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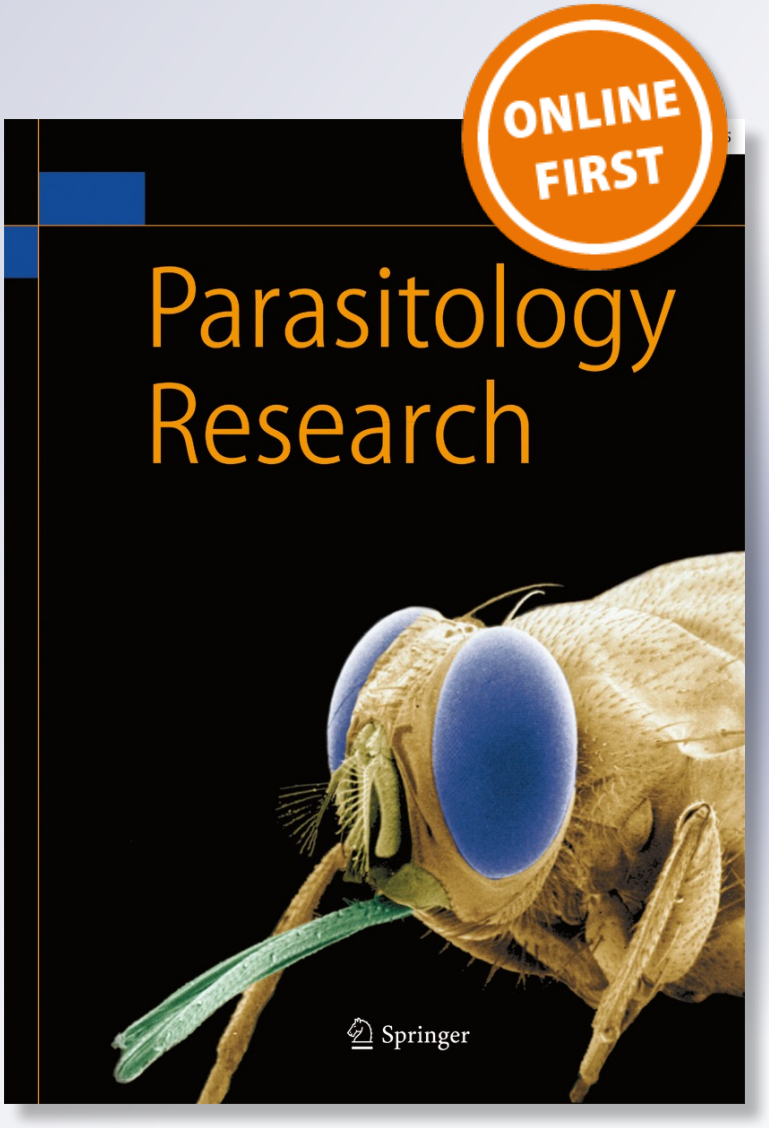

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ORIGINAL PAPER

The potential application of plant essential oils to control *Pediculus humanus capitis* (Anoplura: Pediculidae)

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Abstract The human head louse, *Pediculus humanus capitis* (Anoplura: Pediculidae), is an ectoparasite confined to the scalp and human hairs. The repeated use of insecticides for the control of head lice during past decades has resulted in the development of marked levels of resistance. Natural compounds such as essential oils (EOs) have been suggested as alternative sources for insect control agents. In order to introduce a new pediculicide based on EOs, the effectiveness of the product and their effects on human being must be analyzed. In consequence, the biological activity of EOs from the leaves and fruits of *Schinus areira* (Anacardiaceae) and the leaves of *Thymus vulgaris* (Lamiaceae), *Aloysia polystachya* and *Aloysia citriodora* (Verbenacea) were evaluated against the eggs and adults of *P. humanus capitis* by fumigant and contact toxicity bioassays. Additionally, dermal corrosion/irritation tests were performed on New Zealand albino rabbits. In a fumigant bioassay, EOs from the leaves and fruits of *S. areira* were the most toxic against *P. humanus capitis* adults while these EOs and *T. vulgaris* were the most effective against the eggs. In contact bioassay, the EO from *T. vulgaris* was the most toxic against both stages. In the corrosion/irritation tests, the EOs did not produce dermal effects. According to the results, the essential oils from the leaves of *T. vulgaris* would be a valid tool for the management of *P. humanus capitis.* This EO produces a high knockdown effect in adults (followed by mortality) and toxicity in the eggs when it is applied for 21 min at a low concentration.

Keywords Head lice · Essential oils · Adulticidal · Ovicidal · Skin irritation/corrosion tests

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Introduction

The human head louse, *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae), is a worldwide public health concern (Kurt et al. 2015). This human obligate ectoparasite usually infests school-age children; it is estimated that each year, five million people are newly infested with this parasite (Gratz 1997). Its presence is unpleasant and may cause severe pruritus, but the infestation also generates excoriations on the skin, sleep loss, as well as occasional secondary bacterial infections and inflammatory reactions caused by its bite and saliva (Gratz 1997; Rozendaal 1997; Rutkauskis et al. 2015). In addition to these symptoms, pediculosis can also cause psychological problems as a result of embarrassment and low self-esteem in infested children, particularly aggravated by the exclusionary practices adopted in some schools (Lebwohl et al. 2007).

In a previous work, we evaluated the prevalence of head lice in kindergarten children from Bahia Blanca city, Argentina. We observed an overall prevalence of 42.7 % and a higher frequency in girls than in boys; Clore and Longyear (1990) consider pediculosis as an epidemic illness when 5 % of students or more are infested. We also concluded that more than 65 % of the infested children require pediculicide treatment (Gutiérrez et al. 2012).

Chemical control of head lice has been based on a variety of conventional insecticides, such as DDT and lindane in the

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1940–1950s, carbaryl and malathion in the 1960-1970s, and pyrethroids in the 1980s up to the present. Repeated use of these compounds has led to resistance in several countries (Burgess 2004; Toloza et al. 2006; Bagavan et al. 2011; Combescot-Lang et al. 2015).

Plant essential oils (EOs) have been suggested as an alternative for insect control because they constitute a rich source of bioactive chemicals (Regnault-Roger and Arnason 2013). Some of these natural products are repellents, ovicides, adulticides, feeding inhibitors, or attractants for various insect species (Tapondjou et al. 2005; Mann and Kaufmann 2012), including head lice (Burgess 2004). In this study, we evaluated the contact and fumigant activity of EOs of the leaves and fruits of *Schinus areira* L. (Anacardiaceae) and the leaves of *Thymus vulgaris* L. (Lamiaceae), *Aloysia polystachya* (Griseb.) Moldenke and *A. citriodora* Palau (Verbenacea) in adults and eggs of *P. humaus capitis*. Additionally, the irritating and corrosive effect of these EOs on the skin of albino rabbits was examined.

Materials and methods

Head lice

Adults and eggs were collected from infested children 3–6 years old using a fine-toothed anti-louse comb. Head lice were obtained from kindergartens located in different parts of Bahia Blanca city, Argentina. Lice were examined carefully with a stereoscopic microscope (Olympus SZ 40), and damaged specimens were discarded (WHO 1981). All lice used for the bioassays were tested within 2 hours after collection and were protected from sunlight and heat. Selected eggs corresponded to the medium development stage, showing reddish eyes and outlines of appendages (Mougabure Cueto et al. 2006).

Essential oils

Leaves and mature fruits of *S. areira* and leaves of *A. citriodora* were collected during the summer seasons in Bahia Blanca city, Buenos Aires, Argentina (38°43′ S, 62°16′ W); leaves of *A. polystachya*, in Lamarque and Pomona city, Rio Negro, Argentina (39°24′ S, 65°42′ W) and leaves of *T. vulgaris* were collected from Salta city, Salta, Argentina (24°27′21″ S, 65° 24′ 38″ W). All specimens were authenticated at the herbarium of the Department of Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (BBB). EOs were extracted from fresh material by hydrodistillation using a modified Clevenger apparatus in 3–4 h. With this method, secondary metabolites (mainly terpenes and phenolic compounds) are obtained in a relatively pure fraction excluding most of the primary metabolites (Regnault-Roger and Arnason 2013). Then, EOs were dried over anhydrous sodium sulfate and stored in airtight containers at −4 °C. The chemical composition of the EOs was determined by gas chromatography-mass spectrometry in previous works (Werdin et al. 2008; Werdin González et al. 2010, 2011a, 2011b, 2013) and the physical and chemical properties of the main components were obtained by Advanced Chemistry Development software version 12.0 (ACD/Labs 2008) (Table 1).

Bioassays with *P. humanus capitis*

All bioassays were performed at 28±1 °C, 75±5 % RH and darkness.

Fumigant toxicity bioassay

Toxicity exposure to vapors of EOs was evaluated in an enclosed chamber against adults and eggs in accordance with Yang et al. (2005). Each experimental unit consisted of a glass Petri dish (5.5 cm diameter × 1.2 cm) containing 50 μL of pure

Table 1 Chemical composition and physicochemical variables of the main compounds found in the essential oils studied

Compound	*Sa*L	*Sa*F	*Ap*	*Ac*	*Tv*	Boiling point (°C at 760 mmHg)	Density (g/cm^3)
α-Pinene	x	x				157.9	0.844
Camphene	x	x				158.6	0.842
Myrcene	x	x				167.2	0.794
α-Phellandrene	x	x				171.5	0.846
Limonene	x	x	x	x		175.4	0.841
Carvone			x			230.5	0.969
Citronellal				x		208.4	0.859
α-Curcumeno				x		276.3	0.944
P-cymene					x	177.8	0.857
Thymol					x	233.1	0.968
Carvacrol					x	237.2	0.977
Cariophyllene oxide					x	279.7	1.00

essential oil on a micro coverglass on the bottom and covered using a lid with a fine voile sieve attached over the central hole (4.5 cm diameter).

In adulticidal bioassays, batches of 10 insects were placed over the sieve in order to prevent the direct contact with the test compounds. Each unit was then covered with another Petri dish. Controls were performed without addition of any substance. All treatments were replicated three times. The percentage of knockdown was evaluated every 5 min for 1 h in order to calculate the median knockdown time (KT_{50}). The criterion for knockdown was when an insect remained on its back with limited or no leg movements. After 1 h exposure, the insects were removed from the experimental arena and observed for 4 h to detect the insect's recovery.

In ovicidal bioassays, batches of 10 eggs were exposed to EOs vapors for 24 h; then, the eggs were transferred to a clean Petri dish and kept in an incubator and cultured under the conditions previously informed. Mortality data of the treated eggs were recorded 12 days after the hatching of controls. The percentage of inhibition of hatch (PIH) was calculated from the formula: PIH (%)=$[(C-T)/C]\times 100$, where C is control percentage hatch and T is treated percentage hatch (Yang et al. 2005). The criterion for embryo mortality (abortive eggs) was when louse eggs presented closed operculum and nymphs inside (Di Campli et al. 2012).

Contact toxicity bioassay

To evaluate the contact toxicity of the EOs, filter paper disks (Whatman no 1, 5.5 cm diameter) were treated with 100 μl of EOs hexanic solutions or solvent alone (control). For adults the concentrations ranged from 0.21 to 0.84 mg/cm^2 and for eggs, from 0.013 to 0.21 mg/cm^2. After solvent evaporation, the filter papers were placed on the bottom of a Petri dish. Batches of 10 adults or 10 eggs were added and covered with a lid. All treatments were replicated three times.

In adulticidal bioassays, the percentage of knockdown was evaluated every 5 min for 1 h to calculate KT_{50}. In ovicidal bioassays, the eggs were exposure during 24 h and the PIH was calculated as previously reported.

Acute dermal irritation/corrosion

The evaluation of the irritation and corrosive effects of EOs of *S. areira* (leaves and fruits), *T. vulgaris*, *A. citriodora*, and *A. polystachya* were performed according to OECD Guideline No 404 (OECD 2002). The experimental protocol was approved by the Institutional Animal Care and Use Committee from the Department of Biology, Biochemistry and Pharmacy (UNS) (Protocol No 013/2014, CICUAE-BByF-UNS).

Healthy young adult New Zealand rabbits were provided by the Bioterio from the Department of Biology, Biochemistry and Pharmacy (UNS), and maintained in such facilities under constant conditions of temperature (20±3 °C) and relative humidity (60–70 %), in a 12 h light:12 h dark cycle (lights on at 6:00 hours). Animals had free access to tap water and a standard diet for rabbits (Ganave®, Alimentos Pilar S.A., Argentina) throughout the experiment.

According to the protocol, three rabbits were designed to evaluate each EO: one for the initial test and the other two to confirm the results, 24 h before EO exposure, fur from the dorsal area of the trunk of the animals were carefully removed using an electric shaver (Gama, GM560). In the initial test, three test patches of 6 cm^2 of gauze impregnated with 0.5 ml of undiluted EO were applied sequentially in different sites over the shaved skin of one rabbit. Gauze patches were covered with a non-irritant adhesive film (Tegaderm film, 3M) and were held in place with hypoallergenic tape. Untreated areas of the shaved skin served as control. Each patch was applied and removed sequentially after 3 min, 1 h, and 3 h. After removal, residual EO was carefully cleaned from the exposed area and any sign of local skin reaction was recorded. The presence or absence and the degree of severity of the irritant effects, as erythema or edema, were determined according to a rating scale showed in Table 2. Another two rabbits were exposed to a treated patch as was previously described for a period of 4 h to confirm the responses observed in the initial test. To determine the reversibility of effects or the late appearance thereof, the animals were observed daily up to 14 days after the removal of the patches.

Table 2 Scoring system to describe the formation of erythema and/or edema after dermal exposure to essential oils (OECD 2002)

Scoring	Formation of erythema	Scoring	Formation of edema
0	No erythema	0	No edema
1	Very slight (barely perceptible)	1	Very slight (barely perceptible)
2	Well-defined	2	Slight (edges of area well defined by definite raising)
3	Moderate to severe	3	Moderate elevation (raised approximately 1 mm)
4	Severe, with eschar formation	4	Severe (raised more than 1 mm and extending beyond area of exposure)

Once the assessment on the first rabbit was conducted, confirmatory tests with the second and third animals were done, which consisted of applying a single patch for 4 h. A control patch free of any substance was used in each animal.

Statistical analysis

The KT_{50} were used to compare the toxic effects of the EOs on adults exposed in fumigant and contact toxicity assays. KT_{50} and KT_{99} values were calculated with their respective 95 % confidence interval (CI) using SPSS 15.0 statistical software and were considered significant if 95 % CI values did not overlap. No knockdown effect was found in the controls.

The PIH were used to compare the toxic effects of the EOs on eggs exposed in fumigant and contact toxicity assays. Data from PIH were analyzed by ANOVA and Tukey's HSD.

A simple linear regression analysis was performed to examine relations among KT_{50} values and PIH obtained from fumigant and contact bioassays. For contact, the values used were those obtained from 0.21 mg/cm^2, a common concentration for adults and eggs.

Results and discussion

Efforts have recently been focused on the use of plant-based compounds for the protection of human health as possible alternatives to synthetic insecticides due to their low mammalian toxicity and low persistence in the environment (Khater 2012). Phytochemicals from *Azadirachta indica*, *Artemisia annua*, *Curcuma longa*, *Eucalyptus* sp., *Lawsonia inermis*, *Melia azedarach*, *Syzygium aromaticum*, and essential oils from bergamot and the tea tree have been taken into account for their activity against human head lice and their nits (Carpinella et al. 2007; Soonwera and Wangsapha 2007; Soonwera et al. 2009; Toloza et al. 2010; Bagavan et al. 2011; Abdel-Ghaffar et al. 2012; Campli et al. 2012; Greive and Barnes 2012).

Table 3 KT50 values from fumigant activity of essential oils against adults of *P. humanus capitis*

Essential oil[a]	KT_{50} (min)[b]	95% CI[c]	Slope±SE
S. areira (fruit)	10.80 A	8.43–13.01	2.75±0.48
S. areira (leaf)	12.75 A	6.44–15.51	6.34±0.68
T. vulgaris	18.25 B	15.87–21.72	10.98±0.92
A. polystachya	20.64 B	16.11–24.34	6.32±0.50
A. citriodora	38.35 C	29.97–61.05	1.84±0.38

[a] Essential oils are ordered by decreasing effectiveness

[b] values followed by the different letters within the same column are significantly different

[c] lower and upper limits of the 95 % confidence interval

In previous works, we demonstrated the biological activity of the EOs from *S. areira*, *T. vulgaris*, *A. polystachya*, and *A. citriodora* against insect pests of both agricultural and medical importance (Benzi et al. 2008; Benzi et al. 2009; Benzi et al. 2014; Werdin et al. 2008; Werdin González et al. 2010, 2011a, b).

Fumigant activity

All EOs produced toxicity by fumigant activity in adults of *P. humanus capitis*. The KT_{50} values ranged from 10.8 to 38.35 min (Table 3). On the basis of these values, the toxicity order of EOs was *S. areira* (fruits)=*S. areira* (leaves)> *T. vulgaris*=*A. polystachya*>*A. citriodora*.

The most effective EOs were from *S. areira* fruits and leaves and no differences were found between them; this could be due to the chemical composition of both EOs which shared the main compounds (Table 1). Toloza et al. (2006) evaluated the adulticidal effects of *S. areira* leaves from Cordoba city and obtained KT_{50} values three times higher compared with our study. The variability of the results could be due to the methodology used and the origin of plant material because plants of the same species obtained from different geographical areas may have different chemical compositions (Angioni et al. 2006; Koul et al. 2008; Mondal and Khalequzzaman 2010).

The toxic effect of a substance depends on different toxicokinetic steps, but also on its physicochemical properties. In the case of volatile substances entering through the respiratory system, their toxic effect is strongly associated with their volatility rate which can be estimated by the boiling point. EO components with a low boiling point can volatilize easily and are generally more toxic than those with a high boiling point (Phillips 2009). Indeed, the *S. areira* EO compound had the lowest boiling point values (<172 °C) (except limonene which is a common constituent of all EOs), while those from the other EOs had the highest values (ranged from 175 to 279 °C).

Few studies have evaluated the ovicidal activity on head lice (Rossini et al. 2008). In our work, all EOs were effective against medium development eggs. After 24 h of exposure,

Table 4 PIH values from fumigant activity of the essential oils against eggs of *P. humanus capitis* exposed for 24 h

Essential oil	Hatchability (%)	PIH[a]	
S. areira (fruits)	0	100	A
S. areira (leaves)	0	100	A
T. vulgaris	0	100	A
A. polystachya	10.00	90.00±3.3	B
A. citriodora	23.33	77.00±3.3	C
Control	100	0	D

[a] values followed by the same letters within the same column are not significantly different

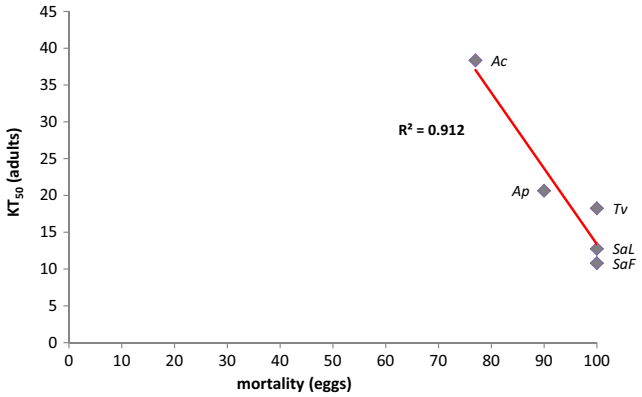

Fig. 1 Simple regression of essential oils fumigant toxicity on adults (KT_{50}) and on eggs (percentage mortality) of *P. humanus capitis*. *SaL S. areira* leaves, *SaF S. areira* fruits, *Tv T. vulgaris*, *Ap A. polystachya*, *Ac A. citriodora*

both EOs from *S. areira* and the EO from *T. vulgaris* exhibited potent ovicidal activity with a PIH of 100 % (Table 4). With the EOs from *A. polystachya* and *A. citriodora*, egg hatch was inhibited to 90 and 77 %, respectively, and significant differences were found between them.

Egg gas exchange between the atmosphere and the embryo depends on the diffusion through the operculum micropyles; thus, EO components can reach embryos through these structures. Previous studies reported on the fumigant effectiveness of some of the components present in our EOs against *P. humanus capitis* eggs (Lahlou and Berrada 2003; Yang et al. 2004).

Regression analysis revealed a significant correlation between estimated KT_{50} values (adults) and PIH (eggs). The resulting model was KT_{50} (adults)=−1.029 PIH+116.2; r^2= 0.91; F=31.38; df=1.4; P<0.05 (Fig. 1). Thus, all EOs act similarly on adults and medium development eggs. It is known that at this point in the embryogenesis, organogenesis has led to an advanced stage and sites of action for EOs might be available to exercise their toxic effect (Gillot 2005; Mougabure Cueto et al. 2006).

Contact activity

In adults, KT_{50} values varied according to EOs and their concentration. At the lowest concentration, the most effective EO was *T. vulgaris* followed by *A. polystachya* and *A. citriodora*; significant differences were found between these oils (P<0.05) (Table 5). At the highest concentration, the EOs from *S. areira* did not produce knockdown effects, so the KT_{50} value could not be calculated.

The contact toxicity depends on several factors which include the lipophilicity of the products and the rate of diffusion through the cuticle (Rossini et al. 2008; Tarelli et al. 2009). Some of the physicochemical variables that may affect the penetration rate are the density and the molecular structure of the EO components. It is known that those with a higher density usually penetrate more easily (Phillips 2009).

EOs from *T. vulgaris*, *A. polystachya*, and *A. citriodora* were the most toxic by contact. These EOs present the components with the higher density values (>0.855 g/cm^3).

Table 5 KT50 values from contact activity of essential oils against adults of *P. humanus capitis*

Essential oil	Concentration (mg/cm^2)	KT_{50} (min)[a]		95% CI[b]	Slope±SE
T. vulgaris	0.84	3.93	a A	1.07–5.65	2.76±0.89
	0.63	6.30	a A	4.94–7.23	3.69±0.65
	0.42	6.49	a A	5.32–7.79	5.87±1.10
	0.21	9.90	b A	8.89–10.99	10.78±2.29
A. polystachya	0.84	5.96	a A	5.24–6.79	8.09±1.63
	0.63	7.18	a A	5.44–8.65	3.44±0.57
	0.42	8.14	a A	6.60–9.54	5.56±1.07
	0.21	12.93	b B	11.03–14.51	5.22±0.81
A. citriodora	0.84	5.13	a A	3.37–6.40	3.53±0.79
	0.63	11.37	b B	8.72–13.26	5.34±1.28
	0.42	16.23	c B	13.78–18.16	6.94±1.48
	0.21	18.01	c C	14.57–20.05	3.29±0.44
S. areira (leaves)	0.84	Not calculated			
S. areira (fruits)	0.84	Not calculated			

[a] values followed by the different uppercase letters indicate significance differences between the EOs at the same concentration; values followed by the different lowercase letters indicate significance differences between concentration of the same EO

[b] lower and upper limits of the 95 % confidence interval

Table 6 PIH values from contact activity of the essential oils against eggs of *P. humanus capitis* exposed for 24 h

Essential oil	Concentration (mg/cm^2)	Hatchability (%)	PIH[a]	
T. vulgaris	0.21	0	100	a A
	0.105	3.70	93.30±3.3	a A
	0.052	43.40	56.60±3.3	b A
	0.026	66.67	33.33±8.1	c A
	0.013	80.00	20.00±0	c A
A. polystachya	0.21	0	100	a A
	0.105	10.00	90.00±0	a A
	0.052	50.00	50.00±5.7	b A
	0.026	73.33	26.66±3.3	c A
	0.013	80.00	20.00±3.3	c A
A. citriodora	0.21	70.00	30.00±3.3	a B
	0.105	80.00	20.00±3.3	b B
	0.052	100	0	c B
	0.026	100	0	c B
	0.013	100	0	c B
S. areira (leaves)	0.21	100	0	C
S. areira (fruits)	0.21	100	0	C
Control (hexano)		100	0	C

[a] values followed by the different uppercase letters indicate significance differences between the EOs at the same concentration; values followed by the different lowercase letters indicate significance differences between concentration of the same EO.

Moreover, the major components of the *S. areira* EO present low density values (<0.855 g/cm^3) and since their boiling points are markedly lower than for other EOs, these compounds could evaporate quickly, so they would have less time in contact with the insect, causing a lower toxic effect as that shown in this work.

Regarding the molecular structure, the aromatic/cyclic compounds such as thymol and carvone (present in *T. vulgaris* and *A. polystachya*) have a low rate of degradation resulting in organisms that are more toxic than linear or acyclic compounds (Phillips 2009; Abdelgaleil et al. 2009).

The ovicidal activity by contact exposure, as measured by PIH, was concentration-dependent (Table 6). At all concentrations, *T. vulgaris* and *A. polystachya* EOs that were the most effective, followed by *A. citriodora* ($P<0.05$). At the highest concentration, the EOs from *S. areira* did not produce ovicidal effects.

Werdin González et al. (2010) demonstrated that EOs from *T. vulgaris* and *A. polystachya* produce the highest toxicity effect on medium development eggs of *Nezara viridula* while the EO from the leaves and fruits of *S. areira* present the lowest activity. Other authors reported toxicity of *T. vulgaris* against eggs of *Acanthoscelides obtectus* (Regnault-Roger and Hamraoui 1994).

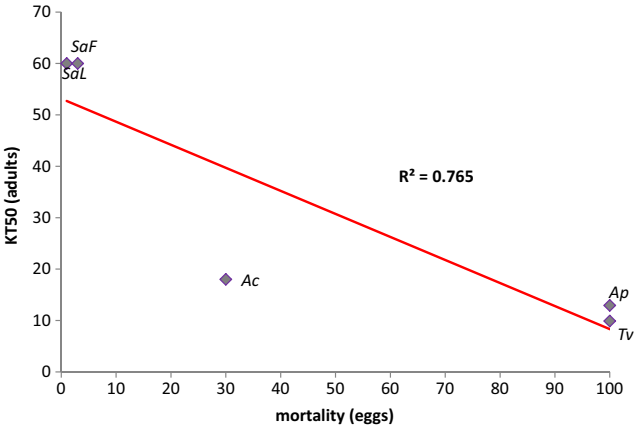

Fig. 2 Simple regression of essential oils contact toxicity on adults (KT_{50}) and on eggs (percentage mortality) of *P. humanus capitis* at 0.21 mg/cm^2. *Sa*L *S. areira* leaves, *Sa*F *S. areira* fruits, *Tv T. vulgaris*, *Ap A. polystachya*, *Ac A. citriodora*

Oxygenation levels are other physicochemical properties that can influence both adulticidal and ovicidal activity. The major compounds present on the EOs of *T. vulgaris*, *A. polystachya*, and *A. citriodora*, such as thymol, carvone, and citronellal, are mono-oxygenated compounds whose structures have a simple alcohol, ketone, or phenol as a functional group proved to be the most active compounds against adults and eggs of *P. humanus capitis*. (Priestley et al. 2006).

Regression analysis revealed a significant correlation between estimated KT_{50} values (adults) and PIH (eggs) at 0.21 mg/cm^2. The resulting model was KT_{50} (adults)= −0.448 PIH+53.14; $r^2=0.77$; $F=10.36$; $df=1.4$; $P<0.05$ (Fig. 2). As in fumigant studies, all EOs act similarly on adults and medium development eggs.

Pediculicide effects of EOs and exposure route

Based on the results previously obtained, we proceeded to compare the effectiveness of EOs by discriminating the route of exposure; therefore, *P. humanus capitis* eggs were exposed to EOs during the KT_{99} value obtained from adults.

For fumigant toxicity, the most effective EOs were *S. areira* fruits and leaves; so eggs were exposed in an

Table 7 Comparative analysis of the pediculicide effects of the essential oils and exposure route

Exposure route	Essential oils	Exposure time (min)	PIH[a]
Fumigant	*S. areira* (leaves)	30	33.3±5.1 A
	S. areira (fruits)	35	26.6±3.3 A
Contact	*T. vulgaris*	21	93.3±3.3 B

[a] values followed by the different letters within the same column are significantly different

Table 8 Effects observed in rabbit skin exposed to essential oils

Essential oils	Rabbit	Exposure Time					
		3 min		1 h		4 h	
		Erythema	Edema	Erythema	Edema	Erythema	Edema
S. areira (leaves)	1	0[a]	0	0	0	0	0
	2	–	–	–	–	0	0
	3	–	–	–	–	0	0
S. areira (fruits)	1	0	0	0	0	0	0
	2	–	–	–	–	0	0
	3	–	–	–	–	0	0
T. vulgaris	1	0	0	0	0	0	0
	2	–	–	–	–	0	0
	3	–	–	–	–	0	0
A. polystachya	1	0	0	0	0	0	0
	2	–	–	–	–	0	0
	3	–	–	–	–	0	0
A. citriodora	1	0	0	0	0	0	0
	2	–	–	–	–	0	0
	3	–	–	–	–	0	0

[a] values determined according to the rating scale showed in Table 2

enclosed chamber (according 2.3.1) to the EO of *S. areira* leaves for 30 min, and 35 min to the EO of *S. areira* fruits. The PIH obtained was 33 and 26 %, respectively. These values were significantly lower than those obtained when eggs were exposed for 24 h (PIH=100 %) ($P<0.05$) (Table 7).

For contact toxicity, the most effective EO at 0.21 mg/cm^2 was *T. vulgaris* so eggs were exposed in a direct contact assay (according 2.3.2) for 21 min. The PIH obtained was 93 % and no differences were observed with PIH for 24 h (PIH=100 %) ($P>0.05$). Moreover, the PIH obtained with *T. vulgaris* during KT_{99} was significantly higher than *S. areira* fruits and leaves ($P<0.05$).

Finally, it can be concluded that treatment by contact with the EO from *T. vulgaris* for 21 min would produce a high adulticidal effect and 92 % mortality in eggs of *P. humanus capitis*.

Acute dermal irritation/corrosion

During the evaluation of acute dermal irritation/corrosion, none of the EOs produced effects on the skin of the albino rabbits studied (Table 8).

Taking into account that the EOs could be used as a pediculicide product, it is important to evaluate the adverse effects that these might cause on the human skin. The results obtained in this study demonstrated that none of the EOs tested caused irritating or corrosive effects on rabbit skin. Whereas the applied doses were larger than those that could be used in pediculicide products, it can be concluded that the use of these EOs on the skin of humans does not involve health risks.

Conclusion

According to the results, the essential oil of *T. vulgaris* could become a valuable tool for use in the management of *P. humanus capitis* at low concentration and applied for 21 min. It can produce an effect of appreciable tumbling in adults (followed by mortality) and toxicity in eggs. On the other hand, this EO did not produce irritation and/or skin corrosion.

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Compliance with ethical standards The evaluation of the irritation and corrosive effects of EOs of *S. areira* (leaves and fruits), *T. vulgaris*, *A. citriodora*, and *A. polystachya* were performed according to OECD Guideline No 404 (OECD 2002). The experimental protocol was approved by the Institutional Animal Care and Use Committee from the Department of Biology, Biochemistry and Pharmacy (UNS) (Protocol No 013/2014, CICUAE-BByF-UNS).

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