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Short Communication

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Pediculicidal Activity of Hydroethanolic Extracts of *Ruta graveolens, Melia azedarach* and *Sambucus australis*

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SUMMARY. **Pediculosis is a worldwide head infestation caused by *Pediculus humanus*. The treatment for this infestation is use of topical insecticides and there are studies which show that the insect may be resistant to chemical active ingredients. The insecticidal properties of *Sambucus australis, Melia azedarach* and *Ruta graveolens* are not known if they have pediculicidal properties as well. The aim of this study is to evaluate the insecticide activity of treatments using extracts of *S. australis, M. azedarach* and *R. graveolens* against the pediculosis. The results showed that lice are sensitive to the treatments used, and *M. azedarach* extract presented the most efficient results.**

INTRODUCTION

Pediculosis is a head infestation caused by the hematophagous lice *Pediculus humanus* capitis (Phthiraptera: Pediculidae). This infestation is common all over the world, especially amongst school children, and it is considered a public health problem [1,2]. Head infestation caused by lice is fast and it is associated to prurits, bite reactions and excoriations [3]. The basic treatment for head lice is manual removal and repeated use of topical insecticides, such as pyrethrins, permethrin and lindane [4]. Besides the side effects, the repeated use of these insecticides has also caused the development of resistance to them [5-7].

In Brazil, the deprived population usually makes use of homemade treatments based on herbs such as: *Ruta graveolens* L. (Rutaceae), *Melia azeradach* L. (Meliaceae) and *Sambucus australis* Cham. & Schltdl. (Caprifoliaceae). The insecticide activities of these plants have already been described in specialized literature; however, there is no evidence of their pediculicidal activities. The insecticide property of these plants can be an interesting option for fighting and eliminating head lice. It is estimated that millions of people are infested each year and this fact shows the importance of an alternative and efficient control over the disease. For this reason, we decided to analyze and compare the insecticidal activities of the species *S.australis, M. azedarach* and *R. graveolens* against the parasite *Pediculus humanus capitis.*

MATERIALS AND METHODS

Plant material

The leaves of *S. australis, M. azedarach* and *R. graveolens* were collected in February, 2008 in the medicinal garden at UNIOESTE in Cascavel, Paraná State, Brazil. The plants were identified by the biologist Dr. Norma Catarina Bueno.

Extraction

The extracts were obtained by maceration, as 300 g of fresh chopped leaves were put in 1 L of hydroethanolic solution 50%. After 24 h, the mixture was filtered and tested.

Characterization of extracts

The plants *S. australis, M. azedarach* and *R. graveolens* have been well investigated chemically and phytochemical studies have reported

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that they have many important secondary metabolites. The pediculicidal treatment used in this study is hidroethanolic extract made up of a complex blend of vegetable substances. Then, pharmacognostical tests were used to investigate some classes of secondary metabolites present in the extracts. The pharmacognostical tests results obtained were analysed through chemical reaction with color alterations. The Shinoda's reaction or Cyanidin reaction [8] was employed in the analysis of flavonoids. The presence of alkaloids in the extracts was indicated by the Dragendorff's reagent and Mayer's reagent. The Liebermann-Burchard'reaction [9] was used in order to investigate the presence of steroids and the tests for tannins were made employing chemical reaction with iron salts [10].

Insect

Head lice were collected from the heads of infested 5 to 10 year-old children who attend nurseries and day care public institutions in Cascavel, Brazil. The head lice were collected by combing the children's scalps and were then pooled in Petri dishes. The in vitro tests were immediately started and all head lice used in bioassay were protected from sunlight and heat.

The method used to assess the pediculicidal activity was adapted from a World Health Organization [11,12]. The bioassay was carried out with hydroetanolic extracts of the three plants separately. For each analysis, one group of ten Petri dishes lined with cotton-wool was used. For dishes one to eight, the cotton-wool was soaked with one of the vegetal extracts and the remaining two dishes had its cotton-wool soaked in water (control group). Groups of 5 head lice each (replications) were transferred and released into each dish, and were observed through a magnifying glass for enough time to see one of the extracts eliminate the head lice 100%.

Statistical analysis

Kruskal-Wallis test was used to verify the presence of differences between the average times whereas the Student-Newman-Keuls comparison test was used to locate differences between the average times. The hypothesis: the absence of difference in treatment time (H_0) and the presence of differences in treatment time (H_1) showed that there are differences over the treatment time in a level of significance of 5%.

RESULTS AND DISCUSSION

The results of pharmacognostical tests to S.australis, M.azedarach and R.graveolens are consigned in Table 1.

Shinoda's reaction was positive for all of the three extracts. In fact, flavonoids are bioactive constituents of *R. graveolens* [13], *M. azeradach* [14] and *S. australis* [13,15]. The Dragendorff's reagent and Mayer's reagent indicated the presence of alkaloids for all the three extracts. Phytochemical studies have reported alkaloids in the plants *R. graveolens* and *S. australis* [13].

Finally, the Liebermann-Burchard's reaction used to investigate the presence of steroids was positive for all of the three extracts and the tannis presented a negative result only for the extract of the plant *R. graveolens*.

Table 2 shows accumulated percentage of head lice killed by the treatment using the hydroetanolic extracts of *S. australis, M. azedarach* and *R. graveolens* for nine minutes. The control groups are not shown because none of the insects were killed during the observation period. The maximum observation time was set at 9 minutes, because that is the period that the hydroetanolic extract of *M. azedarach* needed to eliminate 100% of the lice.

Figure 1 uses the data shown on Table 1 to *demonstrate* the rising numbers of pediculicidal activity.

The results of the Kruskal-Wallis test for treatment with *R. graveolens* ($p = 0.0216 < 0,05$); *M. azedarach* ($p = 0,005 < 0,05$) and *S. australis* ($p = 0.0002 < 0,05$) have shown that there is a time difference between the action time using hydroalcoholic extracts.

The results of the Student-Newman-Keuls test have shown rising numbers over the three different treatments. *M. azedarash* presented considerable difference in performance between

Hydroalcoholic extracts	Alcaloids		Flavonoids	Tannis	Esteroids	Saponines
	Dragendorff	Mayer				
R. graveolens	+	+	+	-	+	-
M. azedarach	+	+	+	+	+	+
S. australis	+	+	+	+	+	+

Table 1. Results of pharmacognostical tests. + = present, - = absent.

Extracts	Time (min)															
	2		3		4		5		6		7		8		9	
	Fi%	SD	Fi%	SD	Fi%	SD	Fi%	SD	Fi%	SD	Fi%	SD	Fi%	SD	Fi%	SD
R. graveolens	22.5	0.8345	37.5	0.71	52.5	1.165	72.5	0.5345	72.5	0.5345	85	0.744	90	0.4629	38	0.4629
M. azedarach	45	1.7525	62.5	1.46	82.5	1.069	85	0.3536	90	0.4629	97.5	0.488	97.5	0	100	0.3536
S. australis	10	1.4142	10	1.41	10	1.4142	10	1.4142	57.5	1.0607	85	0.9161	87.5	0.4629	95	1.165

Table 2. Accumulated percentage of dead head lice Fi% accumulated frequency, SD standard deviation.

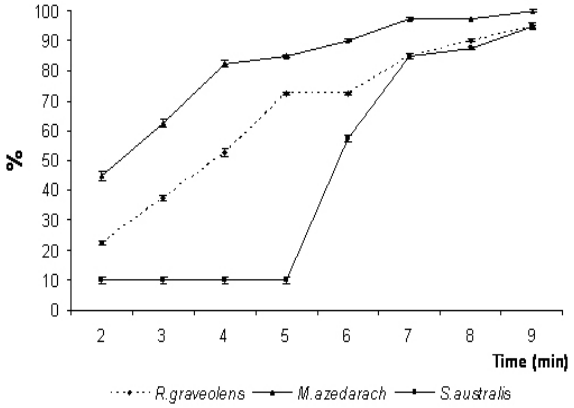

Figure 1. Accumulated frequency of dead lice.

the second and the fifth minute and the fifth and the ninth minute, whereas, *R. graveolens* presented marked differences between min 2 and 4, 5 and 7, and 8 and 9. *S. australis* had the most constant performance, which was low, up to the fifth minute, when suddenly the numbers rose to approximately 80% of mortality.

The hydroalcoholic extracts of *R. graveolens* and *S. australis* were unable to eliminate 100% of head lice in 9 min. The extract of *M. azedarach* proved more efficient than the others, eliminating 90% of the parasites in just 6 min and 100% in 9 min.

CONCLUSION

The results achieved show that head lice are sensitive to different treatment times with the hydroalcoholic extracts of fresh chopped leaves of *R. graveolens, S. australis* and *M. azedarach*.

The mortality of head lice when exposed to *S. australis* was not immediate, but was constant until 6 min, when the mortality increased markedly. The treatment with *R. graveolens* showed a less considerable level of mortality if compared to other treatments and *M. azedarach* had the best results as it was able to eliminate 100% of the parasites in 9 min.

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REFERENCES

1. Burgess, I.F. (2004) *Annu. Ver. Entomol.* **49**: 457-81.
2. Calderón-Arguedas, O., M.E. Solano & C. Sanchez (2003) *Parasitol. Latinoam.* **58**: 177-80.
3. James, S. (2003) A review of the regulation of head lice treatments in Australia. Department of Health and Ageing Therapeutic Goods Administration. Australian Government. Medicines Evaluation Committee by Dr Susan James.
4. Roberts, R.J. (2002) *N. Engl. J. Med.* **346**: 1645-50.
5. Gratz, N.G. (1997) *Human lice: their prevalence, control and resistance to insecticides*. Document WHO/CTD/WHOPES, World Health Organization, Geneva.
6. Jadhav, V., A. Kore & V.J. Kadam (2007) *Fitoterapia* **78**: 470-3.
7. Priestley, C.M., , I.F. Burgess & E.M. Willianson (2006) *Fitoterapia* **77**: 303-9.
8. Henning, S. (1967) *Deutsche Apotheker Zeitung. Berlin* **107**: 1538-40.
9. Nath, M.C., M.K. Chakravorty & S.R. Chawdhury (1946) *Nature* **157**: 103-4.
10. Michelin, D.C., S.M. Iha, D. Rinaldo, M Sannomiya, L.C. Santos, W. Villegas & H.R.N. Salgado (2005) *Rev. Bras. Farmacog.* **15**: 209-11.
11. Cestari I.M., S.J. Sarti, C.M. Waib & A.C. Branco (2004) *Neotrop. Entomol.* **33**: 805-7.
12. World Health Organization (1981) *Instructions for determining the susceptibility or resistence of body lice and head lice to insecticides,* WHO /VBC, Geneva.
13. Ivanova, A., B. Mikhova, I. Najdenshi & I. Tsvetkova (2005) *Fitoterapia* **76**: 344-7.
14. Carpinella M.C, , M.T. Defago, G. Valladares & S.M. Palácios (2006) *Adv. Phitomed.* **3**: 81-123.
15. Clemes, S.M., A.L.B. Zeni & M. Kretzschmar (2008) *Rev. Bras. Farm.* **89**: 10-2.