



Journal of Medical Sciences

ISSN 1682-4474

science
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Research Article

Mortality Effects of *Lepidium latifolium* Alcoholic Extract Against *Pediculus humanus*

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Abstract

Background and Objective: *Pediculus humanus capitis* is a public health problem that is seen in many countries. The treatment of this ectoparasite is based on synthetic chemical insecticides that most which are harmful and toxic to human health. So, finding them safer and more effective alternatives is needed urgently. In the present study, the pediculicidal activity of methanolic extract of *L. latifolium* was evaluated for head lice treatment. **Materials and Methods:** The leaves of the plant *L. latifolium* were isolated and dried under shade. The alcoholic extract was prepared and concentrations of 0.12, 0.25, 0.50 and 0.75 mL/cm² from herbal extract were examined under a World Health Organization (WHO) bioassay protocol. After the collection of head lice, *in vitro* tests were started within 30 min. **Results:** The lice mortalities were evaluated after 5, 15, 30, 45 and 60 min of exposure. After 30 min of exposure to 0.75 mL/cm² concentration, all lice were dead. Permethrin 1% and the solvent that has been used for extraction were used as the positive and negative control, respectively. **Conclusion:** Head lice must be controlled as a serious disease at an early stage. The present study showed that *Lepidium latifolium* can well kill the parasite. Our data suggested that this herbal extract formulation of a native plant in Iran is suitable to be used as pediculicides.

Key words: Pediculosis, *Lepidium latifolium*, methanolic extract, ectoparasite, protocol, mortalities, pediculicides

Citation: Jafari, A., S.M. Razavi, H. Khazraei, A. Babaei and V.R. Ostovan, 2022. Mortality Effects of *Lepidium latifolium* alcoholic extract against *Pediculus humanus*. J. Med. Sci., 22: 99-102.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pediculus humanus capitis (Phthiraptera: Pediculidae), is a kind of ectoparasitic insect commonly known as the head louse that lives at the scalp and neck area¹. Pediculosis is an important public health problem especially in children². It is generally spread through head-to-head contact with an infected human directly or by sharing personal hairbrushes, hats and clothing indirectly. Itching, scars, allergic reaction, inflammation of the scalp and neck, sleep loss as well as secondary bacterial infection are the most manifestations of this problem among school children³. The distribution of pediculosis is more in temperate areas and epidemic results in children are about 2-10% of developed countries in rural and urban areas⁴. Head lice are growing up in Iran because of increasing population, migration to big cities, suburb areas around the cities without hygiene.

Organochlorine (lindane), organophosphate (malathion), carbamate (carbaryl) and pyrethroid insecticides (permethrin and α -phenothrin) are the most common treatment of head lice⁵⁻⁷. In the health system of Iran, permethrin and gamma benzene hexachloride are used for head lice in schools by different formulations. Physical approaches are using a clean comb through hair, picking up the lice and shaving hair⁸. The most effective treatment is shampoo form of insecticide, combing hair and education to the improvement of hygiene⁹. Pediculicides might not kill eggs completely and some of them grow after incubation time and cause an infestation. Unfortunately, many synthetic chemical insecticides failed to treat head lice and resistance of head lice against them have been shown in several countries such as England, Australia and Argentina⁸. Synthetic insecticides are very harmful and toxic for children immune systems and their underdeveloped detoxification mechanisms¹⁰. Malathion disrupts the immune system carbaryl is a human carcinogen and permethrin and phenothrin showed corneal damage after treatment⁵. Moreover, pediculicidal agents currently approved for the treatment of head lice are permethrin, malathions, pyrethrins and lindane that may cause allergic irritants, respiratory depression, dermatitis and seizures⁵. So, looking for safer and more effective alternatives are needed urgently. Particularly, the alternative products are plant-based products like traditionally oil, grease, kerosene and vinegar.

However, there are some plant-based compounds and essential oils from *Eucalyptus* sp., *Cinnamomum zeylanicum*, *Curcuma longa* (Turmeric), *Melia azedarach* (Chinaberry), *Acacia concinna*, *Melaleuca alternifolia*, *Artemisia annua*, *Rosmarinus officinalis*, *Lawsonia inermis* (Henna), *Averrhoa bilimbi*, *Citrus paradisi*, *Tamarindus indica*, *Azadirachta indica*,

Plectranthus amboinicus, *Clitoria ternatea*, *Syzygium aromaticum* and *Myristica fragrans* (Nutmeg) that have been used against head lice and their nits⁸.

Lepidium latifolium was known as a perennial weed for many years that had rapid and substantial vegetative growth⁴. The acaricidal effect of this herb was shown in 2015 and thymol is one of its effective ingredients for the treatment of arthropods¹¹. Its creeping rhizomes with high storage capacity and deep rhizome penetration are the most important characterization of it. In the present study, the pediculicidal activity of methanolic extract of *L. latifolium* was evaluated for head lice treatment.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Parasitology, Shiraz University, Iran from March-August, 2019.

Preparation of methanol extract: The leaves of the *L. latifolium* was isolated and dried under shade (the Agriculture Faculty of Shiraz University has identified the species of plant). After grinding the leaves, 100 g of milled leaves (using a commercial electrical blender) and 400 mL methanol were mixed and left at room temperature for 24 hrs. After filtering the mixture and separating coarser particles, the alcohol was separated in vacuum conditions and finally, the obtained dry powder was placed into sterile containers and stored at -70°C for further use¹².

In vitro study: *In vitro* tests were done within 30 min after the collection of head lice. The method of filter paper contact bioassay was used to evaluate the toxicity¹³. For *in vitro* study, concentrations of 0.12, 0.25, 0.50 and 0.75 mL/cm² from herbal extract were provided. Pieces of filter papers were soaked in this solution and put on separate plates and then 10 head lice were placed on the filter paper. According to time, the mortalities of them were recorded at 5, 15, 30, 45 and 60 min after the exposure. The test was repeated 3 times for each concentration. Permethrin 1% (Gilaranco, Iran) and the solvent that has been used for extraction were used as the positive and negative control, respectively.

RESULTS

The results are illustrated in Table 1. In this study, concentrations of 0.12, 0.25, 0.50 and 0.75 mL/cm² from herbal extract were evaluated and compared with permethrin 1% and the solvent that has been used for extraction as the positive and negative control, respectively. The mortalities of

Table 1: *In vitro* effects of alcoholic extract of *L. latifolium* on head lice, the test was repeated 3 times for each concentration

	Dose (mL/cm ²)	Mean dead number (%) after treatment N = 20				
		5 min	15 min	30 min	45 min	60 min
Alcoholic extract of <i>Lepidium latifolium</i>	0.12	26.67	36.67	48.33	58.33	65.0
	0.25	35.0	41.67	55.0	70.0	83.33
	0.50	46.67	53.33	66.67	88.33	100.0
	0.75	81.67	91.67	100.0	100.0	100.0
Negative control (the solvent that has been used for extraction)		0.0	0.0	1.67	3.33	3.33
Positive control (Permethrin 1%)		58.33	81.67	98.33	100.0	100.0
p-value*		<0.001	<0.001	<0.001	<0.001	<0.001

**Post-hoc* test revealed that just 0.12 and 0.25 mL/cm² in each time was not significantly different and other doses and controls were significantly different from each other

head lice were recorded at different times. After 30 min of exposure to 0.75 mL/cm² concentration, all lice were dead.

DISCUSSION

In Iran, pediculosis is a serious problem in primary school children¹⁴. In our study, the potential of pediculicidal activity of alcoholic extract of *L. latifolium* was evaluated in comparison with chemical medicine for the first time. After 30 min of exposure to 0.75 mL/cm² concentration, all lice were dead.

The acaricidal effect of this herb was shown in 2015 and thymol is one of its effective ingredients for the treatment of arthropods¹¹. In addition, *in vitro* pediculicidal activity has been reported for some plant-based pediculicides such as *Averrhoa bilimbi*, *Cananga odorata*, *Citrus limon*, *Cymbopogon nardus*, *Cymbopogon winteratus*, *Clitoria ternatea*, *Piper*, *Tacca chancieri* and *Zingiber cassumuna*^{7,15-17}.

Sari *et al.*¹³ reported frangipani leaves formulation which showed highly pediculicidal activity. They reported that this formulation indicated the anoxia similar to permethrin 1%. The studies of Abdel-Ghaffar and Semmler¹⁰ neem seed extract and grapefruit extract showed a highly pediculicidal activity against all stages of head lice of naturally infected humans in Egypt. In another study, the combination of paranix, coconut, anise and ylang-ylang oil demonstrated 82% head lice mortality, while a product containing soya oil and coconut oil showed 62% head lice mortality^{18,19}.

Finally, it is worth mentioning that head lice must be controlled as a serious disease at an early stage. *L. latifolium* is a weed that has invaded crops such as cereal grains and alfalfa and can contaminate hay shipments. It could be controlled by intensive grazing, flooding and herbicides, but in this study, it is used for the first time as a pediculicidal. The present study showed that *Lepidium latifolium* can well control the disease. Further studies are needed to be carried out for testing higher doses to decrease medication time and several replications.

CONCLUSION

Head lice must be controlled as a serious disease at an early stage. The present study showed that *Lepidium latifolium* can well kill the parasite. Our data suggested that this herbal extract formulation of a native plant in Iran is suitable to be used as pediculicides.

SIGNIFICANCE STATEMENT

The *Lepidium latifolium* was used in this study for the treatment of head lice for the first time. This study will help the researchers to find the suitable effective compound for the treatment of head lice clinically and it is suggested that the effective ingredients of the herbal extract are defined by chemical analysis and formulated as a new product. Future studies should be performed in this regard.

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