resistance patterns were minimal due to its different mode of action (Breuer M, et al.;2003).which greatly supports the safe use of marjoram extracts as a potent anti-lice agent.

Figure 1.Collection of lice from children **Figure2.**Removal of lice from comb to petri dish

Figure3.Rearing of licein petri dish

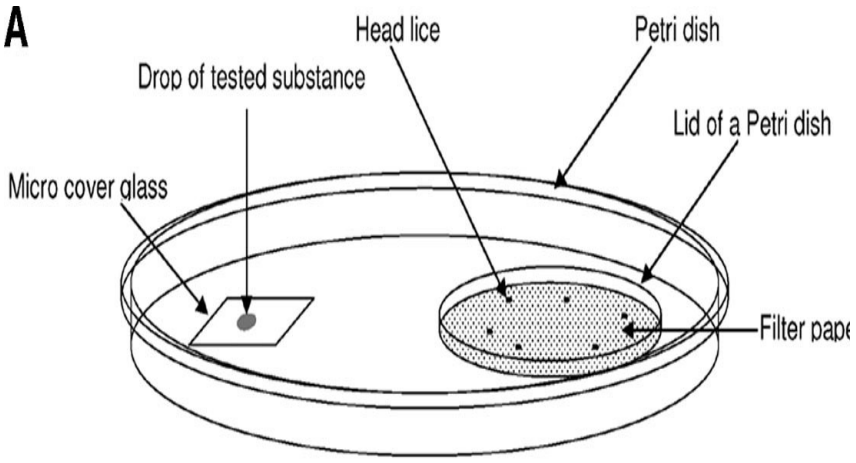

Figure 4.(A) Diagram of arena used to evaluate fumigant properties of vapors from essential oils or their chemical components

Table1. Relative toxicity of Marjoram essential oil, -phenothrin, and pyrethrmn against female *P. humanuscapitis* by using the filter paper contact bioassay at 0.25, 0.125 and 0.0625 mg/cm2

Essential oil	At 0.25 mg/cm^2			At 0.125 mg/cm^2			At 0.625 mg/cm^2	
	LT_{50}^{min}	95% CL	RL	LT_{50}^{min}	95% CL	RL	LT_{50}^{min}	95% CL
Marjoram	11.4	10.67-12.09	2.0	15.1	13.98-16.19	2.2	19.6	18.33-20.92
&- phenothrin	23.1	20.49-25.89	1.0	33.2	28.41-38.73	1.0	>300	-
Pyrethrum	25.3	22.14- 28.55	0.9	35.6	31.24-40.53	0.9	>300	-

For marjoram oil, the number of females tested was 60.
Marjoram oil showing potent pediculicidal activity at 0.25 mg/cm and 0.125 mg/cm are recorded.

Table 2. Fumigant activity of Marjoram oil, &phenothrin, and pyrethrum against female *P. humanuscapitis* at 0.25mg/cm^2

Insecticides	Method	LT_{50} min	95% CL	RT
Marjoram	A	12.6	11.61-13,39	>24
&-phenothrin	A	>300.0		
pyrethrum	B	>300.0		

For each test material, the number of females tested was 60.
A, vapor in close containers; B, vapor in open containers.
Confidence limit.LT_{50} value of method B/LT_{50} value of method A.

Figure 5. Petri dish with dead lice from one head after treatment for 10 min

Table.3 Effects of *Marjoram* oil extracts against *Pediculus humanuscapitis* nits

Test sample	Concentration %	Emergence[a]%	
		Days 6	Days 14
Dis. Water(0.5ml)	-	82.0	92.1
*Hexane fraction	5	22.1	7.2
	10	0	0
	20	0	0
*Ethanol fraction	5	58.3	42.4
	10	53.1	27.3
	20	22.1	12.3
Water extract(0.5ml)	5	71.1	45.2
	10	32.3	15.3
	20	16.8	10.2
Benzoyl	5	6.1	0
benzoate(25%w/v)	10	0	0
(0.5ml)	20	0	0

*Indicate significant (P<0.05) difference compared with control (Dis. water).

Table 4. Effects of *Marjoram* oil extracts against *Pediculus humanusapitis* adults and nymphs

Test sample	Concentration %	Average mortality[a] %
Dist. Water (0.5 ml)	-	9.8
*Hexane fraction	5	52.1
	10	79.8
	20	95
*Ethanol fraction	5	33.5
	10	40.1
	20	58.2
Water xtract(0.5ml)	5	10.1
	10	11.2
	20	16.8
Benzoyl benzoate(25%w/v) (0.5ml)	5	60.9
	10	100
	20	100

[a]n= 3.

*Indicate significant (P<0.05) difference compared with control (Dis. water).

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