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**THE EFFECT OF PYRETHRUM EXTRACT AND NATURAL ESSENTIAL OILS  
ON HEAD LICE: IN VITRO TEST AND CLINICAL EVIDENCE****TAHEREH REZAZADEH<sup>1,6</sup>, ALI KARIMI BAKHSHANDI<sup>\*2,6</sup>, MARYAM KHODAYAR<sup>3,6</sup>,  
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**ABSTRACT**

Currently, common chemical treatments with synthetic drugs for head lice have many harmful side effects and often lead to the development of resistances. In this context, medicinal plants with pediculicide properties are safe and can be targeted against head lice infestation. Therefore, the objective of this study was to determine pediculicide property of pyrethrum and essential oils as herbal formulation and lindane as chemical formulation against head lice using in vivo and in vitro assay. The results showed that herbal formulation was more effective and safe than lindane to remove head lice and their nits. Furthermore, there was a significant difference between efficacy of herbal and chemical formulation in the first week and second week ( $p \leq 0.01$ ), as herbal formulation resulted in a significant effect on resistant head lice when compared with lindane chemical formulation. The findings of this study indicate that herbal formulation is a promising remedy that can be targeted against head lice infestation and for the remedy of other cutaneous to parasite diseases without having any harmful side effects.

**Key words: Chemical formulation, Herbal formulation, Pediculicide effect, Resistance**

## INTRODUCTION

Head lice are obligate ectoparasite of humans that feed generally on human blood (1, 2). Due to bite reaction, they are responsible for a very intense pruritus that may lead to high irritation and even wound infection. Head lice can spread through direct contact (with an infected person's hair) and indirect contact (using personal items of an infected person; such as comb, towel, blanket, pillow and so on (3). Inflammatory reaction and scalp irritation happen due to releasing lice saliva and secretions at the lice biting area (4). It leads to pruritus, urticaria, eczema, conjunctivitis and red and itchy scalp which are main symptoms of pediculosis and making the scalp susceptible to catching secondary bacterial infections such as impetigo and pyoderma (4). Chemical pediculicide compounds have been used extensively to treat head lice. There are different types of neurotoxic synthetic insecticide, but the resistance of head lice against synthetic formulations and their toxic effects on human have been reported [5- 11]. The most common strategy currently available for coping with mentioned problems is using the pediculicide properties potential of plant extracts or essential oils. pediculicide properties have been reported for some essential oils and herbal extracts such as

aniseed, rosemary, sage, cinnamon leaf, thyme red, tea tree oil, *Ageratum conyzoides*, *Andrographis paniculata*, *Arcangelisia flava*, *Butea superba*, *Illicium verum*, *Nigella sativa*, *Azadirachta indica*, *Annona squamosa*, *Rutagra veolens*, *Melia azedarach* and *Sambucusau stralis* [12-16]. Traditionally, pyrethrum (*Chrysanthemum cinerariifolium*) has been used as an insecticide and promising results have been reported for the treatment of head lice and nits [17]. Therefore, the aim of this study was to assess the efficacy of pyrethrum extract and natural essential oils (lavender, citronella and tea tree oil) on head lice as alternative herbal formulations in comparison with lindane chemical formulation.

## MATERIAL AND METHODS

### Plant material and extract preparation

Pyrethrum was gathered from northern mountains of Iran. First, pyrethrum flower and root were converted into powder by mechanical milling and prepared for Soxhlet extraction with chloroform. 500 ml of chloroform was added to 50 g of powder. The obtained solvent extract was evaporated by using rotary Vacuum evaporator. Extract was weighed and percentage was calculated in terms of the air dried weight. Then, 100 mg/ml of stock was prepared for making different dilutions. Essential oil of the

plants was extracted from the above-ground dry biomass by hydro-distillation (250 g DW in 500 ml H<sub>2</sub>O) for 3 hour at 100 °C using a Clevenger type apparatus. The extraction was conducted with 3 replicates per treatment, following the experimental design.

#### **Isolation and culture of human epidermal keratinocytes for the determination of cytotoxicity**

Human neonatal foreskin was used to isolate keratinocyte cells. First, the skin was washed with PBS buffer, and divided into small segments. Next, these segments were placed in Hanks buffer containing 25 u/ml Dispaseenzyme for 12 hours at 4°C. It leads to separate epidermis from the dermis. Then, Trypsin-EDTA (0.25% trypsin, 1 Mm EDTA) were used for the separation of keratinocytes for 15 minutes at 37 °C. Trypsin enzyme was deactivated by using 10 % fetal bovine serum (FBS). Cells were precipitated by centrifuge at 10000 rpm for 5 minutes at 4 °C. Next, keratinocyte serum free medium (K-SFM) was used for the culture of keratinocyte cells. Cells were suspended in K-SFM medium containing 0.1-0.2 ng/ml of epidermal growth factor (EGF) and 25-30 µg/ml of bovine pituitary extract (BPE). Finally, cells were incubated at 37 °C in 5 % CO<sub>2</sub> with 90 % humidity, and allowed to adhere overnight.

#### **Cell Viability and Cytotoxicity Assay**

3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used for the determination of cell viability. 2500 cells were placed in 96 well plates. Then, different concentration of pyrethrum extract (0, 1.5, 2.5, 3.3, 4.3µl/ml), tea tree oil and citronella (0, 10, 20, 30, 40 µl/ml), and lavender oil (0, 1, 1.5, 2, 2.5 µl/ml) were added to cells at 24 hours. 25 µl of MTT solution (5 mg/ml) was added each well. After 4 hours incubation and dissolving of formazan crystals in dimethylsulfoxide (DMSO), the absorbance has been read at the wavelength 570 nm by using a plate reading spectrophotometer. The percentage of cell viability was calculated as the following formula:

$$\text{Cell viability (\%)} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

Generally, cytotoxicity activity of four plant extracts were carried out against keratinocyte cells at different concentrations to determine the IC<sub>50</sub> (50% growth inhibition) by MTT assay.

#### **Herbal Shampoo and Solution Formulations**

To perform the present study, optimum concentration of each extract were added to two formulations: shampoo and solution. Shampoo and solution were used for *in vivo* and *in vitro* assay, respectively (Table 1).

### ***In vivo* efficacy test**

A double-blind, randomized, placebo-controlled, parallel group were used for clinical trial. Infected students have been selected from Shahriar elementary school in Tehran. In order to be assured of head lice, each person's hair was examined by a dermatologist for 10 minutes in the presence of natural light. Then, accuracy and intensity of infestation were confirmed. The number of head lice was calculated by a magnifying glass, and 120 infected students were divided into two equal groups according to the level of contamination (Table 2).

The first group was 60 infested students, and was asked from them to use lindane shampoo 1% and the second group was asked to use herbal shampoo. The training was given to infested students and their family by a researcher. All students were checked 2nd and 3rd days after treatment in the first week, as well as 9th and 10th day after treatment in the second week.

### ***In vitro* assay**

100 head lice and eggs (50 head lice and 50 eggs) were used for their *in vitro* assay of herbal solution, and compared with lindane solution 1 % (Figure 1). 25 of live head lice were separated from infected hair by using fine toothed metal comb, and 5 head lice were put on wet filter paper (Whatmann No. 1; 6 cm diameter) placed

in the bottom of each petri dish. Experiment started within 5 minutes of head lice collection. 100  $\mu$ l of herbal solution was added to each head lice. After 15 minutes, head lice were rinsed with tap water. Next, head lice were then examined under dissecting microscope, and mortalities were recorded every 10 minutes for 4 hours. This method also was repeated for lindane solution.

Minor signs of life and minimal movements were classified as alive and the lice were dead if there were no vital signs at all. Hair shafts with nits attached <1 cm from the scalp were cut and collected. Then, the vitality of eggs was observed by a microscope. Live nits had a uniform ovoid shape. Moreover, the operculum was closed in live eggs. But, dead nits were misshapen and shriveled and had a non-uniform shape. The test was conducted by placing hair shaft containing 5 live eggs on the filter paper (Whatmann No. 1; 6 cm diameter) placed in the bottom of each petri dish. Experiment on nits started within 5 minutes of eggs collection. 100  $\mu$ l of herbal solution was exposed to each egg, and put them in the dark room for 15 days at 32°C and 70 % humidity. The eggs were examined by microscope each day. Each treatment was replicated 3 times.

### **Statistical analysis**

The GLM procedure of SPSS was used to analyze treatments effects.

Difference between two means was determined using the test at the 0.05 probability level.

## RESULTS

IC50 value of pyrethrum extract, tea tree oil, citronella and lavender oil were 3.3  $\mu$ l/ml, 20  $\mu$ l/ml, 20  $\mu$ l/ml and 1.5  $\mu$ l/ml, respectively. The result of *in vivo* efficacy was showed in table 3. Accordingly, 100 and 87 percent of infested students were treated with herbal shampoo and lindane, respectively, as using herbal shampoo formulation removed head lice and their nits in all of the infested students in the first and second weeks. No side effects were reported by using herbal shampoo. However, using lindane led to itching, redness and swelling of scalp on a few number of infested students. In addition, it seems that herbal shampoo resulted in removed resistant headlice, as all nits were

removed as resulted of applied herbal formulation (Table 3). Application of the herbal shampoo was more efficient in second week than lindane shampoo. Analysis of data also showed that there was a significant difference ( $p \leq 0.01$ ) in the efficacy of herbal formulation and lindane in the second week. Assessing of *in vitro* test showed that total head lice were immobilized after 15 minutes of treatment with herbal solution, and no signs of movement were observed after washing. Meanwhile, 72 % of head lice were immobilized after 15 minutes of treatment with lindane solution after washing. Moreover, the head lice mortality has reached 84 % after 4 hours of treatment with lindane. Furthermore, all nits were misshapen and shriveled, and no larvae hatched out from the eggs after treatment of herbal solution.

Table 1: Herbal shampoo and solution formulations

Ingredient	%	Herbal Shampoo	Herbal Solution
CocamidopropylBetaine	10	*	-
sodium lauryl ether sulfate (SLES)	9	*	-
Sodium Lauryl Ether Sulfate (pearly)	6	*	-
Polysorbate 20	0.6	*	-
Cocamide DEA	3	*	-
Pyrethrum extract	0.33	*	*
Lavender oil	0.15	*	*
Tea tree oil	2	*	*
Citronella oil	2	*	*
Phenoxy Ethanol	0.5	*	*
DMDM Hydantoin	0.1	*	-
Deionized Water	To 100	*	-
D-Limonene	To 100	-	*

Table 2: Classification of infestation based on the level of contamination

Category	Quantification	The number of Infested students
Mild infestation	Less than 10 head lice & nits	32
Moderate infestation	10 to 50 head lice a& nits	18
Severe infestation	More than 50 head lice & nits	6
Very severe infestation	Uncountable head lice & nits	4

Table 3: *In vivo* assay for antipediculosis efficacy of herbal and lindane (chemical) shampoos

Category	Number		Week 1				Week 2			
	Before treatment		2 <sup>th</sup> day		3 <sup>th</sup> day		9 <sup>th</sup> day		10 <sup>th</sup> day	
	Herbal	chemical	Herbal	chemical	Herbal	chemical	Herbal	chemical	Herbal	chemical
Infestation	%		%		%		%		%	
No pediculosis	-	-	27a	22b	55a	35b	93a	67b	100a	87b
<10	53	53	58a	50b	43b	51a	5b	28a	0b	10a
11-50	30	30	8a	11a	2b	7a	2a	3a	0b	3a
>51	10	10	5a	10a	0b	5a	0a	2a	0a	0a
Uncountable	7	7	2a	7a	0a	2a	0a	0a	0a	0a
T test	ns		**		**		**		*	

ns, not significant at the 0.05 probability level;

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

<sup>a</sup> Means within a row followed by the same letter do not differ statistically at  $P \leq 0.05$  according to T test.

Table 4: The effect of herbal and chemical formulations on Head lice nits after the 15 and 240 minutes of application

Head lice	Before treatment		After treatment			
	Number		15 minutes		240 minutes	
	Herbal	chemical	Herbal	chemical	Herbal	chemical
Alive lice	%		%		%	
Dead Lice	-	-	100a	72b	100a	84b
T test	ns		**		*	



Figure 1: Head lice and nits on the hair of an infested student

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## DISCUSSION

Overall, the prevalence of head lice related infestation is increasing worldwide and specifically in the developing countries. There are different types of pediculicide chemical drugs such as DDT, lindane, malathion, naphthalene, sulfur, mercury, petroleum and permethrin, to treatment of head lice. The main problem of using these chemical drugs is the resistance of head lice against synthetic formulations and their toxic effects on human (5, 6, 8, 9, 10, 11, 18). Likewise, different side effects such as itching, redness, swelling of scalp, eye irritation have been reported after using permethrin [18]. Previous studies have been illustrated the efficacy of permethrin into control head lice was nearly 100%, but recent studies have been reported that this efficacy has decreased up to 50%. This phenomenon also was seen after using different kind of chemical drugs, and several resistance mechanisms have been reported (24). Lindane also is the most common treatment of head lice which caused several cases of severe seizures in children (18). Severe resistances have been reported for lindane. Amino acid substitutions (leucine-phenylalanine substitution at position 1014) in the alpha subunit of the voltage-gated sodium channel are associated to decrease or delay

the pediculicide effects of permethrin and DDT (19). The problems and disadvantages of chemical treatments (especially their toxic effects), have promoted the research on new generation of alternative treatments with pediculicide activity. The result of in vivo test illustrated that alternative herbal shampoo had significant effect on head lice without harmful effect on infested students, as 13 percent of resistant head lice were not removed from hair by lindane shampoo. In a clinical study that head lice were treated with a small amount of permethrin, they stayed alive after 180 minutes (5). This study showed that head lice were resistant against permethrin, and a substitution of an amino acid in the nervous system of head lice occurred, and could eliminate the sensitivity of head lice against permethrin [5]. The result of in vitro test showed that herbal solution had significant effect on head lice, and total head lice were killed, but 16 percent of resistant head lice were not killed after 4 hours of treatment with lindane solution. The aim of the present study was to synthesize herbal formulations with more and better efficacy which could be used as alternative treatment instead of chemical formulation available in the market. Since toxicity and safety of herbal formulations were necessary for the consumer. Therefore, the optimal

concentration of each extract was determined by MTT assay. In order to assure the efficacy of formulations, *in vivo* and *in vitro* assay were conducted. These formulations were made by natural and herbal ingredients, and unlike many pediculicide drugs in the market, are not toxic for human. One of the effective ingredients in the formulation could be pyrethrum extract. The most important ingredients in this plant are pyrethrin, polysaccharides, lignans, sesamin, terpenes, polyphenols and proteins. Pyrethrin has neurotoxic and paralytic properties for lice. Several researches illustrated that pyrethrum extract acts rapidly on the head lice nervous system producing muscular excitability, convulsions and paralysis (20). Besides, many studies have been reported pediculicide activity for essential oils from natural sources such as eucalyptus, marjoram, spearmint, peppermint, sage, rosewood, clove bud and cinnamon bark (21, 22). Citronella oil also as a potent pediculicide agent was used in herbal formulations. This plant repels head lice, and also treats itching and inflammation in the lice biting area. Many studies showed that lavender and tea tree oil were effective to remove head lice and nits, and also prevent the resistance (3). These oils have also blocked the respiratory

pores, and killed head lice by suffocation [3, 23].

## CONCLUSION

Generally, this study provides scientific insights about the potential pediculicide properties of herbal formulations which can be further targeted against other cutaneous ectoparasite diseases.

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