

# Plant extracts and their components as potential control agents against human head lice

Carmen Rossini · Lucía Castillo ·  
Andrés González



Received: 15 June 2006 / Accepted: 28 August 2006 / Published online: 13 March 2007  
© Springer Science+Business Media B.V. 2007

**Abstract** The head louse, *Pediculus humanus capitis* (Phthiraptera:Pediculidae), is an obligate ectoparasite of humans that causes pediculosis capitis, a nuisance for millions of people worldwide, with high prevalence in children. Pediculosis capitis has been treated by methods that include the physical removal of lice, various domestic treatments and conventional insecticides. None of these methods render complete protection, and there is clear evidence for the evolution of resistance and cross-resistance to conventional insecticides. Non-toxic alternative options are hence needed for head louse treatment and/or prevention, and natural products from plants, especially essential oils (EOs), are good candidates for safer control agents that may provide good anti-lice activity and low levels of evolved resistance. A few EOs have been tested as repellents with promissory results, although often in vitro tests and clinical trials produce contradictory results. A handful of fixed extracts and several EOs and their individual components have also been tested as contact pediculicides or fumigants. The studies have focused mainly on plant families

characterized for the production of EOs. While many EOs and individual compounds showed pediculicide activity, comparing results is difficult due to the diverse bioassay methodologies. Studies of anti-lice activity of individual EO components provide the basis for preliminary conclusions of structure–activity relationships, although no clear patterns can yet be drawn. We here attempt to provide a concise compilation of the available information on anti-lice activity of plant extracts and plant-derived compounds, which we hope may be of help for future developments in this area.

**Keywords** Anti-lice products · *Pediculus humanus capitis* · Monoterpenoids · Pediculicides · Insect repellents

**Index terms** *Pediculus humanus capitis* · Plant extracts · Essential oils · Pediculicides · Head lice · Repellents · Monoterpenoids · Phthiraptera · Anoplura · Pediculidae

## Abbreviation

DDT Dichloro-diphenyl-trichloroethane  
DEET *N,N*-diethyl-*m*-toluamide  
EO(s) Essential oil(s)  
 $K_{ow}$  Octanol-water partitioning coefficients  
LT<sub>50</sub> Lethal time for killing 50% of population

C. Rossini (✉) · L. Castillo · A. González  
Laboratorio de Ecología Química, Facultad de  
Química, UdelaR, Gral. Flores 2124, Montevideo,  
CP 11800, Uruguay  
e-mail: crossini@fq.edu.uy

MW Molecular weight,  
Pv Vapour pressure

## Introduction

Lice are small wingless insects that live as ectoparasites of mammals and birds. They are usually classified within the order Phthiraptera, although some authors have placed them in two separate orders, the Mallophaga, which includes the chewing lice, and Anoplura, the sucking lice. The later includes the three lice that parasite humans: *Pediculus humanus capitis* (the head lice), *P. humanus humanus* (the body lice), both of the family Pediculidae, and the pubic lice, *Phthirus pubis* (family Pthiridae) (Borror et al. 1989).

The head louse is the most prevalent of the three. It is an obligate and permanent ectoparasite that affects millions of people worldwide (Gratz 1997). As all Phthiraptera, *P. h. capitis* is a holometabolous insect, completing its entire cycle on head hair, from eggs (nits) to adults through three nymphal instars, in about 45 days (Takano-Lee et al. 2003).

The main consequence of head lice infestation (pediculosis capitis, hereafter pediculosis) is scalp pruritus of variable intensity, due to sensitization to toxins secreted by the insect in its saliva. Albeit not extremely serious, a few cases of infectious diseases have been associated to scratching, along with secondary excoriations and in extreme cases cervical adenopathy (Burkhart et al. 1998). Unlike the body lice, *P. h. humanus*, which is a vector of the typhus-causing bacteria *Rickettsia prowazekii*, *P. h. capitis* is not known to be a disease vector, although it has been artificially infected in the laboratory with *R. prowazekii*, and it is argued that its role in the transmission of typhus should be further investigated (Murray and Torrey 1975; Gratz 1997; Robinson et al. 2003).

There is a popular notion that associates head lice infestation with bad cleaning habits, which results in a certain degree of embarrassment associated to pediculosis. Such perception seems

not to hold true, inasmuch as *P. h. capitis* prefers clean hair (Veal 1996). The prevalence of head lice does not correlate with either socioeconomic level or gender, although there appears to be greater incidence in children and youth, and possibly in white people (Burkhart et al. 1998; Leung et al. 2005). All things considered, pediculosis does not pose a serious health hazard, but it is nonetheless a nuisance that readily spreads among people, especially children, and therefore it often calls for different forms of treatment, with varying results.

## Conventional treatments for head lice

Head lice have been associated with humans for a long time (Filer 1996). The problem was apparently under control after the development of conventional insecticides such as DDT (dichloro-diphenyl-trichloroethane). However, a few years later there were clear signs of acquired resistance, not only to DDT but also to other control products, including cases of cross-resistance (Gratz 1997; Yoon et al. 2004). Nowadays, available treatments for pediculosis include topical products that are marketed as shampoos, lotions, perfumes or repellents, usually accompanied by the physical removal of nits, nymphs and adults. Another option of recent arrival is the systemic treatment with ivermectin (Burkhart et al. 1998; Jones and English 2003; Develoux 2004; Burkhart and Burkhart 2006), a semi-synthetic macrocyclic lactone derived from the soil microorganism *Streptomyces avermitilis* (Hotson 1982).

The potential world market for products that control pediculosis is quite large. There are no estimations of the current use of pediculicides worldwide, but in the United States alone, despite some products requiring a medical prescription, consumers spend an estimated \$350 millions annually for over-the-counter products (Jones and English 2003). Extensive lists of available products that have been approved for head lice treatment may be found elsewhere (Rozendaal 1997; Jones and English 2003; Burgess 2004; Burkhart and Burkhart 2006); these include organochlorides

(DDT and lindane), natural and synthetic pyrethrins (synergized with piperonyl butoxide) and carbamates. It is noteworthy that none of these products render 100% protection (Burgess 2004), or protection against all developmental instars (from nits to adults) (Burkhart et al. 1998). Moreover, there have been several reports of evolution of resistance to the conventional insecticides that are usually included in formulations (i.e., pyrethroids, lindane) (Burkhart et al. 1998; Picollo et al. 1998; Levot 2000; Jones and English 2003; Yoon et al. 2004). Such resistance appears to be associated to a variety of biochemical traits, including a mechanism based on glutathione *S*-transferase (GST) for resistance to DDT (Miller and Mumcuoglu 1999; Namazi 2005), the cytochrome monooxygenase P450 system for permethrin (Audino et al. 2005), esterase activity for dual resistance to malathion and permethrin (Gao et al. 2006), and sodium channel-mediated resistance to pyrethroids (Tomita et al. 2005). The universe of treatments has been further decreased since some insecticides that had been successfully used in the past (DDT, gamexan, carbaryl) were fortunately withdrawn as anti-lice products in some countries, due to their high toxicity (Burkhart et al. 1998).

The need for alternative options for head lice treatment, combined with the growing tendency to use non-toxic, “natural” products, particularly since children are the usual recipients, has prompted a number of scientists to search for new control products from plants, products that should combine good anti-lice activity, low levels of resistance and safety. A few studies have tested fixed extracts (Ninci 1991; Morsy et al. 2000; Tiangda et al. 2000), but most have focused on plant essential oils (hereafter EOs). EOs are complex natural products, usually mixtures of mono- and sesquiterpenes and/or phenols, which are typically obtained from aromatic plants by steam distillation (Isman 2006). EOs possess characteristics that make them good candidates for new head lice treatment alternatives: they are volatile and hence would leave no residue after treatment, they usually have a pleasant odour, and they are assumed to be harmless to people

while often showing some degree of insecticidal activity (Isman 2006).

In this review we focus on studies of anti-lice activity of plant extracts or plant-derived compounds. Previous reviews have carefully covered other issues related to pediculosis, such as louse taxonomy, epidemiology, diagnosis and general treatment options (Burgess 2004).

### Popular alternative treatments

The well-documented information for traditional head lice treatment, either with ethnobotanicals or other methods, is rather scarce. Products used in popular medicine include vinegar, formic acid, isopropyl alcohol, olive oil, mayonnaise, melted butter, propoleo, copper oleate and petroleum jelly (Takano-Lee et al. 2004). How these products work can only be guessed, and none have been proved to effectively kill lice. Acids may act upon the nits by softening the protective sheath that covers and attaches the egg to the hair (Angel et al. 2000). Oily products may occlude the respiratory spiracles of the lice, or slow the insects making it easier to physically remove them with a fine comb (Takano-Lee et al. 2004). Veal (1996) reviews many home remedies described in recent aromatherapy books. These are all essential oils that are added to a carrier such as castor oil, and include juniper, lavender, rosemary, geranium, tea tree, lemon, rose, cinnamon, lemongrass, thyme, myrtle, oregano and eucalyptus.

### The search for plant extracts with anti-lice activity

Some studies that have searched for potential anti-lice products from plants have focused on repellent activity to prevent head lice infestation. Most studies, however, evaluated the toxicity of plant extracts and plant-derived compounds, in order to treat infestations. In the later the focus has been on toxicity against adults and nymphs combined, and to a minor extent against nits (niticidal products). To our knowledge, no attempts have been made to distinguish between toxicity against adults and nymphs in *P. h. capitis*,

although one early study on the body louse did (Aschner and Mager 1945).

### Repellents

Because head lice infest their hosts by moving easily from one head to another, recurring infestations are relatively common. To avoid this, repellent products may be used preventively, especially after treatment of a previous infestation. Relatively few studies have reported repellent activity of plant extracts against head lice, but some have found repellent activity against body lice. Using a well-known insect repellent as a standard for comparison [DEET: *N, N*-diethyl-*m*-toluamide, (Nentwig 2003)], these studies reported good repellent activity in EOs of eucalyptus, lavender, rosemary, and citronella—as well as some of their individual components (limonene, geraniol, citronellal) (Mumcuoglu et al. 1996)—and piperonal (Peock and Maunder 1993). In the first study, a trend of decreasing repellent activity, although not statistically significant, showed citronella as the best body lice repellent, followed by citronellal, rosemary, geraniol and DEET (Mumcuoglu et al. 1996). In the case of piperonal, a 2% solution was more effective than a 50% DEET solution (Peock and Maunder 1993). Inasmuch as body lice are assumed to be good models for testing activity against head lice (Priestley et al. 2006), these reports provide leads for potentially valuable products to repel head lice. Indeed, piperonal was tested against head lice and found to be repellent in vitro (Burgess 1993), but the result could not be repeated in a double-blind clinical study (Burgess 2004). Besides, another study also used a double-blind clinical trial (Mumcuoglu et al. 2004) to evaluate the repellency of citronella, one of the first plant products to be used as a prophylactic agent against insects (Nentwig 2003). The authors found significant differences between children treated with citronella (12% infested) and placebo trials (51% infested).

In southern South America, there has been a recent increase in the use of ethanolic extracts of wood chips of *Quassia amara* (Simaroubaceae). This plant family is associated with the

production of quassinoids (Polonsky et al. 1975; Connolly and Hill 2005), highly oxygenated triterpenes that possess insecticidal activity against insects (Klocke et al. 1985; Latif et al. 2000). In a double blind placebo trial, however, *Q. amara* extracts showed only prophylactic activity against head lice, rather than toxicity (Ninci 1991).

Although not of plant origin, three synthetic aliphatic lactones have recently been evaluated for head lice repellency in vitro, using for comparison piperonal and EOs of eucalyptus and lavender—both previously reported as repellent (Tolozza et al. 2006). It was found that a  $\gamma$ -lactone, delta-hexalactone-5-methylpentanolide, was as good a repellent as piperonal, and better than both EOs.

In summary, plant extracts with proven head lice repellency include mostly those which are general insect repellents, with some discrepancies between results in vitro and in clinical trials. It is likely that more information on specific repellents for head lice will be the results of further studies.

### Pediculicides

The information on the toxic activity of plant extracts against head lice is rather extensive. Table 1 shows a summary of plant families, species and literature references of the different plant extracts tested against head lice. Plants evaluated include several species that belong to three families commonly associated with the production of EOs (Apiaceae, Lamiaceae, Myrtaceae) and fewer species from other families that are also associated with EO production [Asteraceae (1 species), Lauraceae (2), Myristicaceae (1), Poaceae (3), Pinaceae (1), Rosaceae (1), Rutaceae (1), Zingiberaceae (1)] (Oliveira and Spitzer 1999). To our knowledge, no species from the EO-producing Piperaceae family have been tested.

To a lesser extent, studied species belong to families that have been associated with insecticidal activity of fixed extracts (Meliaceae, Simaroubaceae), although some families that might be included in this category are noticeably absent from the literature [Solanaceae, Asteraceae (*Chrysanthemum*), Fabaceae (rotenoid-producing

**Table 1** Summary of plant species with documented activity against *P. h. capitis*

Family	Species	Reference
Annonaceae	<i>Annona squamosa</i>	Tiangda et al. (2000)
	<i>Cananga odorata</i> <sup>a</sup>	Mumcuoglu et al. (2002)
Apiaceae	<i>Petroselinum sativum</i>	Yang et al. (2004a, b)
	<i>Pimpinella anisum</i>	Yang et al. (2004a, b)
Asteraceae	<i>Tagetes minuta</i>	Cestari et al. (2004)
Cupressaceae	<i>Juniperus oxycedrus</i>	Yang et al. (2004a, b)
Lamiaceae	<i>Salvia officinalis</i>	Yang et al. (2004a, b)
	<i>Rosmarinus officinalis</i>	Lahlou and Berrada (2003); Yang et al. (2004)
	<i>Mentha pulegium</i>	Yang et al. (2004a, b)
	<i>Origanum majorana</i>	Yang et al. (2004a, b)
Lauraceae	<i>Aniba rosaeodora</i>	Yang et al. (2004a, b)
	<i>Cinnamomun zeylanicum</i>	Yang et al. (2005)
Meliaceae	<i>Azadirachta indica</i>	Mulla and Su (1999); Morsy et al. (2000); Heukelbach et al. (2006)
Myrtaceae	<i>Eucalyptus globulus</i>	Yang et al. (2004a, b)
	<i>Eucalyptus saligna</i>	Kambu et al. (1982)
	<i>Eugenia caryophyllata</i>	Yang et al. (2003)
	<i>Myrtus communis</i>	Agoumi and Gourai (1989); Yang et al. (2004a, b)
	<i>Syzygium aromaticum</i>	Yang et al. (2004, b)
Poaceae	<i>Cymbopogon nardus</i>	Mumcuoglu et al. (2004)
	<i>Cymbopogon winteratus</i>	Mumcuoglu et al. (2004)
Simaroubaceae	<i>Quassia amara</i>	Ninci (1991)
Verbenaceae	<i>Lippia multiflora</i>	Oladimeji et al. (2000)
Zingiberaceae	<i>Elettaria cardamomum</i>	Yang et al. (2004a, b)

<sup>a</sup> Reported without activity in (Yang et al. 2004a, b). [Additional plant species with putative activity against head lice are listed in Veal (2003)]

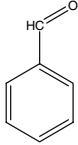
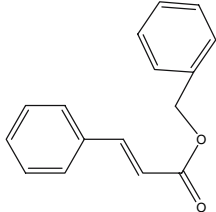
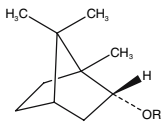
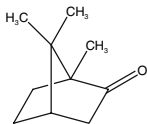
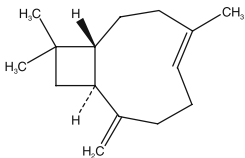
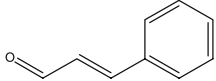
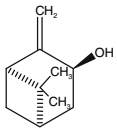
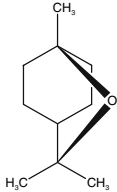
species), and furanocoumarine-producers from the Rutaceae and Apiaceae]. In some of these cases, the toxicity associated to their anti-insectan active principles would prevent their topical use, but such bias deserves further explanation.

Studies of head lice toxicity of fixed extracts include the evaluation of the neem oil (Meliaceae) (Mulla and Su 1999; Morsy et al. 2000; Heukelbach et al. 2006), the petroleum ether extract of *Annona squamosa* (Annonaceae) (Tiangda et al. 2000), and ethanolic extracts of *Q. amara* chips (Ninci 1991). Even though the phytochemical characterization of these three species indicates the presence of limonoid triterpenes, acetogenins and quassinoids, respectively (Connolly and Hill 2005; Isman 2006), no characterization of anti-lice activity has been attempted at the level of individual components.

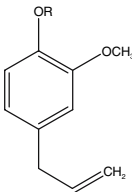
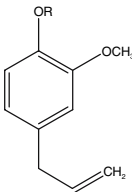
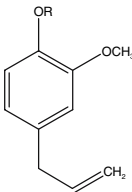
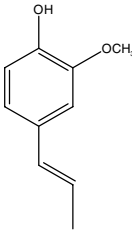
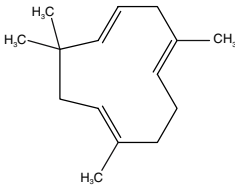
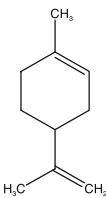
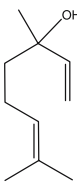
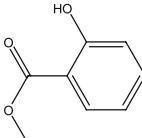
The search for pediculicides from plant EOs and their components has centred its attention on

toxicity against adults, while a few studies have evaluated anti-nit activity. It appears that no general correlation can be made between the two activities, so the different life stages do not seem to be redundant as targets. The bioassays that evaluate these activities are not always comparable. Usually, evaluation of adult toxicity is based on mortality, using parameters such as percent mortality or the time it takes for killing 50% of a population (LT<sub>50</sub>). In tests against nits, percent of hatching failure is the usual parameter. The experimental designs of the bioassays also vary, including assays with direct contact of the lice, either in open or closed environments, with filter paper where the products have been applied; submersion into test solutions, and fumigation assays where nits and adults are exposed to vapour in the open or in closed chambers, with no direct contact with extract or solution (see Table 2 for references).

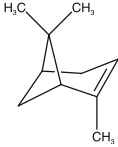
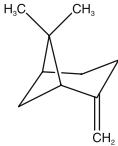
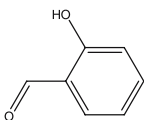
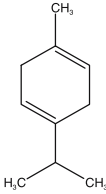
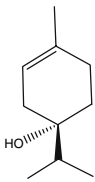
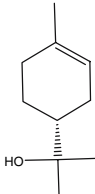
**Table 2** Isolated components tested against *P. h. capitis*

Compound	Structure	Log $K_{ow}^a$	Pv <sup>a</sup>	Activity/Reference
Benzaldehyde		1.64	0.974	C, A (Females) (Yang et al. 2005) C, Nits (Yang et al. 2005)
Benzylcinnamate		3.65	1.1e-5	NoA against A (Yang et al. 2005) C, Nits (Yang et al. 2005)
Borneol (R = H, d-form shown) Bornyl acetate (R = COOCH <sub>3</sub> )		2.71 3.6	0.0398 0.0959	C, Nits (Lahlou and Berrada 2003) C, Nits (Lahlou and Berrada 2003)
Camphor (1 form shown)		2.13	0.225	C, Nits (Lahlou and Berrada 2003)
$\beta$ -Caryophyllene		6.78	0.0128	C, Nits (Lahlou and Berrada 2003) NoA against A (Yang et al. 2003)
E-Cinamaldehyde		2.12	0.0265	C, Nits (Yang et al. 2005) NoA against A (Yang et al. 2005)
Pinocarveol		2.45	0.0283	F, A (Yang et al. 2004) C, A and Nits (Yang et al. 2004a, b)
1,8-Cineole		2.82	1.65	C, Nits (Lahlou and Berrada 2003) C, A and F, A (Yang et al. 2004a, b) F, A (Yang et al. 2004a, b)

**Table 2** continued

Compound	Structure	Log $K_{ow}^a$	Pv <sup>a</sup>	Activity/Reference
Eugenol (R = H)		2.20	0.0104	F, A (Yang et al. 2003) C, A (Female); F and C, Nits (Yang et al. 2003)
Acetyl-eugenol (R = COCH <sub>3</sub> )		2.40	7.9e-3	NoA against A (Yang et al. 2003)
Methyl-eugenol (R = CH <sub>3</sub> )		2.97	0.0272	NoA against A (Yang et al. 2003)
iso-Eugenol		2.45	5.2e-3	NoA against A (Yang et al. 2003)
$\alpha$ -Humulene		7.03	8.1e-3	NoA against A (Yang et al. 2003)
Limonene		4.45	1.54	C, Nits (Lahlou and Berrada 2003)
Linalool		3.28	0.0905	C, A (females) (Leo et al. 2005) C, Nits (Lahlou and Berrada 2003)
Methyl salicylate		2.23	0.0700	F, A (Yang et al. 2003) C, A (Female), F and C, Nits (Yang et al. 2003)

**Table 2** continued

Compound	Structure	Log $K_{ow}^a$	Pv <sup>a</sup>	Activity/Reference
$\alpha$ -Pinene		4.37	3.49	F (Yang et al. 2004a, b) C, Nits (Lahlou and Berrada 2003) C, A and Nits and F, A (Yang et al. 2004a, b)
$\beta$ -Pinene		4.37	2.40	C, A and Nits (Yang et al. 2004a, b) F (Yang et al. 2004a, b)
Salicylaldehyde		1.58	0.329	C, A (Females) (Leo et al. 2005) C, Nits (Yang et al. 2005)
$\gamma$ -Terpinene		4.36	1.08	C, A (Yang et al. 2004a, b) C, Nits (Yang et al. 2004a, b)
Terpinen-4-ol		2.99	0.0478	C, A (Downs et al. 2000)
1- $\alpha$ -Terpineol		2.79	0.0283	C, A (Downs et al. 2000) F, A (Yang et al. 2004a, b) C, Nits (Yang et al. 2004a, b)

Activities are coded as follows: C = contact bioassay, D = submersion bioassay, F = fumigant bioassay. Bioassays run against A: adults, or Nits. NoA: no activity

<sup>a</sup> As reported in SciFinder Scholar (2006) Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994–2006 ACD/Labs)

### Physicochemical basis of anti-lice activity

The development of new pediculicides based on plant EOs bears the problem that these natural products are chemically complex and variable,

even at the intra-specific level. Such characteristics may be beneficial, since complex mixtures are less likely to develop resistance, but may also be problematic, since reliability is crucial for a natural product to find its way in any form of

human treatment. Therefore, it is important to develop standardized methods of extraction, and general knowledge of important (essential) components. Several individual components of EOs, particularly monoterpenes, have been tested against lice, providing data that permit to draw preliminary conclusions about structural requirements and structure-activity relationships for anti-lice compounds (Downs et al. 2000; Mougabure-Cueto et al. 2002; Lahlou and Berrada 2003; Yang et al. 2003, 2004a, b, 2005). The structure, octanol-water partitioning coefficient ( $K_{ow}$ ) and vapour pressure (Pv) of tested compounds (SciFinder Scholar 2006) are shown in Table 2.

The lipophilicity and volatility have been discussed by several authors as two properties that influence insecticide activity (Regnault-Roger and Hamraoui 1995; Veal 1996; Mougabure-Cueto et al. 2002; Kim et al. 2003a, b; Yang et al. 2003). In the case of anti-lice activity, given that bioassay methods are either by direct contact or fumigation, it has been suggested that compounds with greater activity in contact bioassays, which presumably need to penetrate the lipophilic cuticle, will be those with a greater  $K_{ow}$ , while compounds with better activity in fumigant assays would be those with higher Pv.

#### Direct contact activity (lipophilicity)

Yang et al. (2003) discussed their anti-lice results with compounds from the EO of *Eugenia caryophyllata* (Myrtaceae) in terms of the lipophilicity of the tested compounds. However, the  $K_{ow}$ s calculated and reported elsewhere (Griffin et al. 1999) for these compounds do not vary in the same direction of anti-lice contact activity. Eugenol was the most active but has the lowest lipophilicity ( $\log K_{ow} = 2.99$ ) compared to its derivatives, isoeugenol ( $\log K_{ow} = 3.04$ ), and methyleugenol ( $\log K_{ow} = 3.45$ ) (Table 2). In the same line, the same authors (Yang et al. 2004a, b) found no correlation between contact toxicity and lipophilicity of typical compounds of the EO of *Eucalyptus globulus* (Myrtaceae). The contact activity of 1,8 cineol ( $\log K_{ow} = 2.84$ ) against adult females was twice that of  $\alpha$ - and  $\beta$ -pinene, which have greater  $K_{ow}$ s ( $\alpha$ - $\log K_{ow} = 4.44$ ,  $\beta$ - $\log K_{ow} = 4.16$ ). The authors also state that no correlation was found

between contact activity, expressed as  $LT_{50}$ , and either molecular weight or steric effects, though no specifics are provided on how they assess the later. In the case of EO components of *Cinnamomum zeylanicum* (Lauraceae), the toxicity against adults in contact bioassays was greater for benzaldehyde ( $\log K_{ow} = 1.64$ ) than for salicylaldehyde ( $\log K_{ow} = 1.58$ ), followed by linalool ( $\log K_{ow} = 0.0905$ ). Interestingly, the nictidal activity followed the opposite trend, salicylaldehyde being more active than benzaldehyde (Yang et al., 2005). Another study involving the monoterpenes  $\alpha$ -terpineol, terpinen-4-ol and  $\gamma$ -terpinene in contact bioassays with adults found a decreasing trend of activity (as listed) (Downs et al., 2000). Again, these results do not follow the expected trend in regards to lipophilicity at least for the two first compounds (Table 2). Finally, a study of the nictidal activity (by direct contact) of EO components of *Rosmarinus officinalis* (Lamiaceae) showed 100% mortality for linalool, borneol,  $\beta$ -caryophyllene, limonene and camphor, and lower activity for  $\alpha$ -pinene and bornyl acetate (Lahlou and Berrada 2003), which have  $K_{ow}$  values in the range of those of the more active compounds (Table 2).

#### Fumigant activity (vapour pressure)

The fumigant activity of different components of essential oils has been studied in confined settings with several coleopterans [*Acanthoscelides obtectus* (Bruchidae) (Regnault-Roger and Hamraoui 1995), *Tribolium castaneum* (Tenebrionidae), *Oryzaephilus surinamensis* (Silvanidae) (Lee et al. 2003)], as well as with the domestic fly and the German cockroach, *Blattella germanica* (Lee et al. 2003). While the data is still limited, there is some evidence that suggests that ketone monoterpenes are more toxic than other monoterpenoids (Lee et al. 2003). In tests against acari (Kim et al. 2003a, b), the fumigant activity varied as expected with respect to vapour pressure, increasing with the methylation of eugenol (Pv = 0.0104 torr) to methyleugenol (Pv = 0.0272 torr), and decreasing for acetyeugenol (Pv = 0.0079 torr). However, eugenol showed better fumigant action than methylsalicylate against head lice, which does not correlate with a decrease in the Pv (0.0104 torr and 0.0700 torr, respectively) (Yang et al. 2003). In other studies the vapour pressure of monoterpenes appeared as a good, but not flawless,

predictor of fumigant activity, although it may be more suitable for comparisons among monoterpenes with similar functionality. For instance, in one report of fumigant activity against adult head lice females, 1,8-cineole ( $P_v = 1.65$  torr) showed the highest activity, followed by  $\alpha$ -pinene ( $P_v = 3.49$ ), pinocarveol ( $P_v = 0.0283$ ) and  $\alpha$ -terpineol ( $P_v = 0.0283$ ) (Yang et al. 2004a, b). The same group found a similar trend in the fumigant activity of the series benzaldehyde ( $P_v = 0.974$ ), salicylaldehyde ( $P_v = 0.329$ ) and linalool ( $P_v = 0.0905$ ) (Yang et al. 2005).

Studies on structural basis for the activity of monoterpenes against the body lice, *P. h. humanus*, found evidence that monooxygenated terpenes (terpinene-4-ol, pulegone, thymol, menthone, among others) were more active than hydrocarbons (camphene, limonene,  $\alpha$ - and  $\beta$ -pinene). However, further increases in the polarity due to the addition of another oxygenated group or an acid function rendered these monoterpenes inactive. The authors discuss their results taking into account structural aspects of the compounds tested, and conclude that flat structures, compared to extended or bulky compounds, were more toxic toward adults, but not necessarily the best niticidal compounds (Priestley et al. 2006). Of note, the relative toxicity reported by these authors against adult lice does not correlate with higher lipophilicity or vapour pressure for the tested monoterpenes (Table 2).

All together, it appears clear that the available data is not enough yet for any generalization on the relationship between anti-lice activity and structural or physicochemical properties of monoterpenes. Most probably, a combination of structural features affects the activity, and the monoterpenes are certainly a structurally diverse group. For this reason, studies that compare similar compounds that vary in a single structural feature provide valuable information. In this direction, one study showed a correlation of chain length—and hence  $K_{ow}$ —and anti-lice contact activity of aliphatic alcohols, using the submersion bioassay method. (Mougabure-Cueto et al. 2002).

### Final remarks

Plant EOs have great potential for the control of several insects (Isman 2006). They are relatively

harmless to mammals and biodegradable, and in fact many of these products have long been used in cosmetic formulations (perfumes, shampoos, lotions, coldcreams). All of these factors make EOs good alternative candidates for the management of sanitary problems, including not only insects of medical and veterinary relevance but also agricultural pests. In the case of head lice treatment, there is growing evidence that EOs may be a source of new control agents, although our knowledge regarding efficacy and mode of action is still limited. Such limited information is a factor that prevents the widespread recommendation of EOs as alternatives for treatment [for example by the US National Academy of Pediatrics (Jones and English 2003)].

Studies with pure EO components show great variation in anti-lice activity for compounds with rather small structural differences. This may be due to the existence of specific molecular targets for the different compounds (Priestley et al. 2006). In fact, specific modes of action of botanical pediculicides have virtually not been investigated, with the exception of *Malaleuca* oil (Tea tree oil), which anti-lice properties may be attributed to anticholinesterase activity (Mills et al. 2004). In a more general view, the mode of action of EO components against insects has been associated with neurotoxic activity, such as interference with octapamine and GABA-mediated chloride channels (references in Isman 2006). More information on the modes of action of pediculicides may provide the basis for a narrower search of alternatives from plants.

Unfortunately, several studies with individual EO components have yielded contradictory information, the differences probably arising from different bioassay methodologies. One important methodological factor is the source of head lice. Although there have been reports on the maintenance of head lice in the laboratory, the requirements seem rather complicated (Takano-Lee et al. 2003a, b), and most studies use newly-collected lice from environments with high incidence of pediculosis (schools and jails). These natural populations may have developed different degrees of resistance to some of the conventional products, and such resistance may affect the evaluation of anti-lice activity, since cases of cross-resistance have been reported (Yoon et al. 2004).

Therefore, it appears necessary to standardize bioassays, and recommendations to that effect have already been proposed (Burkhart and Burkhart 2001; Burkhart 2004). Indeed, a standardized experiment for monitoring lice resistance has been recommended by the World Health Organization (WHO 1981), a method that could be adopted in future studies in order to avoid artefacts derived from cross-resistance (Burkhart 2004).

Regarding whole EOs, it is quite possible that their chemically complex nature results in a wider spectrum of anti-lice action than the individual components. For instance,  $\alpha$ -terpineol, a component of the EO of *E. globulus*, showed differential activity against nits and adults (inactive against adults and active against nits) (Yang et al., 2004a, b), while other components, i.e., 1,8-cineole, showed good activity against adults but were inactive against nits. These results suggest that complex mixtures of monoterpenes may provide the advantage of simultaneous control of different life stages of lice [contrary to this view, the EO of *C. zeylanicum* showed lower activity than its isolated components (Yang et al. 2005)]. Natural synergisms are likely to play a key role in the activity of whole EOs, as it has been shown in the case of the toxicity of constituents of the EO of Rosemary against mites (Miresmailli et al. 2006). In fact, synergisms have already been used for conventional louse control, although with one exception [sesame oil as a synergist for pyrethrins (Bushland et al. 1944)], none of the synergists so far employed are of plant origin. Pyrethrins have been used for head lice treatment in combination with piperonyl butoxide (Burkhart et al. 1998), and more recently triphenylphosphate was described as a good synergist for permethrin (Picollo et al. 2000). Other compounds have been used as synergists in different preparations against the body louse, *P. h. humanus*. Examples include the addition of piperonyl sulfoxide and piperonyl butoxide to rotenone (Cole and Clark 1958); *N*-isobutylundecylenamide to pyrethrins (Bushland et al. 1944); as well as piperonyl sulfoxide and 1,2-methylenedioxy-4-[2-octylsulfonyl]propyl added to organophosphorates (Eddy et al. 1954).

Future search for anti-lice products from plants should include more work on EOs and a broader search from fixed extracts. In addition, there

should be a diversification of target activities, including more effective repellents, natural synergists, products that may soften the glue that attaches nits to the hair (Burkhart and Burkhart 2005), and products that prevent nit hatching. Unusual developmental features of lice may be targeted for specificity, for example, head lice nymphs bear multiple exoskeleton and are able to shed only the outer one (Meinking 2004), a process that may be selectively disrupted by hormone mimics or other plant compounds. Finally, to our knowledge there have been no studies on lice chemical communication, most likely due to the difficulty of obtaining large numbers of lice under controlled conditions for chemical and behavioural work. Semiochemicals and the disruption of intra-specific communication may provide an additional approach for lice treatment.

**Acknowledgements** We thank the editors for the opportunity to prepare this work, and we also wish to thank financial support by the Program for the Development of Basic Sciences (PEDECIBA, Uruguay), and the Organization for the Prohibition of Chemical Weapons (OPCW).

## References

- Agoumi A, Gourai M (1989) Activity of *Myrtus communis* extracts against *Pediculus humanus capitis*. *Plant Med Phytother* 23(2):95–108
- Angel T, Nigo J, Levy M (2000) Pediatric dermatology: infestations in the pediatric patient. *Pediatric Clin North Am* 47:921–935
- Aschner M, Mager J (1945) Evaluation of materials for louse control. *Ann Appl Biol* 32:143–148
- Audino PG, Barrios S, Vassena C et al (2005) Increased monooxygenase activity associated with resistance to permethrin in *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. *J Med Entomol* 42(3):342–345
- Borror D, Triplehorn C, Johnson N (1989) An introduction to the study of insects. Saunders Coll Publ, New York
- Burgess I (1993) New head louse repellent. *Br J Dermatol* 28(3):357–358 as cited in Burgess 2004
- Burgess IF (2004) Human lice and their control. *Annu Rev Entomol* 49:457–481
- Burkhart CG, Burkhart CN (2006) Safety and efficacy of pediculicides for head lice. *Expert Opin Drug Safety* 5(1):169–179
- Burkhart CG, Burkhart CN, Burkhart KM (1998) An assessment of topical and oral prescription and over-the-counter treatments for head lice. *J Am Acad Dermatol* 38(6):979–983

- Burkhart CN (2004) Head lice revisited: in vitro standardized tests and differences in malathion formulations. *Arch Dermatol* 140(4):488–489
- Burkhart CN, Burkhart CG (2001) Recommendation to standardize pediculicidal and ovicidal testing for head lice (Anoplura: Pediculidae). *J Med Entomol* 38(2):127–129
- Burkhart CN, Burkhart CG (2005) Head lice: scientific assessment of the nit sheath with clinical ramifications and therapeutic options. *J Am Acad Dermatol* 53(1):129–133
- Bushland RC, Eddy GW, Knipling EF (1944) Synergists for pyrethrum against the body louse. *J Econ Entomol* 37:556–557
- Cestari IM, Sarti SJ, Waib CM et al (2004) Evaluation of the potential insecticide activity of *Tagetes minuta* (Asteraceae) essential oil against the head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Neotrop Entomol* 33(6):805–807
- Cole MM, Clark PH (1958) Tests with compounds showing synergistic action with rotenone against body lice. *J Econ Entomol* 51:484–486
- Connolly JD, Hill RA (2005) Triterpenoids. *Nat Prod Reports* 22(2):230–248
- Develoux M (2004) Ivermectin. *Ann Dermatol Vener* 131(6–7):561–570
- Downs A, Stafford K, Coles G (2000) Monoterpenoids and tetralin as pediculocides. *Acta Derm Vener* 80:69–70
- Eddy GW, Cole MM, Marulli AS (1954) Louse powder synergists. *Soap Chem Specialties* 30:121–123
- Filer J (1996) Egyptian bookshelf diseases. Austin, TX, Univ Texas Press cited in Burkhart & Burkhart, 2001
- Gao JR, Yoon KS, Frisbie RK et al (2006) Esterase-mediated malathion resistance in the human head louse, *Pediculus capitis* (Anoplura: Pediculidae). *Pestic Biochem Physiol* 85(1):28–37
- Gratz NG (1997) Human lice: their prevalence, control and resistance to insecticide. A review 1985–1997. World Health Organization, Geneva, Switzerland
- Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. *J Chromatogr A* 864(2):221–228
- Heukelbach J, Oliveira F, 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
- Hotson I (1982) The avermectins a new family of antiparasitic agents. *J S Afr Vet Assoc* 53(2):87–90
- Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann Rev Entomol* 51(1):45–66
- Jones KN, English JC (2003) Review of common therapeutic options in the United States for the treatment of *Pediculosis capitis*. *CID* 36:1355–1361
- Kambu K, Di Phanu N, Coune C et al (1982) Study of the insecticidal and chemical properties of *Eucalyptus saligna* from Zaire. *Plantes Medicinales et Phytotherapie* 16(1):34–38
- Kim E, Kim H, Choi D et al (2003a) Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). *App Entomol Zool* 38:261–266
- Kim E-h, Kim H-K, Ahn Y-J (2003b) Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J Agric Food Chem* 51(4):885–889
- Klocke JA, Arisawa M, Handa SS et al (1985) Growth inhibitory, insecticidal and antifeedant effects of some antileukemic and cytotoxic quassinoids on two species of agricultural pests. *Experientia* 41(3):379–382
- Lahlou M, Berrada R (2003) Composition and niticidal activity of essential oils of three chemotypes of *Rosmarinus officinalis* L. acclimatized in Morocco. *Flavour Frag J* 18:124–127
- Latif Z, Craven L, Hartley TG et al (2000) An insecticidal quassinoid from the new Australian species *Quassia* sp. aff. *bidwillii*. *Biochem Syst Ecol* 28(2):183–184
- Lee S, Peterson C, Coats J (2003) Fumigation toxicity of monoterpenoids to several stored product insects. *J Stored Prod Res* 39:77–85
- Leo NP, Hughes JM, Yang X et al (2005) The head and body lice of humans are genetically distinct (Insecta : Phthiraptera, Pediculidae): evidence from double infestations. *Heredity* 95(1):34–40
- Leung AKC, Fong JHS, Pinto-Rojas A (2005) Pediculosis Capitis. *J Pediatric Health Care* 19(6):369–373
- Levot G (2000) Resistance and control of lice on humans and production animals. *Int J Parasitol* 30(3):291–297
- Meinking T (2004) Clinical update on resistance and treatment of *Pediculosis capitis*. *Am J Manage Care* 10:S264–S268
- Miller J, Mumcuoglu KY (1999) Pyrethroid resistance mechanisms in the head louse *Pediculus capitis* from Israel: Implications for control. *Med Veter Entomol* 13:89–96
- Mills C, Cleary B-J, Gilmer J-F et al (2004) Inhibition of acetylcholinesterase by Tea Tree oil. *J Pharm Pharmacol* 56(3):375–379
- Miresmailli S, Bradbury R, Isman MB (2006) Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. *Pest Manag Sci* 62:366–371
- Morsy T, el-Ela R, Nasser M et al (2000) Evaluation of the in-vitro pediculicidal action of four known insecticides and three medicinal plant extracts. *J Egypt Soc Paras* 30(3):699–708 Abstracts
- Mougabure-Cueto G, Gonzalez-Audino P, Vassena CV et al (2002) Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). *Entomol Soc Am* 39(3):458–460
- Mulla M, Su T (1999) Activity and biological effects of neem products against arthropods of medical and veterinary importance. *J Am Mosq Control Assoc* 15(2):133–152
- Mumcuoglu K, Magdassi S, Miller J et al (2004) Repellency of citronella for head lice: double-blind randomized trial of efficacy and safety. *Isr Med Assoc J* 6(12):756–759

- Mumcuoglu KY, Bach U, Miller J et al (1996) Repellency of essential oils and their components to the human body louse, *Pediculus humanus humanus*. Entomol Experim Appl 78(3):309–314 Abstracts
- Mumcuoglu KY, Miller J, Zamir C et al (2002) The In Vivo Pediculicidal Efficacy of a Natural Remedy. IMAJ 4:790–793
- Murray ES, Torrey SB (1975) Virulence of *Rickettsia prowazekii* for head lice. Ann NY Acad Sci 266:25–34
- Namazi M (2005) The addition of tretinoin to permethrin, pyrethrins, and DDT for production of powerful anti-lice agents. Med Hypotheses 65(5):992
- Nentwig G (2003) Use of repellents as prophylactic agents. Parasitol Res 90:S40–S48
- Ninci M (1991) Prophylaxis and treatment of pediculosis with *Quassia amarga*. Rev Fac Cien Med Univ Nac Cordoba 49(2):27–31 Abstract
- Oladimeji FA, Orafidiya O, Ogunniyi TAB et al (2000) Pediculocidal and scabicial properties of *Lippia multiflora* essential oil. J Ethnopharmacol 72(1,2):305–311
- Oliveira C, Spitzer V (1999) Oleos volateis. In: Oliveira C, Schenkel E, Gosmann G. et al (eds) Farmacognosia da planta ao medicamento. Porto Alegre, UFRGS, pp 397–426
- Peock S, Maunder J (1993) Arena tests with piperonal, a new louse repellent. J R Soc Health 113(6):292–294 Abstracts
- Piccolo MI, Vassena CV, Casadio AA et al (1998) Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura: Pediculidae). J Med Entomol 35(5):814–817
- Piccolo MI, Vassena CV, Cueto GAM et al (2000) Resistance to insecticides and effect of synergists on permethrin toxicity in *Pediculus capitis* (Anoplura: Pediculidae) from Buenos Aires. J Med Entomol 37(5):721–725
- Polonsky J, Baskevitch Z, Gottlieb H et al (1975) Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. XXXI. Carbon-13 nuclear magnetic resonance spectral analysis of quassinoid bitter principles. J Org Chem 40(17):2499–2504
- Priestley C, Burgess IF, Williamson E (2006) Lethality of essential oil constituents towards the human louse, *Pediculus humanus*, and its eggs. Fitoterapia 77(4):303–309
- Regnault-Roger C, Hamraoui C (1995) Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). J Stored Prod Res 31(4):291–299
- Robinson E, Leo N, Prociw P et al (2003) Potential role of head lice, *Pediculus humanus capitis*, as vectors of *Rickettsia prowazekii*. Parasitol Res 90(3):209–211
- Rozendaal J (1997) Vector control. Methods for use by individuals and communities. World Health Organization, Geneva, Switzerland
- SciFinder Scholar (2006) Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994–2006 ACD/Labs)
- Takano-Lee M, Edman JD, Mullens BA et al (2004) Home remedies to control head lice: Assessment of home remedies to control the human head louse, *Pediculus humanus capitis* (Anoplura: Pediculidae). J Pediatric Nurs 19(6):393–398
- Takano-Lee M, Velten RK, Edman JD et al (2003a) An automated feeding apparatus for in vitro maintenance of the human head louse, *Pediculus capitis* (Anoplura: Pediculidae). J Med Entomol 40(6):795–799
- Takano-Lee M, Yoon KS, Edman JD et al (2003b) In vivo and in vitro rearing of *Pediculus humanus capitis* (Anoplura: Pediculidae). J Med Entomol 40(5):628–635
- Tiangda C, Gritsanapan W, Sookvanichsilp N et al (2000) Anti-headlice activity of a preparation of *Annona squamosa* seed extract. Southeast Asian J Trop Med Public Health 31(Suppl 1):174–177
- Tolozza AC, Zygadlo J, Mougabure-Cueto G et al (2006) The fumigant and repellent activity of aliphatic lactones against *Pediculus humanus capitis* (Anoplura: Pediculidae). Memorias Instituto Oswaldo Cruz 101(1):55–56
- Tomita T, Yaguchi N, Mihara M et al (2005) Sodium channel point mutations associated with pyrethroid resistance in the head louse, *Pediculus humanus capitis*. ACS Symp Ser: Environ Fate Safety Manage Agrochem 899:234–243
- Veal L (1996) The potential effectiveness of essential oils as a treatment for headlice, *Pediculus humanus capitis*. Compl Ther Nurs Midwifery 2:97–101 as cited in Veal, 2003
- Veal L (2003) Headlice, [http://www.wingedseed.com/Agora/Lice\\_page.htm](http://www.wingedseed.com/Agora/Lice_page.htm). access year 2006
- WHO (1981) Instructions for determining the susceptibility or resistance of body lice and head lice to insecticides. WHO VBC 81808. W. H. Organization. Geneva, Switzerland
- Yang YC, Choi HY, Choi WS et al (2004a) Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). J Agric Food Chem 52(9):2507–2511
- Yang YC, Lee HS, Clark JM et al (2004b) Insecticidal activity of plant essential oils against *Pediculus humanus capitis* (Anoplura : Pediculidae). J Med Entomol 41(4):699–704
- Yang YC, Lee HS, Lee SH et al (2005) Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculidae). Int J Parasitol 35(14):1595–1600
- Yang YC, Lee SH, Lee WJ et al (2003) Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. J Agr Food Chem 51(17):4884–4888
- Yoon KS, Gao JR, Lee SH et al (2004) Resistance and cross-resistance to insecticides in human head lice from Florida and California. Pesticide Biochem Physiol 80(3):192–201