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ORIGINAL PAPER

Effectiveness of lotions based on essential oils from aromatic plants against permethrin resistant *Pediculus humanus capitis*

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Abstract In Argentina, field populations of the head louse *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae) have developed resistance to permethrin and other pyrethroids. Thus, the aim of this work was the development of a lotion containing essential oils from plants and an alcoholic coadjuvant to improve biological effect. Ethanol + isopropanol (1 + 1 in volume) 50% in water and ethanol 96% were taken as bases for preparation of experimental lotions containing essential oils from plants. We found that experimental lotions containing lavender, peppermint and eucalyptus oils in a 5% composition and the combination of eucalyptus and peppermint in a total concentration of 10%, dissolved in 50% ethanol + isopropanol (1 + 1) in water, showed the best knockdown effect. On the other side, lotion containing peppermint oil and eucalyptus oil (1 + 1) 10%, dissolved in ethanol 96%, showed to be as effective as the best commercial lotion now available in Argentina. Furthermore, addition of 1-dodecanol in all cases increased the effectiveness of all the experimental lotions. This difference is significantly important for 1-dodecanol concentration of 10%, reaching a toxic activity compared to the best commercial lotion available in the market.

Keywords Head lice · Essential oil · 1-dodecanol · Knock down activity · Permethrin resistant

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Introduction

In Argentina, field populations of the head louse *Pediculus h. capitis* De Geer (Phthiraptera: Pediculidae) have developed resistance to permethrin and other pyrethroids after extensive use of insecticides since 1980 [13]. Recently, an extensive survey for resistance in Buenos Aires showed high levels of head lice on children in 24 out of 26 (92.3%) schools. When compared to a previously unexposed reference population, resistance ratios (RR) to permethrin obtained ranged from 13.6 to 68.6, determined by topical application of the insecticide [5]. Similarly, Hemingway et al. [6] found a high resistance level to permethrin (38.7-fold) in *P. h. capitis* from Israel, and Pollack et al. [15] reported that the RR for field populations from United States was 68 times higher than that obtained in Borneo, although in their work they use insecticide-impregnated papers instead of topical application. Moreover, high resistance to malathion was measured for head lice from the UK [3], high resistance to phenothrin was recorded for head lice from Japan [8], and emergence of resistance to carbaryl was found in England [4].

Pyrethroid resistance in *P. h. capitis* is a phenomenon of great concern because of the lack of novel insecticides introduced in the market as new safe products for chemical control of head lice. The modification of pediculicidal formulations to enhance insecticide activity has shown to be one good strategy to optimize the effectiveness of the available products. In this context, the insecticidal effect of aliphatic alcohols (from C2 to C18) has been tested in our lab in experimental pediculicidal lotions. The highest activity was found for the 1-dodecanol and the lowest for 1-octanol [10].

On the other hand, the use of botanical compounds such as essential oils as a natural means of pest control has recently become of great interest because of its repellent, ovicidal,

adulticidal, and feeding inhibition activity against various insect species [7, 9, 16, 17] including head lice [18, 20, 21].

Essential oils are the steam-distillable fraction of plant tissues which are often responsible for a plant's distinctive scent, odor or taste. These oils are of rather complex composition, generally consisting of low-molecular weight monoterpenes (10-carbon) and related phenols.

Thus, the aim of this work was to establish the guides for the development of a lotion containing essential oils from plants with the addition of an alcoholic coadjuvant to improve biological effect.

Materials and methods

Plant oils

Lavender oil (pure essential oil), peppermint oil (pure essential oil), orange oil (pure essential oil), and eucalyptus oil (pure essential oil), were all from Switzerland. A commercial pediculicidal lotion (SUMO, ELEA, Buenos Aires, Argentina), previously found to be the most effective one available in the Argentine market (Picollo, unpublished results), was used as a positive control for knockdown efficacy. Commercial lotion SUMO®, is based on a combination of an insectide (d-phenothrin 0.3%) and a synergistic agent. 1-Dodecanol was from Aldrich, USA.

Experimental lotions

The plant oils were first dissolved in ethanol (96%, p.a. Merck), isopropanol or their mixtures. For lotions containing water, necessary amount of water to reach 100% was added. To prepare lotions containing 1-dodecanol, the plant oil was first dissolved in a mixture of isopropanol and/or ethanol and afterwards 1-dodecanol was added (in an amount necessary to reach final 5 or 10% in volume). Water necessary to reach 100% was added.

Lice

Head lice were collected from 300 infested children at a children home (Hogar Belgrano) located in San Martín, Buenos Aires, Argentina, where permethrin-based pediculicides have been intensively used since 1990. This population RR to permethrin has been reported by our lab in 2004 as 47.2 by topical application [11] and 22.7 by filter paper exposure [14]. Live head lice were obtained using a fine-toothed anti-louse comb (Nopucid, ELEA, Buenos Aires, Argentina) from children aged 6–12 years, according to a protocol approved by the ad-hoc Committee of CIPEIN and archived in our laboratory. The total number of collected lice was around 2,000 and they were collected on 20 different days and every day at least one water control was performed. A mix of adults or third instar nymphs were selected at the laboratory for the bioassays, as no differences in susceptibilities between them were reported by Mumcuoglu [12]. After collection, lice were maintained without feeding in an environmental chamber (Lab-Line Instruments, Melrose Park, IL) at 18 ± 0.5°C and 70–80% RH in the dark for a maximum of 1 h before its use in toxicological bioassays [13].

Toxicological bioassay

The effectiveness of experimental lotions was evaluated by the immersion method [10]. Batches of at least 10 adults or third nymphs (three replicates for each toxicity bioassay) were submerged for 2 min in 1 ml of each experimental lotion. At the end of the exposure period, the treated insects were placed on a wire mesh and washed with 100 ml water. They were then placed on a piece of filter paper, No.1 (Whatman, Maidstone, UK), of 7 cm diameter moistened with 0.5 ml water, that was placed in the bottom of a plastic Petri dish. Knockdown of exposed lice, our end point defined here as inability of lice to walk over a filter paper, was recorded after 10 min, the necessary time for total recovery of control insects. In all experiments, at least three replicates of each lotion were used, and a negative water control was always performed to consider the effects of manipulating the insects (mortalities obtained for these controls were always under 10%). Data were corrected by knockdown of controls using the Abbott formula [1].

Results were presented as % knockdown (KD) ± CL and means were analysed by ANOVA.

Results

Knock down after 10 min was used as the end point of the bioassays because regulations in Argentina requires an exposition time not longer than this after the treatment for pediculicidal lotions and, on the other hand, we had established a good correlation between KD effect and mortality after 18 h in previous work [10]. It must be understood, however, that these insects can appear temporarily dead yet resurrect within a few hours [2].

Our first goal was to evaluate the influence of the alcoholic base of the experimental lotion containing plant oils on its biological activity. For this reason, it was necessary for an alcoholic base not toxic per se but able to solubilize essential oils. Peppermint oil 5% in volume was the plant oil chosen as a model system. Ethanol or isopropanol compositions 25% in volume in water did not show significant knockdown activity compared to controls (Table 1). Unfortunately, solutions 25% of individual alcohols did not allow

Table 1 Influence of the alcoholic composition of the lotion containing peppermint oil on its knockdown activity

Alcoholic composition	% KD ± CL	
	Control	Peppermint 5%
Ethanol 25%	10.0 ± 10.0a	10.0 ± 10.0a
Isopropanol 25%	0.0 ± 0.0a	10.0 ± 10.0a
Ethanol 25% + isopropanol 25%	6.0 ± 6.0a	81.3 ± 4.6b
Ethanol 70%	10.0 ± 10.0a	33.3 ± 3.1a
Isopropanol 70%	40.0 ± 0a	–[a]
Ethanol 96%	13.3 ± 18.9a	50.0 10.0a
Isopropanol 100%	90.0 ± 12.0b	–[a]

[a] The alcoholic base was too active per se so it was not adequate to evaluate the effectivity of essential oils

Values in the same line with the same letter are not significantly different (ANOVA) ($P > 0.05$). Data were corrected by Abbot formula [1]

enough dissolution of essential oils. However, the combination of both the alcohols in a total concentration of 50% (1 + 1 in volume) had the best knockdown efficacy combined with peppermint oil and it was not active by itself (Table 1). Peppermint lotion containing 70% ethanol did not show a good knockdown activity compared to control and the lotion in ethanol 96% showed some activity, although it was not significantly different from control (Table 1). Furthermore, isopropanol 70 and 100% were intrinsically toxic themselves so they were not useful to evaluate the lotion containing peppermint oil.

Taking into account these results, alcoholic compositions based on ethanol + isopropanol (1 + 1) 50% in water and on ethanol 96% were taken as alcoholic bases for preparation of experimental lotions containing essential oils from plants. Oils from peppermint, eucalyptus, lavender and orange were added in 5% proportion to the alcoholic base.

Knockdown activity of compositions based on ethanol + isopropanol (1 + 1) 50% (Table 2) showed that peppermint, lavender, eucalyptus and orange show significant knockdown activity compared to control and that the mixture of peppermint and eucalyptus oil in a total concentration of 10% was the most effective ($P < 0.05$). Knockdown performance of the last composition was similar to that of the commercial lotion.

In the case of the lotions in ethanol 96%, the ones containing peppermint, eucalyptus, lavender and orange, did not show a significant activity compared to control. In this case (Table 3), the mixture of peppermint and eucalyptus in a total concentration of 10% showed the best performance, not significantly different from the commercial pediculicidal lotion ($P > 0.05$). Previous results of our lab demonstrated that short chain aliphatic alcohols exert pediculicidal activity [10] against susceptible and resistant head lice populations. Insecticidal activity systematically increases with increase in carbon atoms in the aliphatic alcohols and maximum activity was observed when the total number of carbon atoms was 12. Thus, we introduced 1-dodecanol in the experimental lotions containing essentials oils in order to increase their effectiveness (Table 4). Results showed that 1-dodecanol increases effectiveness of all the lotions containing different plant oils, and this difference is significantly important for dodecanol concentration of 10%, reaching a knockdown efficacy of 100%, similar to that of the best commercial lotion available in the market.

Discusion

At present, commercial lotions available in the market in Argentina contain pyrethroid insecticide as an active ingredient with the consequent undesirable effect, resistance in head lice that they have developed. It has become evident that resistance to permethrin which crosses with other pyrethroid available as pediculicides, has spread in head lice from Buenos Aires [14, 19]. Also, the use of botanical oils to replace synthetic insecticides is highly desirable owing

Table 2 Comparison of knockdown activity of different essential oils dissolved in ethanol + isopropanol (1 + 1) 50% in water

Plant oil	% KD ± CL
None	6.7 ± 3.4a
Peppermint 5%	81.3 ± 4.6b
Eucaliptus 5%	73.3 ± 9.5b
Lavender 5%	83.0 ± 11.0b
Orange %	39.1 ± 17.5b
Peppermint 5% + eucalyptus 5%	93.3 ± 9.4c
Commercial lotion SUMO*	100c

Values in the same column with the same letter are not significantly different (ANOVA) ($P > 0.05$). Data were corrected by Abbot formula [1]

Table 3 Comparison of knockdown activity of different essential oils dissolved in ethanol 96%

Plant oil	% KD ± CL
None	13.6 ± 8.9a
Peppermint 5%	41.0 ± 29.0a
Eucalyptus 5%	41.0 ± 27.0a
Lavender 5%	49.0 ± 6.9a
Orange 5%	40.0 ± 20.0a
Peppermint 5% + eucalyptus 5%	93.0 ± 9.4b
Commercial lotion SUMO*	100.0 ± 0b

Values in the same column with the same letter are not significantly different (ANOVA) ($P > 0.05$). Data were corrected by Abbot formula [1]

Table 4 Comparison of knockdown activity of different essential oils dissolved in ethanol + isopropanol (1 + 1) 50% in water and containing 1-dodecanol as coadjuvant

Plant oil	% KD ± CL		
	No dodecanol	1-Dodecanol 5%	1-Dodecanol 10%
Peppermint	81.3 ± 4.6a	95.0 ± 5.0a	100.0 ± 0.0a
Eucalyptus	73.3 ± 9.4a	70.0 ± 10.0a	90.0 ± 10.0a
Lavender	83.0 ± 11.0a	86.5 ± 3.5a	100.0 ± 0.0a
Orange	39.1 ± 17.5a	90.0 ± 10b	100.0 ± 0.0b
Eucalyptus 5% + peppermint 5%	93.3 ± 9.4a	100.0 ± 0a	100.0 ± 0.0a
Commercial lotion SUMO*	100.0 ± 0a	–	–

Values in the same line with the same letter are not significantly different (ANOVA) ($P > 0.05$). Data were corrected by Abbot formula [1]

to their lower mammalian toxicity. Thus, the aim of this work was the development of a pediculicidal lotion based on essential oils from plants dissolved in an alcoholic base.

We found that experimental lotions containing lavender, peppermint and eucalyptus oils in a 5% composition and the combination of eucalyptus and peppermint in a total concentration of 10%, dissolved in 50% ethanol + isopropanol (1 + 1) in water, showed the best knockdown effect. On the other side, lotion containing peppermint oil and eucalyptus oil (1 + 1) 10%, dissolved in ethanol 96%, showed to be as effective as the best commercial lotion now available in Argentina. Furthermore, addition of 1-dodecanol in all cases increased the effectiveness of all the experimental lotions. For the practical use of these lotions containing essential oils to proceed, further research is necessary on several issues, including dermal and ocular irritation tests to ensure safety of these lotions for human health.

From these findings, it is concluded that essential oils of peppermint, eucalyptus and lavender may be considered to be used, either in an ethanolic or isopropanol/ethanolic base, or in combination with 1-dodecanol as a good alternative for capilar commercial lotions against lice.

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