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Assessment of Pediculicidal Potential of Formulation Containing Essential Oils of *Mentha piperita* and *Cymbopogan citratus*

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ABSTRACT

Head louse infestation is difficult to control because of increasing lice resistance to synthetic licicidal drugs. Plant's Essential oils have been extensively used in traditional medicine for the eradication of head lice and the insecticidal potency of some essential oils suggest that they may find an application in the control of head lice. The aim of the present study was to investigate the pediculicidal activity (licicidal activity on head lice- *Pediculus humanus Capitis*)of test formulation containing a combination of essential oils of *Cymbopogan citratus* and *Mentha piperita* oil. A filter paper diffusion bioassay method was carried out in order to determine the pediculicidal activity of formulations. The pediculicidal potential of the formulation containing combined essential oils of *Cymbopogan citratus* and *Mentha piperita* oil was found to be excellent and the mean death time observed with this group was 61±3.16 minutes, which was comparable to positive control group (mean death time 51±2.62 minutes, mean ±SEM).

Keywords: Pediculicidal activity, *Pediculus humanus capitis, Cymbopogan citratus, Mentha piperita,* Filter Paper Diffusion Bioassay Method.

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INTRODUCTION

Lice infestation on the human body (also known as pediculosis) is very common. Head lice are tiny six-legged insects that cling to the scalp and neck and feed on human blood. They feed by injecting saliva with vasodilatory properties into the scalp to draw blood and can move quite quickly in the hair, traveling up to 23 cm a minute [1].

The head lice are not a main health hazard or disease vector, but can cause substantial distress due to scalp, excoriation, local erythema, scalp impetigo, papules and cervical and occipital lymphadenopathy, itching, probable secondary infections, pruritis, bite reactions and conjunctivitis are also common manifestations [2]

The head louse is a grey-white animal about 2-3 mm in length (about the size of a sesame seed). The life span of the female louse is about one month. Head-lice transmission is most commonly via direct head-to-head contact. Based on the finding of lice and nits on the scalp, genital area, body, or on clothing, the doctor can suggest home-care techniques and prescribe medications as necessary. No other tests are usually performed to diagnose lice [3, 4].

An alternative to topical head lice treatments is the fine-toothed comb. In developing countries, most conventional Western (synthetic) pediculicides are either unavailable or prohibitively expensive and lice infestations are therefore even more common. Plants containing essential oils have been used for centuries as medicines, fragrances and insect repellents. They consist of numerous different, mostly volatile, low molecular weight (LMW) terpenoids [5].

Constituents of plant volatile oils have long been known to affect the behavioral responses of pests, with the monoterpenoid components appearing most useful as insecticides or anti-feedants [6].

Many modern pediculicides tend to fail because of low efficacy on lice eggs, whereas essential oil constituents are reputed to have good ovicidal capabilities [7]. All are examples of a very large group of *natural products* called *terpenes.* They are responsible for the characteristic odors of plants such as eucalyptus, pine, mint, peppermint, and lemon. Several plant products such as aniseed, coconut, neem and tea tree oils are used in different available compositions for the treatment of head lice infestation.

Lemongrass is a perennial fast-growing aromatic grass with loose panicle having slender branches nodding at the ends, growing to about 1 meter (3 feet) high with long, thin leaves obtained from *Cymbopogan citratus* belonging to the family Poaceae**.** In Ayurvedic medicine. *C. citratus* is used to help bring down fevers and treat infectious illnesses. It is cultivated for the production of its aromatic volatile oil-citronella oil and it is valuable ingredient in perfumes and citrus-type soaps and is also an insect deterrent possessing insecticidal [8] and antimicrobial activities [9].The oil is considered of value in purifying blood and rheumatism. Lemongrass tea

comprising leaves is considered valuable in common ailments of the elderly in the cold season [10-12].The main chemical components of *C. citratus* oil are Citral, Farnesol, Nerol, Citronellal and Myrcene [13].

Mentha, the genus of Labiatae family, includes 20 species that spread all over the world. *Mentha piperita* L. is one of the *Mentha* species. The essential oil consists of *Mentha piperita* belonging to the family *Labiatae* obtained by aqueous steam distillation from fresh leaves and flowering tips [14].

All are examples of a very large group of *natural products* called *terpenes.* They are responsible for the characteristic odors of plants such as eucalyptus, pine, mint, peppermint, and lemon. As essential oil constituents are reputed to have good ovicidal and licicidal capabilities [15]. Both of the plants are a good source of essential oil.

The aim of the present invention is to optimize a composition for the elimination of lice having specific combinations of essential oils of *C. citratus* and *M. piperita*. Both essential oils are extremely effective in killing lice and their eggs. The resulted pediculicide, provided in the present study, is based on natural products, non-toxic, and mild.

So, the present study was done to explore the licicidal potential to investigate a killing effect on lice with the formulation containing essential oil. It was also determined that whether a single compound could suffice to give a licicidal effect, or whether a combination of agents would perform significantly better.

MATERIALS AND METHODS

Plant Materials

The leaves of *C. citratus* and *M. piperita* were collected from local area of Village-Misrod, Bhopal (M.P.) in the month of Dec, 2009. Before use, freshly-collected *C. citratus* and *M. piperita* leaves were identified and authenticated and specimens were submitted at Department of Pharmacy, Bhopal institute of Technology & Science-Pharmacy, Bhopal for future reference.

Experimental Organism

Human head lice *Pediculus humanus capitis* (Trichodectidae) were collected from slum area children between the age of 4-14 years, with the consent of their guardians, residing at Bhopal district. The insects were collected by combing the children scalps. The children had not been treated with any Pediculicidal solution for at least the preceding month, using only the louse comb. After collection the lice were pooled and held on human hair strands in Petri dishes. In an earlier observation independent of present study the lice were found to remain exist for 24-48 hours when detached and kept away from host body.

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EXPERIMENTAL

Extraction of plant materials

The fresh leaves of 500.0 g of fresh *C. citratus* and *M. piperita* were subjected separately to hydro distillation. The essential oil was then collected and stored in amber colored bottle.

Preparation of Pediculicidal Formulations

Based on the concentration found in literatures, three formulations were prepared in coconut oil and coded M1, L1, ML1 as for pediculicidal activity. The composition of formulations is given in table 1.These formulations (M1, L1, and ML1) were investigated for their licicidal activity against Human head lice in various concentrations by filter paper diffusion bioassay [16].

Standard Solution

1% w/w lindane solution prepared in coconut oil was taken as standard to compare the pediculicidal activity.

Methodology

The *in-vitro* tests were started within 1 hr after collection of lice. A filter paper diffusion bioassay was made [16]. After careful selection of lice under a dissecting microscope, a filter paper discs (Whatman No 1; 9-cm diameter) coinciding with internal diameter of petri dish were cut and placed in petridishes. Petridishes containing filter papers impregnated with the formulations and lice were covered with glass lids and incubated under normal maintenance conditions for lice of 28±2 °C, 60±20% relative humidity (RH).

Groups

Five groups each consisting of 10 lice were taken (Table 2).

1. **Control (A):** Lice were placed directly on the filter paper spread with only coconut oil as negative control.
2. **Control (B):** The 1% w/w lindane solution in coconut oil was simultaneously run as a positive control (B) to treat lice infestation [17].
3. **M1 group:** 1.0% v/w *M. piperita* oil in coconut oil was simultaneously run as a third group.
4. **L1 group:** 1.0% v/w *C. citratus* oil in coconut oil was simultaneously run as a fourth group.

5. **ML1 group:** 0.5% v/w *M. piperita* and 0.5% v/w *C. citratus* oil in coconut oil was simultaneously run as a fifth group.

1 ml of each test formulation (M1, L1, and ML1) was spread over the lice and filter paper by using brush in each group.

The lice were judged as dead if there were no vital signs such as movements of antennae or minimal leg movements (with or without stimulation by a forceps). The petridish lids were kept in place during the tests but removed every 15 min so that the lice could be observed and the number of fatalities recorded. Death was defined as lack of movement of limbs and gut, and failure to respond when the legs were stroked with forceps. The test was done in triplicate and average considered [18, 19].

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. The One – way ANOVA was performed by using Microcal Origin Software.

RESULTS AND DISCUSSION

The test formulation L1 showed significant pediculicidal activity ($p< 0.01$) over negative control (Table.2). The mean death time was found to be 67±2.32. The test formulation M1 also showed significant pediculicidal activity ($p< 0.01$) over negative control (Table.2) but the time required for the death was more comparable to L1 group (mean death time 89±2.51).We also assessed the pediculicidal potential of the combined formulation ML1 group and the mean death time observed for this group was 61±3.16 which was comparable to positive control group (mean death time 51±2.62). The ML1 test formulation showed better pediculicidal activity than the other two groups. The observation also appears that initially when the treatment commenced with M1 test formulation, the head lice were showing movements of antennae and leg movements. Thereafter with the L1 test formulation, the movement activity were getting slightly slow. And with the ML1 test formulation the movements of antennae and legs were stopped. The experimental facts obtained in the laboratory level could provide a justification for the customary use of both the oils for controlling head lice due to difficulty in controlling head lice because of their resistance to the currently used anti-louse agents.

CONCLUSIONS

The management and control of lice presents research challenges and prospects for the identification of new, safe and environmentally appropriate and suitable insecticides. The combination formulation of two essential oils showed better effects as compared to that of individual essential oils. It seems that there is some sort of synergistic pediculicidal effects when these two essential oils are used in combination. The experimental confirmation obtained

in the results could present a rationale for the use of the formulation containing *C. citratus* and *M. piperita* for controlling head lice. This formulation can be a good and safe alternative for the lice infestation control.

Table 1. Composition of test Formulations of *C. citratus* and *M. piperita* oil.

S.No.	Ingredients	Test Formulation 1 (M1)	Test Formulation 2 (L1)	Test Formulation 3 (ML1)
1	Mentha Oil (*M. piperita)*	1 ml	-	0.5 ml
2	Lemongrass Oil (*C. citratus)*	-	1 ml	0.5ml
3	Coconut Oil	9.0 ml	9.0 ml	9.0 ml
	Total	10.0 ml	10.0 ml	10.0 ml

Table 2. *In vitro* Pediculicidal activity of Test Formulations of *C. citratus* and *M. piperita*

Groups	Treatment	Death Time (Minutes) (Mean±SEM)
Group 1	Plain coconut oil (Negative control)	----------
Group 2	1 % w/w Lindane solution (Positive control/standard)	51±2.62*
Group 3	M1 group	89±2.51*
Group 4	L1 group	67±2.32*
Group 5	ML1 group	61±3.16*

* P < 0.01 Compared to Negative Control
(Mean±SEM, n=3)

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