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ORIGINAL PAPER

Contact and fumigant toxicity of hexane flower bud extract of *Syzygium aromaticum* and its compounds against *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

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Abstract The head lice, *Pediculus humanus capitis* De Geer is an obligate ectoparasite of humans that causes pediculosis capitis, a nuisance for millions of people worldwide, with high prevalence in children. *P. humanus capitis* has been treated by methods that include the physical remotion 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 lice treatment and/or prevention, and natural products from plants are good candidates for safer control agents that may provide good anti-lice activity. The plant extracts are good and safe alternatives due to their low toxicity to mammals and easy biodegradability. The present study carried out the pediculocidal activity using the hexane flower bud extract of *Syzygium aromaticum* (Myrtaceae) against *P. humanus capitis* examined by direct contact and fumigant toxicity (closed- and open-container methods) bioassay. The chemical composition of *S. aromaticum* flower bud hexane extract was analyzed by gas chromatography-mass spectrometry. The major chemical constituent (58.79%) of flower bud hexane extract *S. aromaticum* was identified as chavibetol (5-allyl-2-methoxyphenol) by comparison of mass spectral data and retention times. The hexane extract of *S. aromaticum* was subjected to gas chromatography analysis, and totally 47 compounds were detected, of which chavibetol was predominantly present. The other major constituents present in the hexane extract were eugenol acetate (phenol,2-methoxy-4-(2-propenyl)-,acetate (15.09%), caryophyllene-(I1) (2,6,10,10-tetramethyl bicyclo [7.2.0] undeca-1,6-diene (13.75%), caryophyllene oxide (3.04%), 2,6,6,9-tetramethyl-1,4,8-cycloundecatriene (1.67%), and copaene (1.33%). The filter paper contact bioassay study showed pronounced pediculicidal activity in the flower bud hexane extract of *S. aromaticum*. The toxic effect was determined for every five in an 80-min treatment. The result showed percent mortality of 40, 82, and 100 at 5, 10, and 20 min, and the median lethal time (LT_{50}) value was 5.83 (0.5 mg/cm^2); 28, 82, and 100 at 5, 10, and 30 min. (LT_{50}=6.54; 0.25 mg/cm^2); and 13, 22, 42, 80, and 100 at 5, 10, 20, 40, and 80 min (LT_{50}= 18.68; 0.125 mg/cm^2), respectively. The vapor phase toxicity was tested at 0.25 mg/cm^2. There was a significant difference in pediculicidal activity of *S. aromaticum* extract against *P. humanus capitis* between closed- and open-container methods. Adult mortalities were determined for every five in 60 min (closed method) and for every ten in 180 min (open method). The closed method showed the percent mortality was 45, 88, and 100 at 5, 10, and 15 min (LT_{50}=5.39), respectively. In the open-container method, the percent mortality was observed 5, 20, 47, 84, and 100 at 10, 20, 60, 120, and 180 min (LT_{50}=47.91), respectively. The mortality was more effective in the closed containers than in open ones, indicating that the effect of hexane extract was largely a result of action in the vapor phase exhibited fumigant toxicity. Studies of anti-lice activity of extract 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.

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Introduction

Head lice (*Pediculus humanus capitis*) are a major irritant to children and their parents around the world. Each year, millions of children are infested with head lice, a condition known as pediculosis, which is responsible for tens of millions of lost school days. Head lice have evolved resistance to many of the currently used pediculicides; therefore, an effective new treatment for head lice is needed (Goates et al. 2006). *P. humanus capitis* infestation is a major concern in public health. Infestations due to unhygienic conditions are prevalent worldwide and especially common among school children in both developed and developing countries (Gratz 1997). *P. humanus capitis* is transmitted through physical contact. Symptoms associated with infestation are constant itching and scalp irritation. When the ectoparasites are associated with poor social conditions and inadequate iron in the diet, the infestation may even lead to anemia (Linardi et al. 1988; Speare et al. 2006). It has been suggested that transmission of *P. humanus capitis* is caused by shared combs, hairbrushes, mats, hats, bed linen, and clothes (Burkhart 2003). Doucet et al. (1997) have reported that an average infestation of 37.82% and 53.07% infestation levels are found in boys and girls in 1993 in Argentina. The *P. humanus capitis* infections cause skin irritation, pruritus, and sleep loss, as well as occasional secondary bacterial infection from scratching (Rozendaal 1997). Accordingly, in the USA alone, 4–8 million children are treated unnecessarily for head lice annually, which amounts to 64% of all lice treatments. In addition, 12–24 million school days are lost annually. The annual economic loss owing to missed workdays by parents who have to stay home with their children adds US$4–8 billion to the country's economy. The policy also results in serious psychological problems for children and their parents (Mumcuoglu et al. 2006). Approximately 6–12 million people, mainly children 3–12 years of age, are treated annually for head lice in the USA (Taplin and Meinking 1987; Alkinson et al. 1986). An increased rate of louse infestation was reported in recent years from a number of countries including North and South America, Europe, Asia, and Australia (Mumcuoglu 1999; Burkhart and Burkhart 2000). The head louse infestation may result in social embarrassment when infested children and their families become mobbed as "dirty" or "antisocial" (Mehlhorn et al. 1995; Mehlhorn and Mehlhorn 2009; Toloza et al. 2010b).The control of human head lice worldwide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (malathion), carbamate (carbaryl), pyrethrin, pyrethroid (permethrin and δ-phenothrin), and avermectin (ivermectinoriginated from *Streptomyces avermitilis*) as insecticides (Gratz 1997; Dolianitis and Sinclair 2002). The most common insecticides applied against head lice contain as active ingredient permethrin, D-phenothrin, cyclic silicones, botanical extracts, benzyl alcohol, vinegar, or essential oils. Moreover, the only prescriptive pediculicide available contains lindane. Unfortunately, most of the over-the-counter products available in Argentina have not been tested against head lice in vitro assays (Toloza et al. 2009). Their repeated use has often resulted in the development of resistance (Downs et al. 1999; Dolianitis and Sinclair 2002; Cueto et al. 2008), and increasing levels of resistance to the most commonly used pediculicides have caused multiple and excessive treatments, fostering serious human health concerns (Hayes and Laws 1991). A major challenge for manufacturers of natural products is to maintain a commercial product of consistent composition related to the expected variation in chemical composition of the essential oils with location, cultivar, season, and over time (Heukelbach et al. 2006a, b). Licatack® smoothens the hair, is skin-safe, and smells well; it offers a very efficient and positive alternative to toxic, gluing, flammable, or skin-irritating products found on the market of anti-louse products (Abdel-Ghaffar et al. 2010a). Some of the proposed bioassays for evaluating pediculicidal activity include immersion and topical application as methods of insecticide exposure (Burkhart and Burkhart 2001; Meinking et al. 2001; Cueto et al. 2002, 2008; Audino et al. 2007; Heukelbach et al. 2008). As head and body lice show similar toxicological phenotypes, a laboratory-reared *P. humanus humanus* is frequently used as a reference colony in insecticide resistance studies for head lice and as a test organism to evaluate the efficacy of potential pediculicides (Kristensen et al. 2006; Priestley et al. 2006; Cueto et al. 2008; Gallardo et al. 2009).The reason for the recent constant spreading of lice is the reduction of the efficacy of available anti-louse compounds since many of them had been used for a very long time. This recent lack of efficacy is surely based on a variety of reasons among which are the incorrect use and development of resistances (Toloza et al. 2009; Yang et al. 2004a).

Plant essential oils have been suggested as an alternative source of materials for insect control because they constitute a rich source of bioactive chemicals and are commonly used as fragrance and flavoring agents for foods and beverages. Little work has been done on pediculicidal activity of plant essential oils, although insecticidal activity of essential oils has been well described by Isman (1999). Because of this, much effort has been focused on plant essential oils or photochemical as potential sources of commercial head lice control agents (Veal 1996; Morsy et al. 2000; Mumcuoglu et al. 2002; Yang et al. 2003). Alternative control agents with novel modes of action and low mammalian toxicity and environmental impact are

badly needed. This lack of efficacy is due to the emergence of resistance by the head louse to synthetic compounds and researchers were aimed on the search of new substitutes. Abdel-Ghaffar et al. (2010b) reported that the product Licatack® proved its efficacy on larvae and adult head lice after its efficacy was shown in intense in vitro screening tests.

The flower bud of *Syzygium aromaticum*, commonly known as clove, clove oils have been widely investigated due to their popularity, availability, and high essential-oil content. In fact, the clove oil of *S. aromaticum* (Syn. *Eugenia caryophyllata*), belonging to the family Myrtaceae, has long been considered to have medicinal properties (Chaieb et al. 2007). Good potencies displayed by the flower bud extract of *S. aromaticum* may serve as antimalarial agents even in their crude form (Bagavan et al. 2011). The biological activity of *E. caryophyllata* oil was shown to inhibit the emergence of *Culex pipiens* larvae (El Hag et al. 1999), display insecticidal activity against *P. humanus capitis* (Yang et al. 2003) and against *Anopheles dirus* (Trongtokit et al. 2005), and isoeugenol compound isolated and suppresses progeny development of *Tribolium castaneum* and *Sitophilus zeamais* (Ho et al. 1994). Clove essential oil has also showed acaricidal activity towards *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* with eugenol being identified as the acaricidal constituent of the oil. Eugenol congeners also exhibited potent acaricidal activity against both mite species (Kim et al. 2003), and the acaricidal activity in vitro of eugenol on *Psoroptes cuniculi*, both by direct contact and by contact only with their vapor phase, was already reported in literature (Perrucci et al. 1995). A more recent study has confirmed that clove oil could be used as an insecticidal activity against the Japanese termite, *Reticulitermes speratus* using a fumigation bioassay (Park and Shin 2005).

Machado et al. (2011) have reported that the anti-Giardia activity of *S. aromaticum* and its major compound eugenol were evaluated on flagellated protozoan *Giardia lamblia* growth, adherence, viability, and ultrastructure. Cheng et al. (1994) reported that the extracts of the flower buds of *S. aromaticum* caused high mortality in *S. zeamais*, and the compound eugenol present in *S. aromaticum* showed toxic effect against *Lymnaea acuminata* (Kumar and Singh 2006). The flavones, kaempferol and myricetin isolated from the crude MeOH extract of *S. aromaticum* demonstrated potent growth inhibitory activity against the periodontal pathogens, *Porphyromonas gingivalis* and *Prevotella intermedia* (Cai and Wu 1996). The toxic effects of *Eucalyptus globulus* leaf oil-derived monoterpenoids (1,8-cineole, L-phellandrene, 2-β-pinene, trans-pinocarveol, γ-terpinene, and 1-α-terpineol) and the known eucalyptus leaf oil terpenoids (β-eudesmol and geranyl acetate) on eggs and females of the human head louse, *P. humanus capitis* were examined using direct contact and fumigation bioassays and compared with the lethal activity of δ-phenothrin and pyrethrum, two commonly used pediculicides (Yang et al. 2004a). Semmler et al. (2010) reported that a combination of an extract of the seeds of *Vitex agnus castus* (monk pepper) and the compound paramenthan-3,8-diol (which is also found in some plants, e.g., *Eucalyptus*) act synergistically and were able to protect human hair for at least 7 h from invasion of head lice (*P. humanus capitis*). The acaricidal and insecticidal effects of a patented neem seed extract tested against house dust mites, poultry mites, harvest mites, *Ixodes* and *Rhipicephalus* ticks, cat fleas (adults, larvae), bed bugs (all stages), head lice and mallophaga, cockroaches, raptor bugs (*Triatoma*), and even food-attacking beetle (*Tenebrio molitor*) might be controlled with this extract, which is available as Tre-san (against house dust mites) and Mite Stop (against mites, ticks, insects of any kind) to become water-diluted or as Wash-Away Louse or Picksan Louse Stop being diluted in a shampoo (Schmahl et al. 2010).

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, cupper 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).

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. Later on, 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. humanus capitis*, although one early study on body louse did (Aschner and Mager 1945). This article describes a laboratory study that assesses the potential of plant extract as pediculicides showed excellent anti-lice activities of *S. aromaticum* flower bud hexane extract against *P. humanus capitis.*

Materials and methods

Collection of plant materials

The flower bud *S. aromaticum* L. (Myrtaceae) was selected on the basis of aromatic smell, bitter taste, ethnopharmacological, and ethnobotanical literature survey (Fig. 1a, b).

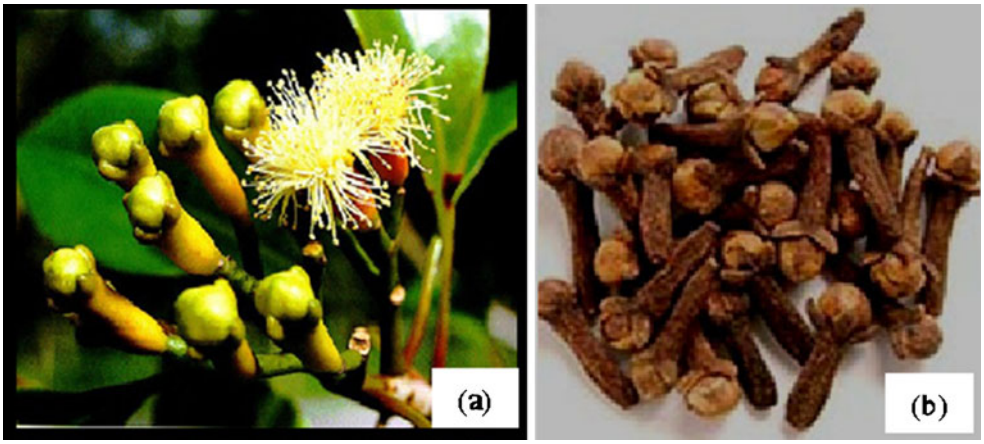

Fig. 1 *S. aromaticum* (Myrtaceae) **a** fresh flower bud and **b** dried flower bud

The plant materials were collected from the tropical region of Nilgiri Mountain, Nilgiri district (11° 29′ 0″ N, 76° 20′ 0″ E, altitude of 880 m), Tamil Nadu, South India in January 2010, and the taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

Preparation of plant extract

The *S. aromaticum* flower bud was air-dried for 7–10 days in the shade at the environmental temperatures (27–37°C day time), and the flower bud (240 g) was powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (2,500 mL, Qualigens) in a Soxhlet apparatus (boiling point range 60–80°C) for 8 h separately until exhaustion. The extract (4.5%) was concentrated under reduced pressure 22–26 mmHg at 45°C, and the residue obtained was stored at 4°C.

Gas chromatography-mass spectrometry analysis of hexane extract of *S. aromaticum*

The chemical composition of *S. aromaticum* flower bud hexane extract was analyzed by gas chromatography-mass spectrometry (GC/MS) using a SHIMADZU QP2010 with a DB-5 column (30 m, film 0.25 μm, ID 0.25 mm). The temperature of the column was programmed from 45°C to 270°C at 5°C/min, and the injector or detector temperature for the analysis was about 250°C. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. The mass spectrometer was operated in electron impact ionization mode with 70 eV energy. The identification of the chemical constituents was based on matching their recorded mass spectra with those obtained from the WILEY8.LIB and NIST08.LIB library spectra provided by the software of GC/MS system (Table 3 and Figs. 2 and 3).

Collection of head lice

Collection of adults head lice of *P. humanus capitis* (Phthiraptera: Pediculidae) were collected from a population of children between the ages of 3 and 12 years, with the approval of their guardians, by raking a metal louse comb through sections of the scalp. Adults were obtained and pooled by carefully removing them from the metal teeth of the comb into clean plastic boxes. Once collected, head lice were transported to our laboratory (Picollo et al. 1998; 2000). The children had not been treated with any pediculicide solution for at least the preceding month, using only the louse comb. The head lice were identified by Dr. A. Sangaran, Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu.

Pediculocidal activity

Filter paper contact toxicity bioassay

A filter paper contact bioassay (Yang et al. 2003) was used to evaluate the toxicity of flower bud hexane extract *S. aromaticum* tested against *P. humanus capitis*. Lice were exposed to three concentrations of extract (0.125, 0.25, and 0.5 mg/cm^2), each of which was dissolved in 80 μl of acetone and applied to filter papers (Whatman no. 1, 5 cm diameter). Control filter papers received 80 μl of acetone only. After drying in a fume hood for 2 min, each filter paper was placed on the bottom of a Petri dish. Groups of ten lice (7–9 days old), fed with a human blood meal 4 h prior to the test, were placed on each Petri dish containing a few strands of human hair and covered with a lid. Treated and control (acetone only) lice were held at the same conditions used for colony maintenance. Adult mortalities were determined for every five in 80 min treatment. All treatments were replicated three times. The median lethal time (LT_{50}) values were calculated by probit analysis (SPSS

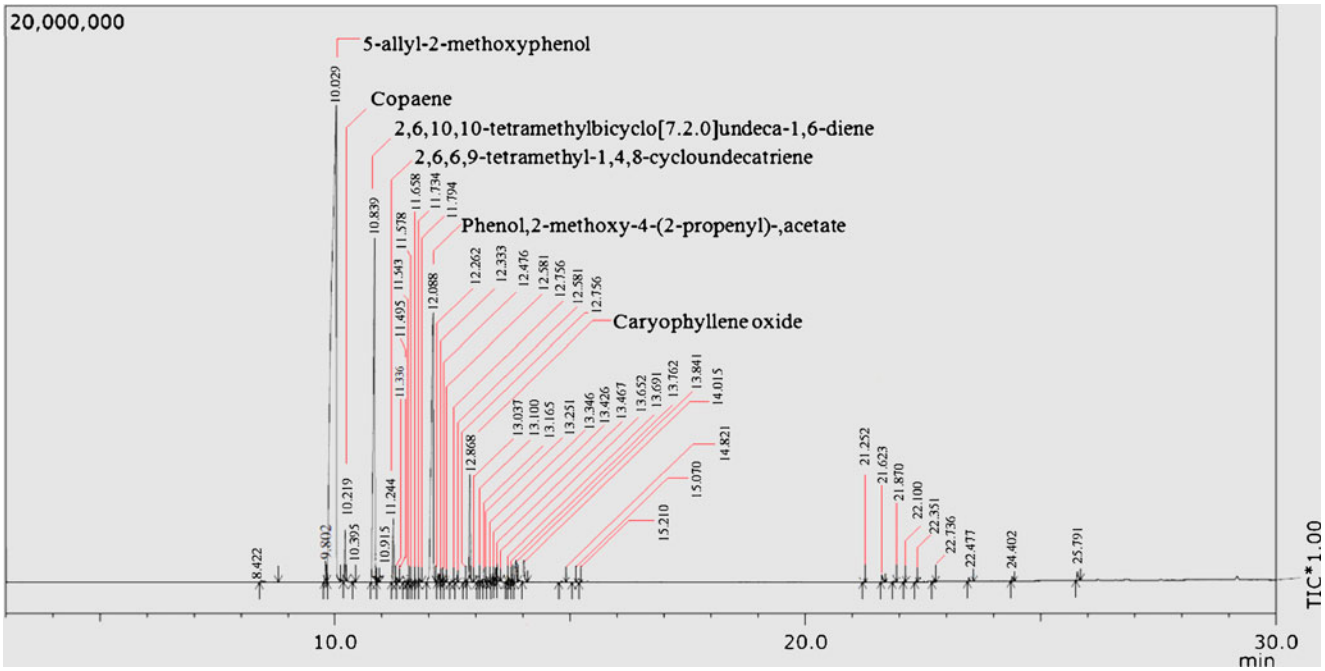

Fig. 2 GC/MS analysis of flower bud hexane extract of *S. aromaticum*

2007). The toxicity was considered significantly different when 95% confidence limit levels of the LT_{50} values failed to overlap.

Vapor phase toxicity bioassay

Fumigant toxicity of the extract tested against *P. humanus capitis* was investigated according to the method of Yang et al. (2003). Briefly, groups of ten lice (7–9 days old) were placed on the bottom of a Petri dish and covered using a lid with a fine wire sieve (4.7 cm diameter) attached over the central hole (4.5 cm diameter). Each filter paper (5 cm diameter), treated with 0.25 mg/cm^2 of the hexane extract of *S. aromaticum*, was dissolved in 80 µl of acetone and placed over the wire sieve. This prevented direct contact of the experimental lice with the test extract. Each Petri dish was then either covered with another lid (closed method) to investigate the potential vapor phase toxicity of the hexane extract or left uncovered (open method). Control filter papers received 80 µl of acetone only. Treated and acetone-treated control lice were held at the same conditions used for colony maintenance. In closed method, the adult mortalities were determined for every five in 60 min and in open method, the experiment was conducted for every ten in 180 min. Adults were considered dead if appendages did not move when they were prodded with a wooden dowel. All the Petri dishes were set aside in a dark chamber at 26±0.5°C and 70±1% humidity. The elapsed time was recorded for each test agent as the "knockdown" time. The death of the louse was confirmed when there was cessation of motility or waggling of the appendages on touching with a needle. Ten lice were used for each determination (Oladimeji et al. 2000).

Statistical analysis

All treatments were replicated three times. The median lethal time (LT_{50}) and the average parasite mortality data

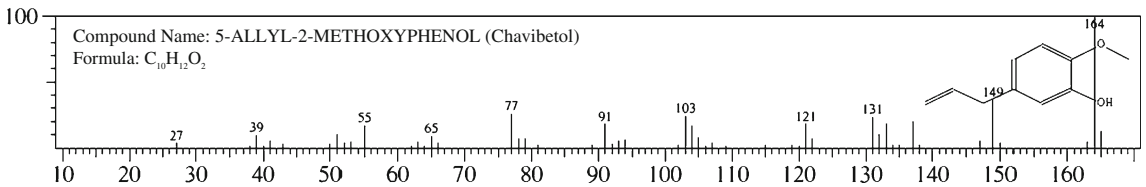

Fig. 3 GC/MS analysis of flower bud hexane extract of *S. aromaticum* showing the major constituents (retention time, 10.025; mass peaks, 108; raw mode, averaged 10.017–10.033(843–845); base peak, 164.05 (3198181); background mode, calculated from peak)

were subjected to Probit analysis for calculating LT_{50}, LT_{90}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, slope, and chi-square values were calculated by using the software developed by Reddy et al. (1992) and (SPSS 2007). Results with $p<0.05$ were considered to be statistically significant.

Results

The contact toxicity bioassay study showed the pediculicidal activity was more pronounced in flower bud hexane extract against *S. aromaticum*. A filter paper contact bioassay study showed the pediculicidal activity was more pronounced in flower bud hexane extract against *S. aromaticum* determined every 5 min for an 80-min treatment. The result showed percent mortality of 40, 82, and 100 at 5, 10, and 20 min (LT_{50}=5.83 and LT_{90}=11.45; 0.5 mg/cm^2); 28, 82, and 100 at 5, 10, and 30 min (LT_{50}=6.54 and LT_{90}=11.79; 0.25 mg/cm^2); and 13, 22, 42, 80, and 100 at 5, 10, 20, 40, and 80 min (LT_{50}=18.68 and LT_{90}=58.72; 0.125 mg/cm^2), respectively. The toxic effects of extract against *P. humanus capitis* were evaluated and the LT_{50}, LT_{90} values estimated from direct contact toxicity bioassay (Table 1). No mortality was observed in acetone-treated (control) lice over the observational interval of the contact toxicity bioassay.

With the pediculicide route of action, the fumigant toxicity of flower bud hexane extract of *S. aromaticum* against adult *P. humanus capitis* was investigated using a vapor phase toxicity bioassay in closed container and open container. The responses varied with the treatment and experimental method. The vapor phase toxicity of median lethal time ($LT_{50,}$ LT_{90}) values at 0.25 mg/cm^2. There was a significant difference in pediculicidal activity of *S. aromaticum* extract against *P. humanus capitis* between closed and open container. Adult mortalities were determined for every five in 60 min (closed method) and for every ten in 180 min (open method). The closed method showed the percent mortality was 45, 88, and 100 at 5, 10, and 15 min. (LT_{50}=5.39 and LT_{90}=9.76), respectively. For the open-container method, the percent mortality was observed 5, 20, 47, 84, and 100 at 10, 20, 60, 120, and 180 min (LT_{50}=47.91 and LT_{90}=147.35; Table 2), respectively. The mortality was more effective in the closed containers than in open ones, indicating that the effect of hexane extract was largely a result of action in the vapor phase exhibited fumigant toxicity.

The results showed that the pediculicidal activity of the major chemical constituent of flower bud hexane extract *S. aromaticum* was identified as chavibetol (5-allyl-2-methoxyphenol; 58.79%; Fig. 2) by comparison of mass spectral data and retention times. The other major constituents present in the hexane extract were eugenol acetate (phenol,2-methoxy-4-(2-propenyl)-,acetate (15.09%), caryophyllene-(I1) (2,6,10,10-tetramethylbicyclo[7.2.0] undeca-1,6-diene (13.75%), caryophyllene oxide (3.04%), 2,6,6,9-tetramethyl-1,4,8-cycloundecatriene (1.67%), and copaene (1.33%) are presented in Table 3 and Figs. 2, 3, and 4.

Discussion

Elucidation of mode of action of natural insecticidal products and insecticides is of practical importance for insect control because it may give useful information on the most appropriate formulation, delivery means, and

Table 1 Evaluation of hexane flower bud extract of *S. aromaticum* against *P. humanus capitis* using the filter paper contact toxicity

Dose (mg/cm^2)	Time/min	% Mortality [a] ± SD	Slope	LT_{50}	UCL–LCL	LT_{90}	UCL–LCL	x^2 (df=4)
0.125	5	13±0.49						
	10	22±1.35						
	20	42±1.02	0.78	18.68	21.92–16.44	58.72	70.50–46.93	17.1
	40	80±1.41						
	80	100±0.00						
0.25	5	28±1.36						
	10	82±1.50	0.29	6.54	7.13–5.96	11.79	13.65–9.95	5.4
	30	100±0.00						
0.5	5	40±1.67						
	10	82±1.85	0.23	5.83	6.46–5.21	11.45	13.09–9.81	1.6
	20	100±0.00						

[a] Mean value of three replicates, control–nil mortality, significant at $P<0.05$ level

LT_{50} median lethal time that kills 50% of the exposed adults, *LT_{90}* median lethal time that kills 90% of the exposed adults, *UCL* upper confidence limit, *LCL* lower confidence limit, $\chi 2$ Chi-square, *df* degrees of freedom

Table 2 Fumigant activity of hexane flower bud extract of *S. aromaticum* against *P. humanus capitis* using the vapor phase toxicity

Method	Dose (mg/cm^2)	Time/min	% Mortality[a] ± SD	Slope	LT_{50}	UCL–LCL	LT_{90}	UCL–LCL	x^2 (df=4)
Closed container	0.25	5	45±1.79						
		10	88±1.36	0.16	5.39	5.94–4.83	9.76	10.91–8.60	2.3
		15	100±0.00						
Open container	0.25	10	5±0.63						
		20	20±0.89						
		60	47±0.80	1.73	47.91	53.85–42.46	147.35	174.29–120.40	15.5
		120	84±2.93						
		180	100±0.00						

[a] Mean value of three replicates, control–nil mortality, significant at $P<0.05$ level

LT_{50} median lethal time that kills 50% of the exposed adults, *LT_{90}* median lethal time that kills 90% of the exposed adults, *UCL* upper confidence limit, *LCL* lower confidence limit, $\chi 2$ Chi-square, *df* degrees of freedom

resistance management. The head lice toxicity of fixed extracts include the evaluation of the neem oil (Mulla and Su 1999; Morsy et al. 2000; Heukelbach et al. 2006a), the petroleum ether extract of *Annona squamosa* (Tiangda et al. 2000), and ethanolic extracts of *Quassia amara* (Ninci 1991). Adulticidal activity against lice has been reported for some essential oils such as aniseed, cinnamon leaf, thyme red, tea tree, and nutmeg oils (Veal 1996); anise and ylang ylang oils (Mumcuoglu et al. 2002); the cade, cardamon Ceylon, clove bud, eucalyptus, marjoram, myrtle, pennyroyal, rosemary, rosewood, and sage oils (Yang et al. 2004b). The ovicidal activity has been also reported in clove bud and leaf oils (Yang et al. 2003) and *Eucalyptus globules* leaf oil (Yang et al. 2004a). The most effective essential oils were *Cinnamomum porphyrium*, followed by *Aloysia citriodora* (chemotype 2), and *Myrcianthes pseudomato* with KT_{50} values of 1.12, 3.02, and 4.09 showed the fumigant activity against head lice (Toloza et al. 2010a). The fumigant and repellent properties of essential oils tested against permethrin-resistant head lice, and the most effective oil was from *Myrcianthes cisplatensis* (Myrtaceae) with a time to 50% knockdown (KT_{50}) of 1.3 min, followed by *Eucalyptus cinerea*, *Eucalyptus viminalis*, and *Eucalyptus saligna* with KT_{50} values of 12.0, 14.9, and 17.4 min, respectively (Toloza et al. 2006). Heukelbach et al. (2006b) and Abdel-Ghaffar and Semmler (2007) have reported that the product containing a neem seed extract was highly effective in vivo and in vitro tests against head lice. Carpinella et al. (2007) reported the pediculicidal activity of extract and oil from *Melia azedarach* fruits showed high levels of mortality on adult lice, with values ranging between 62.9% and 96.5% at 20% ripe fruit extract with 10% ripe fruit oil. Sunilson et al. (2009) observed the petroleum ether extracts possess excellent anti-lice activity with values ranging between 50.3% and 100% whereas chloroform and methanol extracts showed moderate pediculicidal effects. Essential oils obtained from aromatic plants like *Eucalyptus sideroxylon*, *Eucalyptus globulus* spp *globulus*, and *E.globulus* spp *maidenii* were good and safe alternatives due to their low toxicity to mammals and easy biodegradability with knockdown time of 50% (KT_{50}) values of 24.75, 27.73, and 31.39 min (Toloza et al. 2010b). Semmler et al. (2009) reported that the pediculicide shampoos based on neem extracts such as "Wash-Away Louse" and "Picksan Luizenstop" were shown to be highly effective in in vitro and in vivo tests against head lice from Egypt and Germany. Plant essential oils are highly acceptable to the public as they are natural and pleasant smelling (Williamson et al. 2007). They are widely used in traditional medicine for their insecticidal and repellent activity against many species of insects, including lice. Such oils consist of numerous different, mostly volatile low-molecular-weights, and terpenoids (Dewich 2002; Priestley et al. 2006; Williamson 2007; Williamson et al. 2007). Essential oils, in particular, pennyroyal, tea tree, and anise, have potent insecticidal activity for killing head lice and their eggs (Williamson 2007). The botanical extracts as lousicides, tobacco (*Nicotiana tobaccum*), (*Derris philippinensis*), makabuhay (*Tinosphora rumphi*), and neem (*Azadirachta indica*) at concentrations of 10%, 20%, and 40% in oil emulsion induced more than 90% mortality in Carabao louse in vitro, whereas in vivo experimentation showed that only tobacco and Makabuhay induced 45.91% and 79.67% reduction in louse infestations, respectively (Robles 2004).

The toxicity of *E. caryophyllata* bud and leaf oil-derived compounds against eggs and females of *P. humanus capitis* was examined using direct contact application and fumigation methods and compared with those of the widely used delta-phenothrin and pyrethrum. In a filter paper diffusion bioassay with female *P. humanus capitis*, the pediculicidal activity of the Eugenia bud and leaf oils was comparable to

Table 3 Chemical constituents of flower bud hexane extract of *S. aromaticum* identified by gas chromatograph-mass spectrometer (GC/MS) analysis

Number	RT[a] (min)	Compounds	Relative (%)
1	8.422	4-Allylphenol	0.19
2	9.802	Alpha-cubebene	0.53
3	10.029	5-Allyl-2-methoxyphenol	58.79
4	10.219	Copaene	1.33
5	10.395	Germacra-1(10),4(15),5-triene, (−)-	0.03
6	10.839	2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-1,6-diene	13.75
7	10.915	Germacra-1(10),4(15),5-triene,(−)-	0.03
8	11.244	2,6,6,9-Tetramethyl-1,4,8-cycloundecatriene	1.67
9	11.336	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	0.07
10	11.495	1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	0.20
11	11.543	6-Alpha-cadina-4,9-diene, (−)-	0.01
12	11.578	Germacra-1(10),4(15),5-triene,(−)-	0.02
13	11.658	1,5-Dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene	0.02
14	11.734	1-beta-Cadin-4-en-10-ol	0.21
15	11.794	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl	0.11
16	12.088	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	15.09
17	12.198	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene	0.23
18	12.262	Germacra-1(10),4(15),5-triene,(−)-	0.01
19	12.333	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.06
20	12.476	(2R, 5E)-Caryophyll-5-en-12-al	0.02
21	12.581	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.01
22	12.756	(Z,Z)-Alpha-farnesene	0.02
23	12.868	Caryophyllene oxide	3.04
24	13.037	2,5-Dimethyl-3-vinyl-hexa-1,4-diene	0.03
25	13.100	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	0.02
26	13.165	Humulene oxide	0.33
27	13.251	3-Methyl-2-(1',1',5'-trimethyl-5'-hexenyl)-2-cyclopropenyl methyl ketone	0.06
28	13.346	1-Isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydro-4a(2H)-naphthalenol	0.21
29	13.426	4,8a-Dimethyl-4a, 5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	0.42
30	13.467	4,4-Dimethyltetracyclo[6.3.2.0E2,5.0E1,8]tridecan-9-ol	0.94
31	13.652	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol	0.04
32	13.691	3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-pentyn-3-ol	0.12
33	13.762	2,2,6,7-Tetramethyl bicyclo(4.3.0)nona-4,9(1)-dien-8-ol	0.01
34	13.841	3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-pentyn-3-ol	0.65
35	14.015	2',3',4' Trimethoxyacetophenone	0.67
36	14.821	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	0.06
37	15.070	2,2-Dimethyl-2-methylene-1-(3'-methyl- 4'-pentenyl)-3-cyclohexen-1-ol	0.01
38	15.210	l-Alanine, *N*-(4-butylbenzoyl)-, pentyl ester	0.01
39	21.252	*N*-[2-(4-tert-butyl phenoxy)ethyl]-3-(2-furyl)-2-propenamide	0.02
40	21.623	3-[4-(4-Methylphenyl)-1,3-thiazol-2-yl]-4,5,6,7-tetrahydro-1-benzothien-2-ylamine	0.23
41	21.870	Phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy-	0.03
42	22.100	1,2-Bis(4-methoxy-phenyl)-*N*,*N*,*N*',*N*'-tetramethyl-ethane-1,2-diamine	0.02
43	22.351	1-(10-Hydroxy-6,9,10-trimethylspiro[4.5]dec-6-en-2-yl)ethanone	0.02
44	22.736	1-(10-Hydroxy-6,9,10-trimethylspiro[4.5]dec-6-en-2-yl)ethanone	0.07
45	23.477	Phenol, 2-methoxy-4-propenyl-, (Z)-	0.13
46	24.402	*N*-Tetracontane	0.14
47	25.791	*n*-Hexatriacontane	0.32

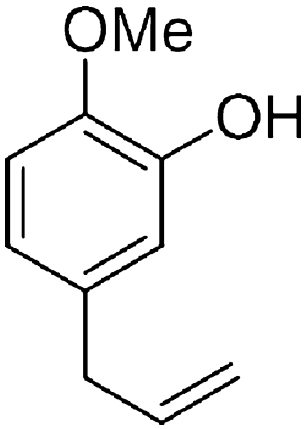

Fig. 4 Chemical structures of chavibetol (5-allyl-2-methoxyphenol)

those of delta-phenothrin and pyrethrum on the basis of LT_{50} values at 0.25 mg/cm (2). At 0.25 mg/cm (2), the compound most toxic to female *P. humanus capitis* was eugenol followed by methyl salicylate. The eugenol and methyl salicylate were more effective in closed cups than in open ones, indicating that the effect of the compounds was largely due to action in the vapor phase (Yang et al. 2003). Studies on structural basis for the activity of monoterpenes against the body lice, *P. humanus humanus*, found evidence that monooxygenated terpenes (terpinene-4-ol, pulegone, thymol, menthone, among others) were more active than hydrocarbons (camphene, limonene, a- and b-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 (Rossini et al. 2008; Priestley et al. 2006). Yang et al. (2005) reported that the pediculicidal constituents of *Cinnamomum zeylanicum* bark essential oil were identified as benzaldehyde, *(E)*-cinnamaldehyde, and linalool by GC/MS analysis. The compounds benzaldehyde, linalool, and salicylaldehyde were found to be more potent adult pediculicides than either D-phenothrin or pyrethrum. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids (Coats et al. 1991). Jayaseelan et al.(2011) have reported that the pediculocidal activity of aqueous leaf extracts and synthesized AgNPs of *Tinospora cordifolia* have the highest mortality observed in synthesized AgNPs against *P. humanus capitis* (LC_{50}=12.46 mg/L; r^2= 0.978). The anti-parasitic activities to determine the efficacies of synthesized zinc oxide nanoparticles (ZnO NPs) prepared by wet chemical method using zinc nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent against the head louse *P. humanus capitis*. In the pediculicidal activity, the results showed that the optimal times for measuring mortality effects of synthesized ZnO NPs were 38% at 10 min, 71% at 30 min, 83% at 1 h, and 100% after 6 h against *P. humanus capitis*. One hundred percent lice mortality was observed at 10 mg/L treated for 6 h. The mortality was confirmed after 24 h of observation period. The synthesized ZnO NPs showed the LC_{50} and r^2 values against the *P. humanus capitis* (11.80 mg/L; 0.966; Kirthi et al. 2011).

Some earlier workers have reported phenolics like chavibetol (53.1%) and chavibetol acetate (15.5%) isolated by the capillary GC analysis of the major constituents of Philippine *Piper betle* oil (Rimando et al. 1986), and counter-current chromatography was used to isolate chavibetol from the essential oil from the leaves of *Pimenta pseudocaryophyllus* (dos Santos et al. 2009). The chemical composition of the leaf oil of *P. betle* collected at Masjid Tanah, Melaka, Malaysia, is reported to have chavibetol (69.0%), eugenyl acetate (8.3%), and chavicol (6.0%) as the major components (Jantan et al. 1994). Bhattacharya et al. (2007) reported the inhibitory properties of an ethanolic extract from leaves of *P. betel* against the photo-sensitization-induced damage to lipids and proteins of rat liver mitochondria, indicating that this activity was mainly correlated to its phenolic constituents such as chavibetol and 4-allylpyrocatechol. Bioassay-directed isolation and purification of the hexane extract of *Apium graveolens* seeds led to the characterization of three compounds, β-selinene (1), 3-*n*-butyl-4,5-dihydrophthalide (2), and 5-allyl-2-methoxyphenol (3). Compounds, 1–3 demonstrated 100% mortality on fourth-instar *Aedes aegypti* larvae at 50, 25, and 200 μg mL^{-1}, respectively, in 24 h (Momin et al. 2000). Ally (1960) reported that the chavicol (4-hydroxyallyl-benzene) is a major component and is strongly antiseptic. Hydroxychavicol (allylpyrocatechol, 3-4-dihydroxyallylbenzene) is present (and contains the carbon skeleton of safrole) as is its four-methyl ether, chavibetol, classically known as betel phenol, and in the use of betel nut, there have been some preliminary studies made into its pharmacological properties. The essential oil extracted from the dried flower buds of clove *E. caryophyllata* is used as a topical application to relieve pain and the main constituents of the essential oil are phenylpropanoids such as carvacrol, thymol, eugenol, and cinnamaldehyde, and the biological activity of *E. caryophyllata* has been investigated on several microorganisms and parasites, in addition to its antimicrobial, antioxidant, antifungal, and antiviral activity; clove essential oil possesses antiinflammatory, cytotoxic, insect-repellent, and anesthetic properties (Chaieb et al. 2007).

Several compounds, such as mayonnaise, petroleum jelly, and olive oil, have erroneously been reported to kill head lice, based on the less stringent criteria of efficacy, but in reality these caused only a transient period of stasis (Burkhart and Burkhart 2001, 2004, 2006a, 2006b). In the

present study, test materials were more effective against adult *P. humanus capitis* in closed containers than in open ones. These results indicate that the mode of delivery of the extract oil nature was likely by vapor action via the respiratory system, although the exact mode of action remains unknown. For the practical use of flower bud hexane extract *S. aromaticum* test as novel fumigants to proceed, further research is required on the safety issues of these materials for human health. Other areas requiring attention are pediculicide mode of action and formulations to improve potency and stability and to reduce cost.

References

Abdel-Ghaffar F, Semmler M (2007) Efficacy of neem seed extract shampoo on head lice of naturally infected humans in Egypt. Parasitol Res 100:329–332

Abdel-Ghaffar F, Semmler M, Al-Rasheid K, Klimpel S, Mehlhorn H (2010a) Efficacy of a grapefruit extract on head lice: a clinical trial. Parasitol Res 106(2):445–449

Abdel-Ghaffar F, Semmler M, Al-Rasheid K, Mehlhorn H (2010b) Comparative in vitro tests on the efficacy and safety of 13 anti-head lice products. Parasitol Res 106:423–429

Alkinson I, Clore ER, Kisel BE, Posch J (1986) Internal and external parasites. Pediatrics 1:1–7

Ally MM (1960) Preliminary observations on the pharmacology of Betel leaf. Proc Pan Indian Ocean Sci Congr, 4th, Karachi, Pakistan Sect G p. 31. CA 61:7363e

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 GP, 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 299:389–392

Bagavan A, Rahuman AA, Kaushik NK, Sahal D (2011) In vitro antimalarial activity of medicinal plant extracts against *Plasmodium falciparum*. Parasitol Res 108(1):15–22

Bhattacharya S, Mula S, Gamre S, Kamat JP, Bandyopadhyay SK, Chattopadhyay S (2007) Inhibitory property of *Piper betel* extract against photosensitization-induced damages to lipids and proteins. Food Chem 100:1474–1480

Burkhart CN (2003) Fomite transmission with head lice: a continuing controversy. Lancet 361(9352):99–100

Burkhart CG, Burkhart CN (2000) Clinical evidence of lice resistance to over-the-counter products. J Cutan Med Surg 4(4):199–201

Burkhart CG, Burkhart CG (2001) Recommendation to standardize pediculicidal and ovicide testing for head lice (Anoplura: Pediculidae). J Med Entomol 38:127–129

Burkhart CN, Burkhart CG (2004) Head lice revisited: *in vitro* standardized tests and differences in malathion formulations. Arch Dermatol 140(4):488–489

Burkhart CG, Burkhart CN (2006a) Asphyxiation of lice with topical agents, not a reality … yet. J Am Acad Dermatol 54 (4):721–722

Burkhart CG, Burkhart CN (2006b) Safety and efficacy of pediculicides for head lice. Expert Opin Drug Saf 5(1):169–179

Cai L, Wu CD (1996) Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. J Nat Prod 59(10):987–990

Carpinella MC, Miranda M, Almirón WR, Ferrayoli CG, Almeida FL, Palacios SM (2007) In vitro pediculicidal and ovicidal activity of an extract and oil from fruits of *Melia azedarach* L. J Am Acad Dermatol 56(2):250–256

Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A (2007) The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytother Res 21(6):501–506

Cheng LPL, Ho SH, Sim KY, Tan HTW (1994) Potential of cloves (*Syzygium aromaticum* L.) Merr. And Rerry as a grain protectant against *Tribolium castaneum*(Herbst) and *Sitophilus zeamais* Motsch. Postharvest Biol Technol 4:179–183

Coats JR, Karr LL, Drewes CD (1991) Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In: Hedin PA (ed) Naturally occuring pest bioregulators ACS symposium series 449. American Chemical Society, Washington, DC, pp 305–316

Cueto GM, Gonzalez Audino P, Vassena CV, Picollo MI, Zerba EN (2002) Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). J Med Entomol 39:457–460

Cueto GM, Zerba E, Picollo MI (2008) Evidence of pyrethroid resistance in eggs of *Pediculus humanus capitis* (Phthiraptera: Pediculidae) from Argentina. J Med Entomol 45:693–697

Dewich M (ed) (2002) Medicinal natural products: a biosynthetic approach, 2nd edn. Wiley, Chichester, p 507

Dolianitis C, Sinclair R (2002) Optimal treatment of head lice: is a no-nit policy justified? Clin Dermatol 20:94–96

dos Santos BC, da Silva JC, Guerrero PG Jr, Leitão GG, Barata LE (2009) Isolation of chavibetol from essential oil of *Pimenta pseudocaryophyllus* leaf by high-speed counter-current chromatography. J Chromatogr A 1216(19):4303–4306

Doucet M, Miranda M, Arce MA (1997) Prevalences of *Pediculus humanus capitis* and *Sarcoptes scabiei* in primary schools of Córdoba, Argentina. Analysis in relation to sex, age and socioeconomic conditions. Res Rev Parasitol 57:67–69

Downs AM, Stafford KA, Coles GC (1999) Head lice: prevalence in school children and insecticide resistance. Parasitol Today 15:1–4

El Hag EA, El Nadi AH, Zaiton AA (1999) Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phytother Res 13:388–392

Gallardo A, Mougabure Cueto G, Picollo MI (2009) *Pediculus humanus capitis* (head lice) and *Pediculus humanus humanus* (body lice): response to laboratory temperature and humidity and susceptibility to monoterpenoids. Parasitol Res 105:163–167

Goates BM, Atkin JS, Wilding KG, Birch KG, Cottam MR, Bush SE, Clayton DH (2006) An effective nonchemical treatment for head lice: a lot of hot air. Pediatrics 118(5):1962–1970

Gratz NG (1997) Human lice, their prevalence, control and resistance to insecticides; a review, 1985–97. Geneva, Switzerland: World Health Organization, Division Control Tropical Diseases, WHO Pesticide Evaluation Scheme, 1997. Report WHO/CTD/WHOPES/97.8, 7: 176–179

Hayes JB Jr, Laws ER Jr (1991) Handbook of pesticide toxicology, vol 1. Academic, San Diego

Heukelbach J, Oliveira F, Speare R (2006a) A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in vitro. Parasitol Res 99:353–356

Heukelbach J, Speare R, Canyon D (2006b) Natural products and their application to the control of head lice: an evidence-based review. In: Brahmachari G (ed) Chemistry of natural products: recent trends and development. Kerala, India, pp 1–26

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 22:264–272

Ho SH, Cheng LPL, Sim KY, Tan HTW (1994) Potential of cloves (*Syzygium aromaticum*) (L.) Merr. and Perry as a grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Posthar Vest Biol Technol 4:179–183

Isman MB (1999) Pesticides based on plant essential oils. Pestic Outlook 68–72

Jantan IB, Ahmad AR, Ahmad AS, Ali NAM (1994) A comparative study of the essential oils of five *Piper* species from Peninsular Malasya. Flav Fragr J 9:339–342

Jayaseelan C, Rahuman AA, Rajakumar G, Vishnu Kirthi A, Santhoshkumar T, Marimuthu S, Bagavan A, Kamaraj C, Zahir AA, Elango G (2011) Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heartleaf moonseed plant. *Tinospora cordifolia* Miers. Parasitol Res. doi:10.1007/s00436-010-2242-y

Kim Eh, Kim HK, Ahn YJ (2003) Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Agric Food Chem 51(4):885–889

Kirthi AV, Rahuman AA, Rajakumar G, Marimuthu S, Santhoshkumar T, Jayaseelan C, Velayutham K (2011) Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. Parasitol Res. doi:10.1007/s00436-011-2277-8

Kristensen M, Knorr M, Rasmussen AM, Jespersen JB (2006) Survey of permethrin and malathion resistance in human head lice populations from Denmark. J Med Entomol 43:533–538

Kumar P, Singh DK (2006) Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. Chemosphere 63(9):1568–1574

Linardi PM, Botelho JR, Maria M (1988) Crendices e falsos conceitos que dificultam acoes profilaticas contra o piolho e a pediculose capitis. Pediatria 64:248–255

Machado M, Dinis AM, Salgueiro L, Custódio JB, Cavaleiro C, Sousa MC (2011) Anti-Giardia activity of *Syzygium aromaticum* essential oil and eugenol: Effects on growth, viability, adherence and ultrastructure. Exp Parasitol 127(4):732–739

Mehlhorn B, Mehlhorn H (2009) Louse alarm. Düsseldorf University Press, Düsseldorf

Mehlhorn H, Eichenlaub D, Löscher T, Peters W (1995) Diagnosis and therapy of human parasites, 2nd edn. G. Fischer, Stuttgart

Meinking T, Entzel P, Villar ME, Vicaria M, Lemard G, Porcelain S (2001) Comparative efficacy of treatments for *Pediculosis capitis* infestations. Arch Dermatol 137:287–292

Momin RA, Ramsewak RS, Nair MG (2000) Bioactive compounds and 1,3-Di[(cis)-9-octadecenoyl]-2-[(cis, cis)-9, 12-octadecadienoyl] glycerol from *Apium graveolens* L. seeds. J Agric Food Chem 48 (9):3785–3788

Morsy TA, el-Ela RG, Nasser MM, Khalaf SA, Mazyad SA (2000) Evaluation of the in-vitro pediculicidal action of four knock insecticides and three medicinal plant extracts. J Egypt Soc Parasitol 30(3):699–708

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 KY (1999) Prevention and treatment of head lice in children. Paediatr Drugs 1(3):211–218

Mumcuoglu KY, Miller J, Zamir C, Zentner G, Helbin V, Ingber A (2002) The in vivo pediculicidal efficacy of a natural remedy. Isr Med Assoc J 4(10):790–793

Mumcuoglu KY, Meinking TA, Burkhart CN, Burkhart CG (2006) Head louse infestations: the "no nit" policy and its consequences. Int J Dermatol 45(8):891–896

Ninci M (1991) Prophylaxis and treatment of pediculosis with *Quassia amarga*. Rev Fac Cien Med Univ Nac Cordoba 49 (2):27–31

Oladimeji FA, Orafidiya OO, Ogunniyi TA, Adewunmi TA (2000) Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. J Ethnopharmacol 72(2):305–311

Park IK, Shin SC (2005) Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus*). J Agric Food Chem 53:4388–4392

Perrucci S, Macchioni G, Cioni PL, Flamini G, Morelli I (1995) Structure/activity relationship of some natural monoterpenes as acaricides against *Psoroptes cuniculi*. J Nat Prod 8:1261–1264

Picollo MI, Vassena C, Casadio A, Massimo J, Zerba E (1998) Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura: Pediculidae). J Med Entomol 35:814–817

Picollo MI, Vassena C, Mougabure Cueto G, Vernetti M, Zerba E (2000) Resistance to insecticides and effect of synergists on permethrin toxicity in *Pediculus capitis* (Anoplura: Pediculidae) from Buenos Aires. J Med Entomol 37:721–725

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

Reddy PJ, Krishna D, Murthy US, Jamil K (1992) A microcomputer FORTRAN program for rapid determination of lethal concentration of biocides in mosquito control. Comput Appl Biosci 8:209–213

Rimando AM, Han BH, Park JH, Cantoria MC (1986) Studies on the constituents of Philippine *Piper betle* leaves. Arch Pharm Res 9:93–97

Robles AP (2004) Comparative efficacy of tobacco (*Nicotiana tobaccum*), makabuhay (*Tinosphora rumphi*), tubli (*Derris philippinensis*), and neem (*Azadirachta indica*) against carabao louse (*Haematopinus tuberculatus*). In: Maala CP, Landicho EF, Sarabia AS, Del Barrio AN, Ataby EP (eds) Abstract of Researches on the Philippine water buffalo. Philippine Carabao Center, The Philippine, pp 139–140

Rossini C, Castillo L, González A (2008) Plant extracts and their components as potential control agents against human head lice. Phytochem Rev 7:51–63.

Rozendaal JA (1997) Methods for use by individuals and communities. World Health Organization, Geneva

Schmahl G, Al-Rasheid KA, Abdel-Ghaffar F, Klimpel S, Mehlhorn H (2010) The efficacy of neem seed extracts (Tre-san, MiteStop) on a broad spectrum of pests and parasites. Parasitol Res 107 (2):261–269

Semmler M, Abdel-Ghaffar F, Al-Rasheid K, Mehlhorn H (2009) Nature helps: from research to products against blood-sucking arthropods. Parasitol Res 105(6):1483–1487

Semmler M, Abdel-Ghaffar F, Al-Rasheid K, Klimpel S, Mehlhorn H (2010) Repellency against head lice (*Pediculus humanus capitis*). Parasitol Res 106(3):729–731

Speare R, Canyon DV, Melrose W (2006) Quantification of blood intake of the head louse: *Pediculus humanus capitis*. Int J Dermatol 45:543–546

Sunilson JSAJ, Suraj R, Rejitha G, Anandarajagopal K, Vimala AG, Husain HA (2009) In vitro screening of anti-lice activity of *Pongamia pinnata* leaves. Korean J Parasitol 47(4):377–380

Takano-Lee M, Edman JD, Mullens BA, Clark JM (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

Taplin D, Meinking TL (1987) Pyrethrin and pyrethroids for the treatment of scabies and pediculosis. Semin Dermatol 6:125–135

Tiangda CH, Gritsanapan W, Sookvanichsilp N, Limchalearn A (2000) Anti-headlice activity of a preparation of *Annona squamosa* seed extract. Southeast Asian J Trop Med Public Health 31(1):174–177

Toloza AC, Zygadlo J, Cueto GM, Biurrun F, Zerba E, Picollo MI (2006) Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus* capitis (Anoplura: Pediculidae) from Argentina. J Med Entomol 43(5):889–895

Toloza A, Vassena C, Gallardo A, González-Audino P, Picollo MI (2009) Epidemiology of *Pediculosis capitis* in elementary schools of Buenos Aires, Argentina. Parasitol Res 104(6):1295–1298

Toloza AC, Lucía A, Zerba E, Masuh H, Picollo MI (2010a) *Eucalyptus* essential oil toxicity against permethrin-resistant *Pediculus humanus capitis* (Phthiraptera: Pediculidae). Parasitol Res 106(2):409–414

Toloza AC, Zygadlo J, Biurrun F, Rotman A, Picollo MI (2010b) Bioactivity of Argentinean essential oils against permethrinresistant head lice, *Pediculus humanus capitis*. J Insect Sci 10:185

Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C (2005) Comparative repellency of 38 essential oils against mosquito bites. Phytother Res 19:303–309

Veal L (1996) The potential effectiveness of essential oils as a treatment for headlice, *Pediculus humanus capitis*. Complement Ther Nurs Midwifery 2:97–101

Williamson EM (2007) The medicinal use of essential oils and their components for treating lice and mite infestations. Natural Prod Commun 2:1199–1336

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 78:521–525

Yang YC, Lee SH, Lee WJ, Choi DH, Ahn YJ (2003) Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. J Agric Food Chem 51:4884–4888

Yang YC, Choi HY, Choi WS, Clark JM, Ahn YJ (2004a) Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). J Agric Food Chem 52:2507–2511

Yang YC, Lee HS, Clark JM, Ahn YJ (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, Clark JM, Ahn YJ (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