Parasitology Research (2018) 117:603–609
https://doi.org/10.1007/s00436-017-5722-5

SHORT COMMUNICATION

In vitro efficacy of five essential oils against *Pediculus humanus capitis*

Kerdalidec Candy[1,2] · **Patrick Nicolas**[3] · **Valérie Andriantsoanirina**[1] · **Arezki Izri**[1,2,4] · **Rémy Durand**[1,4,5,6]

Received: 14 August 2017 / Accepted: 12 December 2017 / Published online: 20 December 2017

Abstract

Treatment of head lice has relied mainly on the use of topical insecticides. Today, conventional topical pediculicides have suffered considerable loss of activity worldwide. There is increasing interest in the use of natural products such as essential oils for head louse control, and many of them are now incorporated into various over-the-counter products presented as pediculicides, often without proper evaluation. The aim of the present study was to assess the in vitro efficacy of five essential oils against adults of *Pediculus humanus capitis* using a contact filter paper toxicity bioassay. The chemical composition of the essential oils from wild bergamot, clove, lavender, tea tree, and Yunnan verbena was analyzed by gas chromatography-mass spectrometry. All treatments and controls were replicated three times on separate occasions over a period of 11 months. In all, 1239 living lice were collected from the scalp of 51 subjects, aged from 1 to 69 years. Clove oil, diluted either in coco oil or sunflower oil, demonstrated the best adulticidal activity, reaching > 90% mortality within 2 h in lice submitted to a 30-min contact. Yunnan verbena oil diluted in coco oil showed also a significant efficacy. Other essential oils showed a lower efficacy. The oil's major component(s) differed according to the tested oils and appeared chemically diverse. In the case of clove oil, the eugenol appeared as the main component. This study confirmed the potential interest of some of the essential oils tested, but not all, as products to include possibly in a pediculicidal formulation.

Keywords *Pediculus humanus capitis* · Head lice · Essential oils · Contact · Filter paper · Bioassay

Introduction

Head louse infestation due to *Pediculus humanus capitis* (De Geer) is the most prevalent human ectoparasitic disease worldwide (Falagas et al. 2008). Head lice are hematophagous, wingless insects belonging to the order of Anoplura (Chosidow 2000). They are obligatory ectoparasites that feed exclusively on human blood. These parasites affect over 100 million people worldwide each year. Although all age groups may be affected, it is particularly frequent among children 3 to 11 years of age. The infestation by head lice, *Pediculosis capitis*, is generally considered as a benign condition. *Pediculosis capitis* may cause skin irritation, secondary infection from scratching, social stigmatization, and psychological distress. Economic implications are also substantial for families who want to rid themselves of the problem.

For the past 50 years, treatment has relied mainly on the use of topical insecticides, such as topical pyrethroids or malathion (Burgess 2009). The large selection pressure induced by neurotoxic insecticides led to the emergence and spreading of resistance to organochlorines, pyrethroids, and organophosphorous compounds in many parts of the world (Durand et al. 2012; Koch et al. 2016). Indeed, clinical, parasitological, and molecular data showed that conventional topical pediculicides have suffered considerable loss of activity worldwide. Other options are now available, including topical non-neurotoxic agents such as dimeticone (Burgess 2009). There is also increasing interest in the use of natural products

✉ Rémy Durand
remy.durand@aphp.fr

1 Parasitology-Mycology Department, AP-HP, Avicenne Hospital, Bobigny, France

2 UMR 190, Unité des virus émergents, Faculté de Médecine-Timone, Université Aix-Marseille, Marseille, France

3 Unit of Pharmacology, AP-HP, Avicenne Hospital, Bobigny, France

4 UFR SMBH, Université Paris 13, Bobigny, France

5 UMR216, Mère et enfant face aux infections tropicales, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France

6 Laboratoire de Parasitologie-Mycologie, Hôpital Avicenne, 125 rue de Stalingrad, 93009 Bobigny, France

for head louse control. Essential oils are blends of many different plant metabolites which are usually extracted from plants through steam distillation (Bakkali et al. 2008). These metabolites are volatile molecules of low molecular weight. Essential oils usually contain two or three major terpene or terpenoid components (Bakkali et al. 2008). They may act at multiple and novel target sites, thereby reducing potential for resistance (Choi et al. 2010). There are several reports showing the pediculicidal activity of some essentials oils, used alone or in combination with other products, either in in vitro assays or clinical trials (Yang et al. 2004; Yang et al. 2005; Heukelbach et al. 2006; Priestley et al. 2006; Gonzalez-Audino et al. 2007; Williamson et al. 2007; Heukelbach et al. 2008; Barker and Altman 2010; Choi et al. 2010; Bagavan et al. 2011; Barker and altman 2011; Gonzalez-Audino et al. 2011; Di Campli et al. 2012; Gallardo et al. 2012a; Gallardo et al. 2012b; Yones et al. 2016). In France, as in many countries, head louse infestation is no longer considered as a disease, and the status of pediculicides changed from medicinal products to medical devices or natural products, requiring less stringent registration procedures. Thus, many essential oils are now incorporated into various over-the-counter products presented as pediculicides without proper evaluation. They are generally well-tolerated, but they may have undesirable effects. For example, lavender oil and tea tree oil have been suspected to be endocrine disrupters (Henley et al. 2007).

The aim of the present study was to assess the in vitro efficacy of five essential oils, already included or not in products marketed as pediculicides, against adults of *Pediculus humanus capitis*.

Materials and methods

Ethical clearance

The protocol was reviewed and approved by the *Comité de Protection des Personnes* (institutional review board) of the ethics committee CPP-Ile-de-France X (2017-02). Informed consent was obtained from all patients.

Patients

Adult head lice were collected in patients consulting at Avicenne Hospital or from schoolchildren in Bobigny, France, between January and November 2015. The patients had not been treated with any anti-louse products for at least 2 weeks before the assays. Fine toothed anti-louse combs were used for collection. Lice were kept in Petri dish (8.5-cm diameter). Unharmed adult lice of both sexes were pooled and distributed randomly for different tests. Experiments started within 2 h of head louse collection.

Essential oils tested

Essential oils and diluent oils were purchased from different suppliers (Table 1). All oils were guaranteed to be pure and free of adulterative substances. All essential oils were diluted with their respective diluent oil to the working concentration on the day of experiments. The diluent oils (sunflower and coconut oil) were selected because they are present in various over-the-counter pediculicides already available in the market (Table 2).

Gas chromatography/mass spectrometry

The main components of the essential oils were analyzed by gas chromatography/mass spectrometry (GC/MS) with an Agilent 5977A Series GC/MSD system. The ability of the columns to separate compounds depends on their stationary phases. According to this principle and considering the potential composition of the oils, we used a DB-1 MS column (30 m × 0.25 mm × 0.25 μm) or an HP-INNOWax column (30 m × 0.25 mm × 0.25 μm) to analyze the different oils. Helium was the carrier gas. The chemical structure of each component was identified by comparing its fragmentation patterns seen in mass spectra with standard library data. The relative percentage of the oil components was calculated from the GC peak areas.

Table 1 Essential oils and diluents used in the study

Common name	Systematic name	Company
Wild bergamot	*Monarda fistulosa*	Aroma Zone, Cabrières, France
Clove	*Eugenia caryophyllus*	Pranarom, Ghisenghien, Belgium
Lavender	*Lavandula augustifolia*	Pranarom, Ghisenghien, Belgium
Tea tree	*Melaleuca alternifolia*	Pranarom, Ghisenghien, Belgium
Yunnan verbena	*Litsea cubeba*	Florame, Saint Rémy de Provence, France
Coconut	*Cocos nucifera*	Bio Planete, Bram, France
Sunflower	*Helianthus annuus*	Rustica, Ivry-sur-Seine, France

Table 2 Products containing essential oils and/or vegetable oils marketed as pediculicides in France

Product names	Galenic form	Essential and vegetable oil components [a]	Company
Puressentiel®	Lotion	***Lavandula angustifolia* (lavender), *Syzygium aromaticum* (clove), *Melaleuca alternifolia* (tea tree)**, *Pelargonium x asperum* (geranium), ***Cocos nucifera* (coconut)**, *Calophyllum inophyllum* (calophyllum), *Simmondsia chinensis* (jojoba), *Prunus amygdalus* (almond)	Puressentiel (France)
	Shampoo	***Lavandula angustifolia* (lavender)**, *Lavandula* hybrida grosso (lavender), *Pelargonium graveolens* (geranium), *Cananga odorata* (ylang ylang)	
	Repellent spray	*Eucalyptus citriodora* (eucalyptus)	
Apaisyl® poux	Lotion	**Coconut oil derivatives**	Merck (France)
Parasidose® poux-lentes	Shampoo	***Lavandula angustifolia* (lavender)**	Gilbert (France)
ITEM® K.O poux	Cream gel	**Coconut oil derivatives**	Item dermatologie (Switzerland)
ITEM® K.O lentes	Balm	*Mentha viridis*, *Eucalyptus dives*, *Rosmarinus officinalis*, *Myrtus communis*, *Salvia officinalis*, *Thuja occidentalis*, *Hyssopus officinalis*, *Artemisia pallens*, *Tagetes erecta*, *Prunus armeniaca*	Item dermatologie (Switzerland)
Aromapoux®	Lotion	***Melaleuca alternifolia* (tea tree)**, *Lavandula hybrida* (lavender), *Illicium verum* (star anise), *Cananga odorata* (ylang ylang), **cocos nucifera (coconut)**, *Prunus armeniaca* (apricot)	Pranarom (Belgium)
	Shampoo	***Melaleuca alternifolia* (tea tree)**, ***Eugenia caryophyllus* (clove)**, *Cananga odorata* (ylang ylang), *Lavandula hybrida* (lavender), *Eucalyptus radiata* (eucalyptus), *Mentha pulegium* (mentha), *Myrtus communis* (myrtle), *Origanum heracleoticum* (oregano)	
Institut phyto®	Lotion	***Cocos nucifera* (coconut oil), *Helianthus annuus* (sunflower)**	Sarbec cosmetics (France)
	Shampoo	***Cocos nucifera* (coconut oil)**	
Capipoux®	Gel	***Cocos Nucifera* (coconut), *Helianthus annuus* (sunflower), *Lavandula angustifolia* (lavender)**, *Ocimum basilicum* (basil), *Pelargonium graveolens* (geranium)	Melvita (France)
P'tit dop®	Lotion	**Lavender**, **copra oil (coconut oil)**	L'Oréal (France)
Quies® anti-poux et lentes	Cream	***Cocos nucifera* (coconut oil)**	Quies (France)
	Shampoo	***Cocos nucifera* (coconut oil)**	
Ecrinal® poux	Lotion	***Cocos nucifera* (coconut oil)**	Asepta (Monaco)
	Shampoo	**Lavander**, cedrus, santal, eucalyptus	
Marie Rose®	Lotion	**Coconut oil derivatives**, ***Helianthus annuus* (sunflower)**	Marie rose (France)
	Shampoo	***Cocos nucifera* (coconut oil)**	
Phytosun Arôms®	Lotion	**Coconut oil derivatives**, *Illicium verum* (anise), *Cananga odorata* (ylang ylang)	Omega Pharma (France)
	Shampoo	***Lavandula angustifolia* (lavender)**	
Pediakid® Balépou	Repellent spray	***Lavandula angustifolia***, ***Melaleuca alternifolia***, *Cedrus atlantica*, *Citrus aurantium* dulcis, *Pelargonium graveolens*, *Ravensara aromatica*	Ineldea (France)
	Shampoo	***Lavandula angustifolia***, ***Melaleuca alternifolia***, *Cedrus antlantica*, *Pelargonium graveolens*, *Ravensara aromatica*, *Citrus aurantium dulcis*	

[a] Essential oils or vegetable oils in bold were tested in the present study

Filter paper contact toxicity bioassay

A filter paper contact bioassay was used to evaluate the toxicity of wild bergamot, clove, lavender, tea tree, and Yunnan verbena essential oils tested against *P. humanus capitis*. Lice were exposed to a unique concentration (1.75 mg/cm^2) of each essential oil. Briefly, 100 μL of the essential oil were diluted in 900 μL of coconut or sunflower oil, and the mixture was shaken (vortex) to facilitate dispersion. Then, the oil mixture was spread evenly across the surface of a filter paper (Whatman No. 1, 8.5 cm in diameter) that was placed in the bottom of a plastic Petri dish (8.5 cm in diameter, 1.5 cm in height). Filter paper of diluent controls received 1 mL of coconut or sunflower oil only. Negative control lice were placed directly on the filter paper moistened with 1 mL of distillated water. Groups of ~ 30 unharmed lice (adults, both sexes) were placed on the bottom of the filter paper in each essential oil group and in control groups. After a contact time of 30 min and to simulate treatment of an infested host, head lice were washed with a neutral shampoo, Neutrapharm™, Laboratoire Gilbert, Hérouville Saint-Clair, France; rinsed with tap water; and dried with absorbent paper. Lice were then examined under stereo microscope, and mortalities were recorded every 30 min for 3 h. If any minor signs of life, such as movements of antennae or minimal leg movements, were observed (with or without stimulation by a forceps), the lice were categorized as alive. The lice were judged as dead if there were no vital signs at all. Treated and control head lice were held at the same conditions of 25 ± 2 °C and 20–50% relative humidity. All treatments and controls were replicated three times on separate occasions over a period of 11 months, and the averages were considered.

Statistical analysis

The data were analyzed by Kaplan-Meier survival curves, and the median lethal times (LT_{50}) of head lice were calculated using JMP 13.0 software. The statistical differences between data obtained with each essential oil and the control for each experiment were measured by log-rank test. P value of ≤ 0.05 was considered significant. For multiple-comparison testing, the Bonferroni correction was used to avoid the inflation of the nominal risk α. Here, the nominal risk was selected as α/k, where k is the number of 2 × 2 comparisons and α is 0.05.

Results

In all, 1239 living lice were collected from the scalp of 51 subjects, aged from 1 to 69 years. The major components of the essential oils analyzed by GC/MS are presented in Table 3. The survival curves of adult lice exposed to essential oils or to diluent oils by direct contact are shown in Fig. 1. All diluted essential oils showed significant lethal activity in comparison with the activity of the diluents alone, coco or sunflower oil (Fig. 2). The LT_{50} values estimated from direct-contact toxicity bioassay of essential oils, at a concentration of 1.75 mg/cm^2 in the two different diluents, are given in Table 4. Clove oil, diluted either in coco oil or sunflower oil, demonstrated the best adulticidal activity, reaching > 90% mortality within 2 h in lice submitted to a 30-min contact. Yunnan verbena oil diluted in coco oil showed also a significant efficacy. Other oils showed a lower efficacy, diluted either in coco oil or sunflower oil. Lavender and tea trea in coco oil, and lavender, wild bergamot and tea tree in sunflower oil killed less than 50% of adult lice during the time of observation, and thus, LT_{50} values could not have been determined in those bioassays. All essential oils showed a higher pediculicidal activity when they were diluted in coco oil in comparison with sunflower oil (Fig. 1). Sunflower oil and coco oil showed a weak pediculicidal activity, when used alone in comparison with control groups (sunflower vs. control, $p = 0.0165$; coco vs. control, $p < 0.0001$). That activity was higher for coco oil than for sunflower oil ($p = 0.0141$).

Table 3 The major components of the five essential oils tested

Major components (%)[a]	Wild bergamot	Clove	Lavender	Tea tree	Yunnan verbena
Acetyl eugenol		16.3			
Eugenol		74.6			
Geranial					36.7
Geraniol	91.7				
Limonene					14.2
Linalol			33		
Linalyl acetate			44.1		
Neral					28.9
α-Terpinene				9.2	
γ-Terpinene				15.6	
Terpinen-4-ol				37.1	
α-Terpineol				5	

[a] Only compounds representing a relative percentage of the oil component ≥ 5%, calculated from the gas chromatography peak areas, are shown

Discussion

We chose to use head lice caught on patients because tests using laboratory-reared body lice may be less representative as they do not identify individual variations of drug susceptibility. The used methodology, including a time contact of 30 min with the diluted essential oils, a shampoo, and a washout of lice with tap water, was

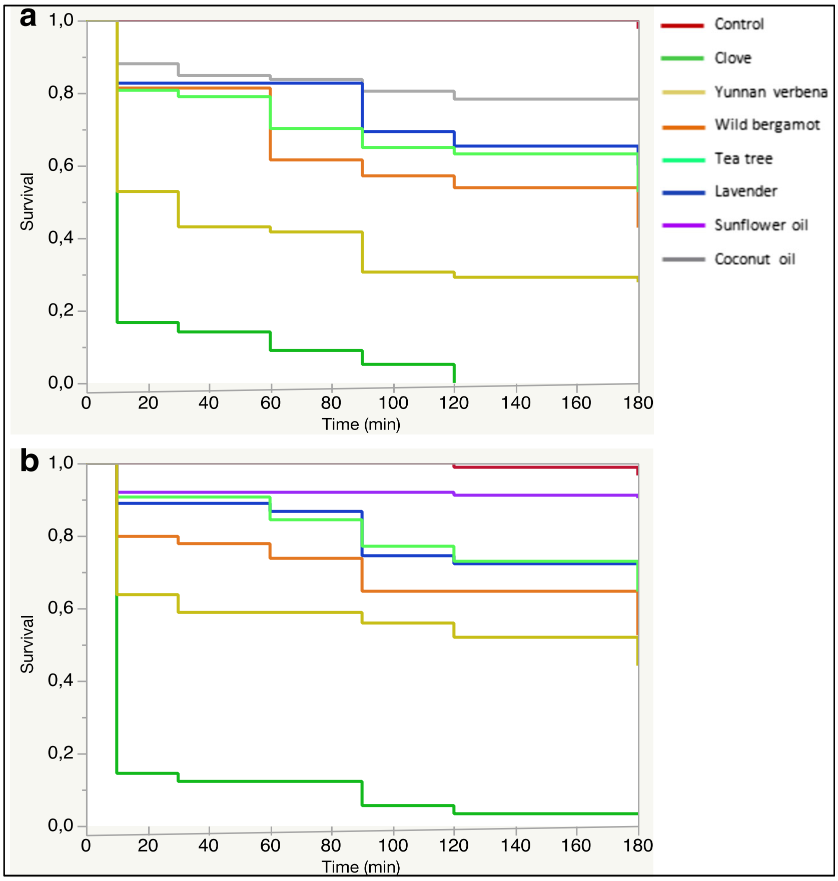

Fig. 1 Survival curves of *Pediculus humanus capitis* adult forms exposed to essential oils. **a** Essential oils diluted in coconut oil. **b** Essential oils diluted in sunflower oil

designed to simulate what head lice may encounter during a real treatment. Our results confirmed the potential interest of clove oil to control head louse infestation. The LT_{50} values observed in our study were comparable to those found in two other recent studies where head lice were laid on the impregnated filter papers during all the experiments (Bagavan et al. 2011; Yones et al. 2016). The second most active essential oil was extracted from *Litsea cubeba* (Yunnan verbena), showing a LT_{50} value of 30 min in both diluent oils. To our knowledge, essential oil from *Litsea cubeba* has not been previously investigated for pediculicidal activity. Other oils showed a lower efficacy, failing to kill more than 50% of lice under the conditions tested, except for the wild bergamot oil diluted in coconut oil. In previous studies, tea tree oil may have shown a better efficacy than what we observed in our work, though results were variable according to the tested formulations (Heukelbach et al. 2008). It may be underlined that the best results with tea tree oil were obtained when ethanol or another alcohol was present in the formulation, which was not the case in our study. Previous

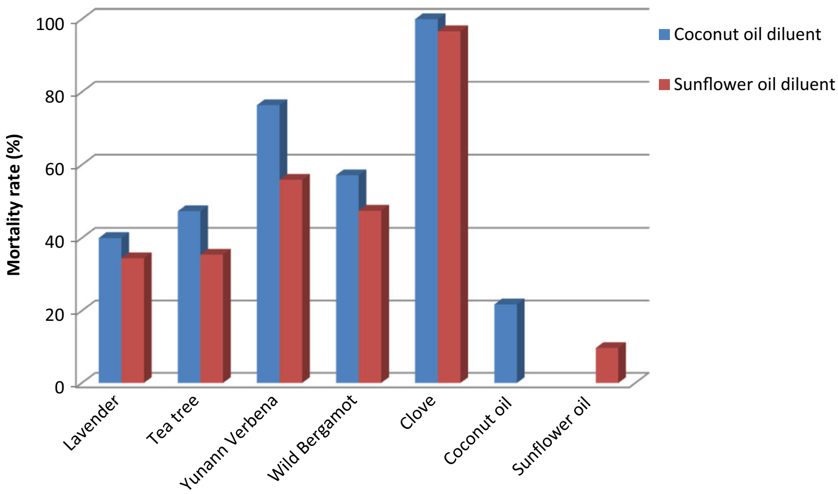

Fig. 2 Evaluation of the activity of five essential oils and their diluents against *P. humanus capitis* using the filter paper contact toxicity assay

Table 4 Comparison of the median lethal times (LT_{50}) of the essential oils tested

Essential oil	LT_{50} (min)	Comparison	Log-rank	*P* value
Clove[a]	10	Clove vs. wild bergamot	93.1752	< 0.0001
Lavender[a]	NA			
Wild bergamot[a]	180	Clove vs. Yunnan verbena	32.1309	< 0.0001
Tea tree[a]	NA			
Yunnan verbena[a]	30	Yunnan verbena vs. wild bergamot	8.8269	0.0030
Clove[b]	10	Clove vs. Yunnan verbena	63.3298	<0.0001
Lavender[b]	NA			
Wild bergamot[b]	NA			
Tea tree[b]	NA			
Yunnan verbena[b]	180			

NA not applicable

[a] Diluted in coconut oil

[b] Diluted in sunflower oil

studies showed that ethanol and other alcohols may have effects on lice due to their own toxicity (Gonzalez-Audino et al. 2007; Meinking et al. 2010; Mougabure Cueto and Picollo 2010; Gonzalez-Audino et al. 2011). It has already been reported that particular formulations may improve the efficacy of a pediculicide. Actually, formulations containing tea tree oil currently marketed often include some alcohols and other essential oils such as lavender oil, for example.

Similarly, lavender oil did not show a major in vitro efficacy in our study. The interesting results previously reported for that essential oil were obtained in association with another essential oil (Barker and Altman 2010) or dissolved in ethanol ± isopropanol (Gonzalez-Audino et al. 2007).

Data about the in vitro efficacy of wild bergamot oil are lacking in the current literature. In our study, wild bergamot oil showed a non-negligible efficacy, mainly in coconut oil, higher than that of tea tree or lavender but lower than that of clove and Yunnan verbena oils. The pharmaceutical market for head louse products in many countries is swamped with poorly tested and/or ineffective products. As essential oils may constitute interesting alternative to insecticide treatment options, there is an urgent need to document the efficacy of the different products. Among the essential oils tested in the present study, clove, tea tree, wild bergamot, and lavender oils, but not Yunnan verbena oil, were already included, in association with other products, in formulations marketed as pediculicides. According to our study, lavender, wild bergamot, and tea tree may not be the best essential oils to include in a pediculicidal formulation. Clove oil and, in a lesser extent, Yunnan verbena oil appeared more interesting.

The mode of action of many essential oils or their components is largely unknown. The insecticidal efficacy of essential oils is often attributed to the oil's major component(s), and there is also evidence that the various oil components may work in synergy (Yang et al. 2004). As shown in this study by CPG-MS, the oil's major component(s) differed according to the tested oils and appeared chemically diverse. In the case of clove oil, a component, the eugenol, appeared predominant. Eugenol was also previously reported as a major component of *Mentha spicata* (spearmint) oil, one of the most efficacious essential oils tested by other authors (Yones et al. 2016). In our study, other oils were more balanced in their composition and it appeared difficult to identify a major active component.

In conclusion, this study confirmed the potential interest of some of the essential oils tested, but not all, as products to include possibly in a pediculicidal formulation. The efficacy of essential oils against *Pediculus humanus capitis* is variable and depends largely on the aromatic plants from which they are extracted and on the quality of the extraction (Bakkali et al. 2008). Further studies are warranted to select the best products and also to evaluate their toxicity in humans.

Compliance with ethical standards The protocol was reviewed and approved by the *Comité de Protection des Personnes* (institutional review board) of the ethics committee CPP-Ile-de-France X (2017-02). Informed consent was obtained from all patients.

References

Bagavan A, Rahuman AA, Kamaraj C, Elango G, Zahir AA, Jayaseelan C, Santhoshkumar T, Marimuthu S (2011) Contact and fumigant toxicity of hexane flower bud extract of *Syzygium aromaticum* and its compounds against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). Parasitol Res 109(5):1329–1340. https://doi.org/10.1007/s00436-011-2425-1

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46(2):446–475. https://doi.org/10.1016/j.fct.2007.09.106

Barker SC, Altman PM (2010) A randomised, assessor blind, parallel group comparative efficacy trial of three products for the treatment of head lice in children—melaleuca oil and lavender oil, pyrethrins and piperonyl butoxide, and a "suffocation" product. BMC Dermatol 10(1):6. https://doi.org/10.1186/1471-5945-10-6

Barker SC, Altman PM (2011) An ex vivo, assessor blind, randomized, parallel group, comparative efficacy trial of the ovicidal activity of three pediculicides after a single application—melaleuca oil and lavender oil, eucalyptus oil and lemon tea tree oil, and a "suffocation" pediculicide. BMC Dermatol 24:11–14

Burgess IF (2009) Current treatments for pediculosis capitis. Curr Opin Infect Dis 22(2):131–136. https://doi.org/10.1097/QCO.0b013e328322a019

Choi HY, Yang YC, Lee SH, Clark JM, Ahn YJ (2010) Efficacy of spray formulations containing binary mixtures of clove and eucalyptus oils against susceptible and pyrethroid/malathion-resistant head lice (Anoplura: Pediculidae). J Med Entomol 47(3):387–391. https://doi.org/10.1093/jmedent/47.3.387

Chosidow O (2000) Scabies and pediculosis. Lancet 355(9206):819–826. https://doi.org/10.1016/S0140-6736(99)09458-1

Di Campli E, Di Bartolomeo S, Delli Pizzi P, Di Giulio M, Grande R, Nostro A, Cellini L (2012) Activity of tea tree oil and nerolidol alone or in combination against *Pediculus capitis* (head lice) and its eggs. Parasitol Res 111(5):1985–1992. https://doi.org/10.1007/s00436-012-3045-0

Durand R, Bouvresse S, Berdjane Z, Izri A, Chosidow O, Clark JM (2012) Insecticide resistance in head lice: clinical, parasitological and genetic aspects. Clin Microbiol Infect 18(4):338–344. https://doi.org/10.1111/j.1469-0691.2012.03806.x

Falagas ME, Matthaiou DK, Rafailidis PI, Panos G, Pappas G (2008) Worldwide prevalence of head lice. Emerg Infect Dis 14(9):1493–1494. https://doi.org/10.3201/eid1409.080368

Gallardo A, Mougabure-Cueto G, Vassena C, Picollo MI, Toloza AC (2012a) Comparative efficacy of new commercial pediculicides against adults and eggs of *Pediculus humanus capitis* (head lice). Parasitol Res 110(5):1601–1606. https://doi.org/10.1007/s00436-011-2668-x

Gallardo A, Picollo MI, González-Audino P, Mougabure-Cueto G (2012b) Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). J Med Entomol 49(2):332–335. https://doi.org/10.1603/ME11142

Gonzalez-Audino P, Vassena C, Zerba E, Picollo M (2007) Effectiveness of lotions based on essential oils from aromatic plants against permethrin resistant *Pediculus humanus capitis*. Arch Dermatol Res 299(8):389–392. https://doi.org/10.1007/s00403-007-0772-7

Gonzalez-Audino P, Picollo MI, Gallardo A, Toloza A, Vassena C, Mougabure-Cueto G (2011) Comparative toxicity of oxygenated monoterpenoids in experimental hydroalcoholic lotions to permethrin-resistant adult head lice. Arch Dermatol Res 303(5):361–366. https://doi.org/10.1007/s00403-010-1110-z

Henley DV, Lipson N, Korach KS, Bloch CA (2007) Prepubertal gynecomastia linked to lavender and tea tree oils. N Engl J Med 356(5):479–485. https://doi.org/10.1056/NEJMoa064725

Heukelbach J, Oliveira FA, Speare R (2006) A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in vitro. Parasitol Res 99(4):353–356. https://doi.org/10.1007/s00436-006-0146-7

Heukelbach J, Canyon DV, Oliveira FA, Muller R, Speare R (2008) In vitro efficacy of over-the-counter botanical pediculicides against the head louse *Pediculus humanus var capitis* based on a stringent standard for mortality assessment. Med Vet Entomol 22(3):264–272. https://doi.org/10.1111/j.1365-2915.2008.00738.x

Koch E, Clark JM, Cohen B, Meinking TL, Ryan WG, Stevenson A, Yetman R, Yoon KS (2016) Management of Head Louse Infestations in the United States—a literature review. Pedriatr Dermatol 33(5):466–472. https://doi.org/10.1111/pde.12982

Meinking TL, Villar ME, Vicaria M, Eyerdam DH, Paquet D, Mertz-Rivera K, Rivera HF, Hiriart J, Reyna S (2010) The clinical trials supporting benzyl alcohol lotion 5% (Ulesfia): a safe and effective topical treatment for head lice (pediculosis humanus capitis). Pediatr Dermatol 27(1):19–24. https://doi.org/10.1111/j.1525-1470.2009.01059.x

Mougabure Cueto G, Picollo MI (2010) Response of *Pediculus humanus humanus* (Pediculidae: Phthiraptera) to water or 70% ethanol immersion and determination of optimal times for measuring toxic effects. Parasitol Res 106(6):1503–1506. https://doi.org/10.1007/s00436-010-1833-y

Priestley CM, Burges IF, Williamson EM (2006) Lethality of essential oil constituents towards the human louse, *Pediculus humanus*, and its eggs. Fitoterapia 77(4):303–309. https://doi.org/10.1016/j.fitote.2006.04.005

Williamson EM, Priestley CM, Burgess IF (2007) An investigation and comparison of the bioactivity of selected essential oils on human lice and house dust mites. Fitoterapia 78(7-8):521–525. https://doi.org/10.1016/j.fitote.2007.06.001

Yang YC, Lee HS, Clark JM, Ahn YJ (2004) Insecticidal activity of plant essential oils against *Pediculus humanus capitis* (Anoplura: Pediculidae). J Med Entomol 41(4):699–704. https://doi.org/10.1603/0022-2585-41.4.699

Yang YC, Lee HS, Lee SH, Clark JM, Ahn YJ (2005) Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculicidae). Int J Parasitol 35(14):1595–1600. https://doi.org/10.1016/j.ijpara.2005.08.005

Yones DA, Bakir HY, Bayoumi SA (2016) Chemical composition and efficacy of some selected plant oils against *Pediculus humanus capitis in vitro*. Parasitol Res 115(8):3209–3218. https://doi.org/10.1007/s00436-016-5083-5