

in vitro Pediculicidal Activity of Juice of *Citrus limon*

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Abstract: Head lice, *Pediculus humanus* var *capitis* are a global health concern and direct head-to-head contact is needed for their transmission. Head-lice infestations are ranked third after diarrhoea and conjunctivitis as the most reported conditions. Herbal medicines, can be promising as alternative pediculicides as these do not have residual activity. *Citrus limon* (commonly known as lemon) is a species belonging to family Rutaceae. Lemon has been used for both culinary and medicinal purposes. In an attempt to assess the lethality of *Citrus limon* juice towards human head lice, the effect of juice was tested in *in vitro* toxicity model against human adult lice and the lice were observed for lack of response to stimuli over 3 hour period. The results confirmed that raw *Citrus limon* juice and ½ diluted juice showed pediculicidal efficacies of 95±5 per cent and 90±10 per cent respectively (P< 0.05 over control).

Keywords: *Citrus limon*, Pediculicidal activity, *Pediculus humanus* Capitis, Percent morbidity.

Introduction

Head lice, *Pediculus humanus* var *capitis*, are wingless insects that have been obligate parasites of humans for thousands of years. ¹ *Pediculus humanus capitis* (human head lice), are parasites found in all areas of the world and in all socio-economic classes. Contrary to popular belief, pediculosis infests anyone regardless of race, economic status, family background or personal habits (Pan American Health Organization). ² ^{3,4} Head lice rarely, if ever, cause direct harm, and they are not known to transmit infectious agents from person-to-person. Thus, they are not considered as a medical or a public health problem. They feed by injecting saliva with vasodilatory properties into the scalp to draw blood. ⁵

The greatest harm associated with head lice results from the well-intentioned but misguided use of caustic or toxic substances to eliminate the lice. ⁶

The life span of the female louse is about one month. During this time, she will produce between 7 to 10 eggs ("nits") per day and attaches them firmly to the hair shaft region close to the scalp or body. Following 6 to 10 days, the nits produce nymphs and grew adults in 10 days. ⁷ The mode of transmission is most

commonly via direct head-to-head contact, sharing pillows, combs/brushes, caps, headphones. The clinical manifestations of head lice include pruritus, excoriation, local erythema, scalp impetigo, papules and cervical and occipital lymphadenopathy. ^{8, 9} Currently in many countries, the mainstay of head lice treatments are topical chemical insecticides, such as permethrin, allethrin, deltamethrin, lindane or benzyl benzoate. ¹⁰ Lindane, an organochlorine, was found to be a more effective pediculicide than DDT. ¹¹ Lindane is still used today in some countries but there remain concerns about its safety.

The main problems associated with these insecticides (mainly Permethrin) are resistance which is particularly frequent in countries where head lice are common and where chemical insecticides are intensively used, mainly in developed countries. ¹² Thus, there is an urgent need for the development of new effective pediculicidal products for the market. Wet combing is a safe and effective method of eradication, especially when combined with a conventional or natural pediculicide. Many herbal medicines and their constituents, such as essential oils appear to be effective at eradicating both the adult lice

and their eggs. They are gaining popularity due to both concern over the use of chemicals and the incidence of increasing resistance.

Citrus limon commonly known as lemon belong to family Rutaceae. Lemon is a small evergreen tree originally belongs to Asia, and contains oval yellow fruits.¹³ Fresh lemon juice is obtained by squeezing lemons. Lemon juice is about 5% (approximately 0.3 moles per litre) citric acid, which gives lemons a sour taste, and a pH of 2 to 3.¹⁴ The Lemon has many important natural chemical components, including citric acid, ascorbic acid, minerals and flavonoids. Although their health-related properties have always been associated with their content of vitamin C, it has recently been shown that flavonoids also play a role in this respect.¹⁵ Lemon fruits not only have their delicious flavors but also have their antioxidant capacity with health benefits.^{16 17} The aim of the present study was to assess the **pediculicidal** activity of lemon juice and the effect of dilution on the activity.

Experimental

Materials

Plant Material

Fresh fruits of lemon (*C. limon*) were purchased from local market of Bhopal. The fruits were identified authenticated and submitted at Department of Pharmacognosy, Bhopal Institute of Technology and Science -Pharmacy, Bangrasia, Bhopal (M.P.).

Experimental Organism

Human head lice *Pediculus humanus capitis* were collected from tribal children between the age of 3-12, with the approval of their parents, residing in Bangrasia near Bhopal (M.P.). The insects were collected by fine wet combing the children scalps.

Methodology

Preparation of Lemon Juice

The juice preparations used in this study were raw lemon juice. Fresh Lemon fruits were peeled and squeezed by hand. The juice was then allowed to stand for 10min at 25 °C. After filtering through Cheese cloth, raw lemon juice was obtained. The juice yield was also calculated. TSS and TA of citrus juice were also measured.¹⁸

Test Solutions

Test solutions of lemon juice were prepared by diluting with double distilled water.1:1, 1:3 and 1:6 dilution of lemon juice in double distilled water were prepared.

Standard Solution

1 % w/w lindane solution was purchased from P.K.Scientific Glassware Pvt.Ltd. Bhopal and used as standard.

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The *in-vitro* tests were started within one hour after collection. A filter paper diffusion bioassay was made. After careful selection of lice under a dissecting microscope, a filter paper discs (Whatman No 1) matching with internal diameter of petri dish were cut and placed in petri dishes. 0.5 ml of each test solution was spread over the lice and filter paper. Negative control lice were placed directly on the filter paper spread with only distil water. The 1 % w/w lindane solution was simultaneously run as a positive control. Each group consisted of 10 lice. Always separate dish was taken for every test solution, control and standard solution. The ***In vitro* pediculicidal** activity was investigated with *fresh lemon juice* and 1:1, 1:3 and 1:6 dilutions of lemon juice. The lice were considered dead if there were no vital signs such as movements of antennae or minimal leg movements upon stimulation with or without a forceps. The test was done in triplicate and average considered.

Statistical Analysis

The data were subjected to statistical analysis. One-way ANOVA. followed by Dunnett post-test was employed to identify pairs of results with significantly different means. Both ANOVA and post-test were performed by Graph- Pad InStat computer package

Results

The results demonstrate that *Citrus limon* juice show pediculicidal efficacies as shown in table 1. The morbidity rate was evaluated until three hours after treatment with various juice in different groups. Undiluted lemon (group I) showed the highest percentage morbidity after three hours (95±5) and after 25 minutes to 3 hours no movement was observed. In group II restricted movement was observed till 30 minutes and afterwards no movement was seen. While in group III and group IV movement was observed among most insects even after 90 minutes. Test group 1 and test group 2 showed significant differences over negative control (P< 0.05). Thus Raw *Citrus limon* juice showed significant pediculicidal activity. Decrease in morbidity of lice was observed with increasing dilutions of lemon juice.

Table 1: Pediculicidal activity of lemon juice and effect of dilution.

Group	Treatments	Mean time in (minutes±SD)	Percent Morbidity after 3 hours	P value Vs Negative control
Negative control group	0.5 ml of Distill water	-----	0	-
Test Group 1	undiluted lemon juice	25±5.5	95±5.77	P< 0.05
Test Group 2	0.5 ml of 1:1 diluted lemon juice	40±8.2	90±10	P<0.05
Test Group 3	0.5 ml of 1:3* diluted lemon juice	103 ± 5.22	30± 5	P>0.05
Test Group 4	0.5 ml of 1:6 diluted lemon juice	110 ± 3.19	30± 5.77	P>0.05
Positive control group	0.5 ml of 1 % w/w Lindane solution	50±2.12	95±11.54	P<0.05

*[1:1, 1 part lemon: 1 part distilled water, (1:3) 1 part lemon juice: 3 parts distilled water]

The test was done in triplicate and average considered. Values are presented as the mean ± SD; Number of insect in each group was 10. One way ANOVA followed by Dunnett compare all vs. control test.

Conclusion

The control of lice presents research challenges and prospects for the identification of new, safe and environmentally acceptable insecticides. The experimental evidence obtained in the laboratory model could provide a rationale for the use of *Citrus limon* juice for controlling head lice which are difficult to control because of their resistance to the currently

used anti-lice agents. The overall results of the trials indicate that the *Citrus limon* juice is highly effective against adult head lice.

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