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Formulation of pediculicidal hair cream from the petroleum ether leaf extract of *Millettia pinnata* (L.) Panigrahi (Fam. Fabaceae)

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Abstract

Pediculosis, commonly referred to as head louse (*Pediculus humanus capitis*) infestation, is common and endemic worldwide. However, exposure to the current standard treatment such as permethrin and pyrethrin can cause neurotoxicity especially when misused and resistance to these chemicals has been a concern. This study aimed to develop a pediculicidal hair cream using the petroleum ether leaf extract of *Millettia pinnata* to address the problems associated with head louse infestation by providing and developing a better and safer alternative pediculicide. This study involved the formulation of an acceptable hair cream and the evaluation of pediculicidal activity of different treatments using filter paper diffusion method. Permethrin (Kwell®), the positive control, had the greatest anti-lice activity of all the treatments. In the determination of minimum effective concentration, only 80% bani hair cream was found to be effective. However, the 5% bani hair cream was selected as the final formulation based on both efficacy and quality. The final formulation exhibited greater pediculicidal activity than the commercially available herbal pediculicide, quassinoids (Oilganics®), and the 60% makabuhay hair cream formulation. Replicate observations should be performed to determine any significant difference among the different treatments.

Keyword: In vitro pediculicidal activity, formulation, hair cream, *M. pinnata*, filter paper diffusion

1. INTRODUCTION

Pediculosis, commonly referred to as head lice (*Pediculus humanus capitis*) infestation is common and endemic worldwide, and affects persons of all socioeconomic backgrounds and ages (Greive and Barnes, 2012). It is a prevalent and contagious condition afflicting mostly children between three to ten years old (Samuel et al 2009). It usually affects school-age children and can be transmitted through direct head-to-head contact. It is not generally associated with morbidity apart from secondary bacterial infections but they may cause social stigma, embarrassment, low self-esteem, lost productivity and frustration among all involved (Greive and Barnes, 2012).

Approved topically applied chemical agents are recommended as treatment once head lice infestation is detected. The current standard treatment for this condition includes neurotoxin-based chemical agents such as pyrethrin and permethrin. However, exposure to some of these chemical agents can cause neurotoxicity and anemia especially when misused. In addition to this, resistance to topical pediculicides has been documented in a number of countries. Alternative management of the condition such as the use of oral agents and wet combing has limited data to support either of the methods to be a primary treatment for head lice (CPS, 2004). Natural remedies, for instance the use of volatile oils, have been use for the management of head lice infestation. A study by Mumcuoglu et al. (2002) reveals that a natural remedy containing oils of coconut,

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anise and ylang-ylang was effective in controlling the infestation with no serious side effects.

In the Philippines, studies have also been conducted in the screening of herbal pediculicides. In the study conducted by Balotro (2011), *T. crispa* was formulated as hair cream to improve its convenience of use and to prolong contact time with the hair and scalp. Other plants that have pediculicidal activity that is readily available the Philippines include *M. pinnata* which is commonly referred to as bani. In the study conducted by Samuel et al. (2009), findings revealed that the petroleum ether extract of *Millettia pinnata* leaves possesses excellent pediculicidal activity that was comparable to that of benzoyl benzoate (25% w/v). The results obtained from this study demonstrate the potential of *M. pinnata* leaf extract as a promising effective alternative anti-lice agent.

This study would address the problems associated with head louse infestation by providing and developing a better and safer alternative pediculicide using *M. pinnata* in a form that will be convenient and easy to use.

2. MATERIALS AND METHODS

2.1. Plant Material

2.1.1. Collection

The plant material was collected in January and March 2014 from a single location: University of the Philippines-Los Banos, where it grows wildly and is not cultivated. It was authenticated as *Pongamia pinnata* Merr. (Fam. Leguminosae), its former name, by Manuel D. Ching OIC of the Varietal Improvement Section of the Bureau of Plant Industry. The plant is currently known as *Milletia pinnata* (L.) Panigrahi and locally known as *bani*.

2.1.2. Extraction

The coarse powdered leaves of M. *pinnata* were extracted with petroleum ether and methanol separately by maceration. All the extracts were concentrated using rotary vacuum evaporator and then exposed to the fume hood to let the residual solvent evaporate. The collected extracts were kept in a desiccator until further studies.

2.1.3. Thin Layer Chromatography (TLC)

TLC of the petroleum ether extract obtained from the leaves was performed using 65:30:5 hexane:ethyl acetate:methanol mobile phase.

2.2. Test Organism

Adult *P. humanus capitis* was obtained from a small community in Linao St., Brgy. 743, Zone 80, San Andres, Malate, Manila. Convenient sampling was employed to select 10 subjects as source of head lice for the study. Live adult lice were obtained by either combing the hair of the patients with fine toothed comb or by direct extraction from the hair. All subjects have not been treated with any anti-lice products for the preceding 3 months. All lice collected were examined for activity and morphological integrity under a dissecting microscope. Only fully active and intact adult lice having sizes ranging from 2-3mm long were used irrespective of sex.

2.3. Formulation

2.3.1. Preformulation Studies

The compatibility of the extract to 10 different excipients was evaluated. The selected excipients include: glyceryl monostearate (emulsifier), cetostearyl alcohol (emulsion stabilizer), mineral oil (emollient), sodium lauryl sulfate (surfactant), propylene glycol (humectant), glycerin (humectant), butylated hydroxytoluene (antioxidant), methylparaben (preservative) and peppermint oil (fragrance). Different sets of vials were prepared by mixing the extract to the different excipients in a 1:1 ratio except for the excipients acting as preservative, antioxidant, colorant or fragrance which used the ratio of 1:20, in sealed stoppered vials. The sealing of the vials was done using melted white wax. The vials were stored in different conditions: Set 1 was stored in ambient temperature (30°C) with light, Set 2 was stored in ambient temperature without light, Set 3 was stored in elevated temperature (40°C), Set 4 was stored in decreased temperature (5°C) and the last set was stored in ambient temperature

but prepared with additional 5% moisture. The compatibility was assessed by observing the presence of discolouration and odour and determining the TLC profile of the sample after a period of 1 month. The TLC profiles of the extract-excipient mixtures were compared with the TLC profile of the petroleum ether leaf extract alone.

2.3.2. Formulation Studies

Four trial formulations containing different concentration of extracts were prepared: 5%, 25%, 60% and 80%. The finished products were subjected to quality tests of homogeneity, color, odor, pH, and spreadability and *in vitro* pediculicidal activity test. The cream formulation with the acceptable physicochemical characteristics and pediculicidal activity was chosen for reproducibility studies. After successful reproducibility studies, the cream formulation proceeded to scale up production. The quality of the formulated hair cream was continuously evaluated every week for a period of one month.

2.4. Evaluation of Pediculicidal Activity

2.4.1. Determination of Minimum Inhibitory Concen-tration

The four trial formulations of *M. pinnata* differing in extract concentrations prepared in the formulation studies were used. Pediculicidal efficacy of each trial formulation was determined using the filter paper diffusion bioassay method of Heukelbachet, al. 2008.

Stringent criteria were used to evaluate pediculicidal activity. Death of a head louse was defined as the complete absence of any vital signs such as gut movement and movement of antennae or legs, with or without stimulation using forceps. This status was defined as 'no vital signs' for the present study. On the other hand, lice were defined as 'active' if no changes in their levels of activity or behavior was observed after treatment. Evaluation was conducted 30, 60, 90, 120, 150 and 180 min post-treatment.

2.4.2. Comparison of Formulated Hair Cream to Existing Herbal Pediculicide Preparations

Four experimental groups were compared, 2 treatment groups (5% Bani hair cream and 60% Makabuhay hair cream) and commercially available Oilganics®, positive control (Kwell®), and negative control (cream base formulation). For each of the experimental groups, 25±3 adult lice were used. The same procedure and criteria as in the determination of minimum inhibitory concentration were employed.

2.5. Safety Testing

Acute dermal irritation test of the finished product was conducted on the intact skin of albino rabbits. A sample of the formulated 5% bani hair cream was submitted to the Standards and Testing Division, Industrial Technology Development Institute of the Department of Science and Technology for the acute dermal irritation test. The agency utilized modified OECD guidelines.

2.6. Data Analysis

The parameters such as homogeneity, color, odor, pH, and spreadability specified for the assessment of the quality of the finish product were analyzed based on its conformance to pharmacopeial specifications. The minimum inhibitory concentration was considered as the concentration of extract in the cream formulation which elicited a percent mortality of at least 80%. The pediculicidal activity of each experimental group was presented as percentage of lice killed (% mortality). The differences in percent mortality of the formulated *M. pinnata* hair cream to herbal anti-lice preparations were compared. The evaluation of the skin reaction was analyzed based on the scores obtained after the acute dermal irritation testing.

3. RESULTS

3.1. Thin Layer Chromatography

The extract produced a characteristic chromatogram consisting of four spots when viewed under 366nm UV (Table 1) using 65:30:5 hexane:ethyl acetate:methanol mobile phase.

Table 1. Rf values and characteristics of spots on the chromatogram of petroleum ether extract using 65:30:5 hexane:ethyl acetate:methanol mobile phase

Spot	Rf	Visible light	Under 366nm
1	0.2750-0.2875	-	Blue
2	0.3875-0.4000	-	Yellow Green
3	0.6000-0.6125	Green	Neon Orange
4	0.7000	Yellow	Dark Orange

3.2. Compatibility Studies

The Rf values of the excipient-extract mixtures are summarized in Table 2 and Table 3.

3.3. Formulation Studies

Ten (10) suitable excipients were selected based on the results of the compatibility studies and these were used to prepare the trial formulations. Information on the functions, accepted amount of use and incompatibilities of these excipients utilized in the formulation were obtained from the Handbook of Pharmaceutical Excipients.

Table 2. Results of one month compatibility test of petroleum ether extract with excipient mixture as measured by Rf values (part 1).

Excipient	30°C											
	with light				without light				with moisture			
	1st Spot	2nd spot	3rd spot	4th spot	1st Spot	2nd spot	3rd spot	4th spot	1st spot	2nd spot	3rd spot	4th spot
Butylated hydroxy toluene	0.2875	0.4125	0.5500	0.6500	0.3125	0.4250	0.6250	0.7500	0.2875	0.4250	-	-
Cetostearyl alcohol	0.2500	0.5000	-	-	0.3000	0.5500	-	-	0.2375	0.3500	0.5375	-
DMDM Hydantoin	0.2875	0.5375	0.6875	-	0.3000	0.3875	0.5625	0.7500	0.2875	0.300	0.6375	-
Glycerin	0.2750	0.3875	0.6125	0.7250	0.3000	0.4000	0.6000	0.7500	0.2500	0.3750	0.6000	-
Glycerin monostearate	0.1500	0.2750	0.3500	0.5000	0.2750	0.3500	0.5125	-	0.3750	-	-	-
Methylparaben	0.3000	0.4750	0.6375	0.7500	0.3125	0.4125	0.6375	0.7625	0.2500	0.3750	0.6125	0.7500
Mineral oil	0.2625	0.3500	0.4875	0.5250	0.2750	0.3500	0.4875	0.5375	0.3275	0.3500	0.5250	0.6000
Propylene glycol	0.3000	0.3875	0.6250	-	0.3125	0.4250	0.6125	0.7625	0.2875	0.5375	-	-
Sodium lauryl sulphate	0.3000	0.4250	0.6375	-	0.3000	0.4000	0.6125	0.7500	0.2625	0.4000	0.6500	-
Peppermint oil	0.2625	0.6000	-	-	0.3125	0.5375	-	-	0.6250	0.7000	-	-

Table 3. Results of one month compatibility test of petroleum ether extract with excipient mixture as measured by Rf values (part 2)

Excipients	5°C				40°C			
	1st spot	2nd spot	3rd spot	4th spot	1st spot	2nd spot	3rd spot	4th spot
Butylated Hydroxy Toluene	0.2875	0.4250	0.6250	0.7500	0.2500	0.3875	0.5250	0.6125
Cetostearyl alcohol	0.2000	0.3250	0.4500	0.6750	0.1500	0.2875	0.4500	-
DMDM Hydantoin	0.2875	0.4000	0.6625	0.7625	0.6375	-	-	-
Glycerin	0.2875	0.4000	0.6250	0.6625	0.2750	0.3875	0.5625	0.7375
Glycerin monostearate	0.2750	0.3625	0.5500	0.7375	0.1125	0.2375	0.3000	0.5000
Methylparaben	0.2875	0.3875	0.6125	0.7375	0.2875	0.3875	0.4625	0.6250
Mineral oil	0.2250	0.3000	0.4250	0.4750	0.2500	0.3125	0.4500	0.5125
Propylene glycol	0.3125	0.4375	0.6625	0.7625	0.2750	0.4000	0.5500	0.7250
Sodium lauryl sulphate	0.3000	0.4250	0.3000	-	0.3000	0.4125	0.6250	-
Peppermint oil	0.2250	0.5500	-	-	-	0.5250	-	-

All trial formulations were smooth and homogeneous, possessed a characteristic leafy odor, and were in the pH range of 4.0-4.5. However, formulations with extract concentrations of 25%, 60% and 80% stains the skin upon application. Only the preparation with 5% of the leaf extract did not demonstrate considerable staining and was water washable. The product development then focused on the formulation with 5% extract due to its acceptable characteristics as hair cream.

The optimum formulation was obtained after trial formulation 2, increasing the proportion of water in the formulation to achieve the desired consistency of the preparation. The details of the trial formulations for the 5% bani hair cream are presented on Table 4. Trial 2 formulation was an olive green, homogeneous cream with minty odor and a spreadability value of 7 cm.

Table 4. Trial formulations of the 5% bani hair cream

Ingredient	Trial 1		Trial 2	
	(%)	(g)	(%)	(g)
Extract	5.00	0.50	5.00	0.50
Cetostearyl alcohol	1.90	0.19	1.66	0.17
Glyceryl monostearate	19.00	1.90	16.63	1.66
Mineral oil	20.90	2.09	18.30	1.83
Propylene glycol	1.90	0.19	1.66	0.17
Glycerin	1.90	0.19	1.66	0.17
Sodium lauryl sulphate	1.90	0.19	1.66	0.17
Butylated Hydroxytoluene	0.19	0.02	0.17	0.02
Methylparaben	0.19	0.02	0.17	0.02
DMDM Hydantoin	0.01	0.01	0.09	0.01
Peppermint oil	q.s.	q.s.	q.s.	q.s.
Water	47.00	4.70	52.00	5.20
TOTAL	100.0	10.00	100.00	10.00

Reproducibility studies were performed using the trial formulation 2 of the 5% bani hair cream. Quality control results of the three trials prepared were found to be consistent (Table 5) and consequently the formulation was tested to be produced on a larger scale.

3.4. Dermal Irritation Test

The sample tested on the skin of male albino rabbit nos. 1 and 2 produced erythema (+2) on day 1 up to day 4 and desquamation on day 7 up to day 11 which disappeared on day 12. Sample application on the skin of Rabbit no. 3 produced erythema (+3) on day 1 up to day 4 and desquamation was observed on day 7 up to the 14 days period of observation. The control (distilled water) did not produce any effect on the site of application.

3.5. Evaluation of Pediculicidal Activity

All the lice exposed to permethrin (Kwell®), the positive control, were killed instantly after 30 minutes. The percentage of lice killed after exposure to the petroleum ether extract remained constant at 72.00% after 60 minutes through 180 minutes. The exposure to the methanolic extract caused 43.48% of the lice treated being killed (Figure 1).

Table 5. Quality control test results for the reproducibility studies of the 5 % bani pediculicidal hair cream and the up-scaled formulation

Test Parameter	Trial 1	Trial 2	Trial 3	Up-scaled formulation
Color	Olive green	Olive green	Olive green	Olive green
Odor	Minty odor	Minty odor	Minty odor	Minty odor
pH	5	5	5	5
Spreadability	7cm	7.5cm	7cm	7cm
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogenous

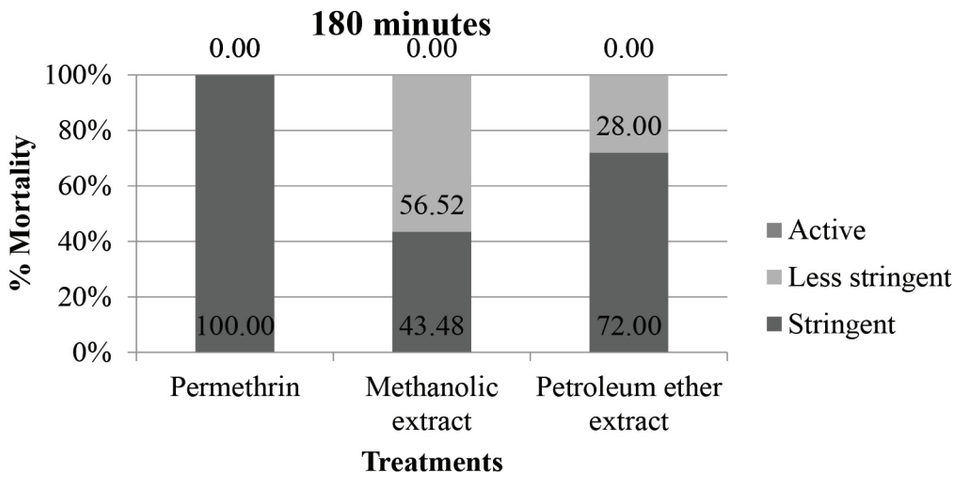

Figure 1. Comparison of pediculicidal activity of permethrin, methanolic extract, and petroleum.

Post-exposure to the different formulated hair creams with varying concentrations of the petroleum ether extract showed an increasing trend in their ability to kill lice based on the percentage of lice killed. Post-exposure to the 80% petroleum ether extract remained

constant at 95.45% throughout the duration of the observation (Figure 2). All lice treated with the formulated base cream remained active while mortality rate after exposure to quassinoids (Oilganics®) remained constant at 4.35% throughout the duration of the observation.

The final hair cream formulation (5% bani hair cream) was compared to commercially available herbel pediculicide, quassinoids (Oilganics®) and an existing hair cream formulation, 60% *T. crispa* hair cream. As shown in Figure 3, the 5% bani hair cream has greater pediculicidal activity than the two other treatments.

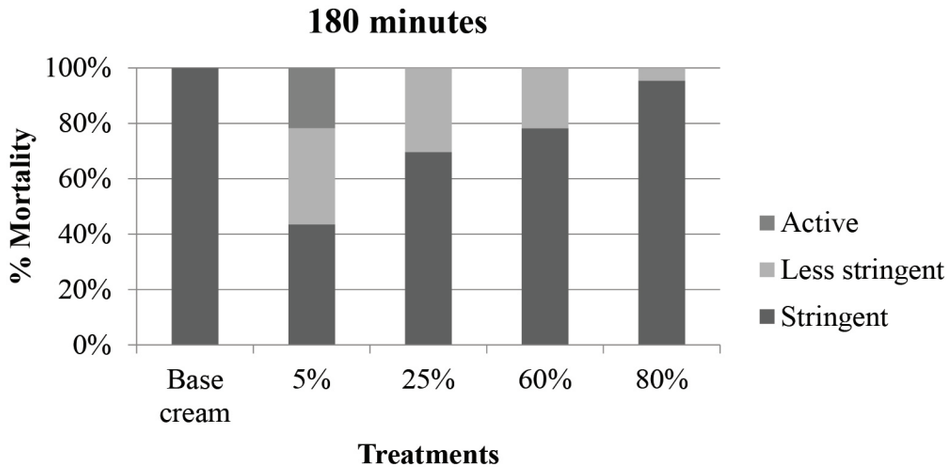

Figure 2. Results for the determination of minimum effective concentration.

4. DISCUSSION

4.1. Compatibility Studies

Based on the compatibility tests, all ten excipients were compatible with the petroleum ether extract throughout the one month observation period. Excipient-extract mixtures were stored in different conditions (5°C, 30°C with light, 30°C without light, 30°C with moisture, and 40°C) and then Rf values of the chromatograms of these mixtures were the basis of the compatibility test. The Rf values were compared to the extract alone as standard. The selected excipients for the formulation were glyceryl monostearate, cetostearyl alcohol, mineral oil, sodium lauryl sulfate, propylene glycol, glycerin, butylated hydroxytoluene, methylparaben and peppermint oil.

4.2. Formulation Studies

The different concentrations (5%, 25%, 60%, and 80%) of the extract were formulated into creams and were tested for their pediculicidal efficacy. The formulation with the highest percentage of lice killed (80% cream) did not have acceptable properties to be developed into a hair cream because of undesirable skin staining, which is also true for formulations with 25% and 60% extract. Only the preparation with 5% extract produced a hair cream with suitable properties for a hair cream. Hence, despite having the lowest pediculicidal activity among the trial formulations, it was considered to be the most appropriate formulation for the *M. pinnata* pediculicidal hair cream.

4.3. Pediculicidal Activity

Lice are capable of physiologically shutting down and going into stasis, and then recovering from an apparently morbid state. Hence, in evaluating head lice treatments, the end-point observation must be taken after the maximum duration of stasis. However, this end-point cannot be determined through observation at the gross level because cessation of finer body functions, such as gut movement, can only

be determined by microscopy (Heukelback et al. 2008). In the present study, stringent criteria was used in the evaluation of pediculicidal activity to reduce the probability of misidentifying mortality due to the transient period of stasis or sham death. However, the pediculicidal activity based on less stringent criteria was also observed to detect any effect of the different treatments on the level of activity of the lice.

Based on the evaluation of pediculicidal activity, all treatments excluding the formulated base cream exhibited an effect on the activity levels of the lice treated. The positive control, permethrin (Kwell®), was able to kill all of the lice after 30 minutes indicating possible absence of resistance to permethrin. At each time point, the percentage of lice killed after exposure to petroleum ether extract was consistently higher than the percentage of lice killed after exposure to methanolic extract. Hence, the petroleum ether extract was selected in the formulation of the hair cream over the methanolic extract.

As the concentration of the petroleum ether extract increased in the formulated creams, the mortality of lice also increased. It was recommended by Heukelbach et al. (2008) that mortality rate of at least 80.00% is defined as effective, thus the formulated hair cream with 80% petroleum ether extract is considered effective due to a mortality rate of 95.45%. All the formulated hair creams demonstrated higher mortality compared to the commercially available herbal pediculicide, quassinoids (Oilganics®). The formulated base cream had no effect on the activity of the lice since all lice treated with it remained active even after the 3 hour duration of observation.

According to Samuel et al. (2009), the oil components in the *Millettia pinnata* extract are responsible for its anti-lice activity. The oil components are trapped in respiratory system causing death. In addition to the oil components, the presence of sterol derivatives enhances the penetration of these oil components into the body of the louse. Results show that the formulated 80% petroleum ether bani hair cream demonstrated greater pediculicidal activity compared to the pure petroleum ether extract. Lipophilicity of a substance has a significant influence on insecticide activity. In the case of pediculicidal activity, it is suggested that compounds with greater K_{ow} have greater activity due to an enhanced penetration of the lipophilic cuticle of the lice (Rossini et. al 2008). In addition to the sterol derivatives present, the lipophilic components of the base cream can also have possibly enhanced the penetration of the oil components. This can also be the possible explanation for the greater activity of the petroleum ether extract compared to the methanolic extract.

In a similar study conducted by Balotro (2011), the formulated 60% makabuhay hair cream exhibited 96.43% mortality. However, in the present study, the demonstrated pediculicidal activity of the formulated 60% makabuhay hair cream was relatively lower at a mortality of only 39.13%. This difference can possibly be attributed to the difference in the method of extraction. The previous study utilized continuous extraction using Soxhlet apparatus while the present study employed maceration for 24 hours as the extraction technique. Continuous extraction technique can exhaust the extractives from the makabuhay leaves more efficiently than maceration for 24 hours.

5. CONCLUSION AND RECOMMENDATIONS

The results for the evaluation of pediculicidal activity demonstrated that the commercially available permethrin (Kwell®) has the greatest pediculicidal activity. The petroleum ether extract had greater anti-lice activity than the methanolic extract. Hence, the petroleum ether extract was selected as the extracting solvent.

Based on the compatibility testing done, the following excipients: glyceryl monostearate, cetostearyl alcohol, mineral oil, sodium lauryl sulfate, propylene glycol, glycerin, butylated hydroxytoluene, methylparaben and peppermint oil were demonstrated to be compatible to the petroleum ether extract.

The final formulation was selected based on pediculicidal activity and quality of the hair cream produced. Although only the

formulated 80% bani hair cream was found to be effective, its quality control test results revealed that it was not suitable for an acceptable hair cream. The 5% bani hair cream was chosen for final formulation instead due to more desirable and acceptable quality control test results. Its anti-lice activity was greater compared to the 60% *T. crispa* hair cream and the commercially available herbal pediculicide, quassinoids (Oilganics®).

The results of the dermal irritation test indicate that adequate warning should be placed on the label of the product due to possible occurrence of irritation.

It is recommended that replicate observations of at least 5 trials be performed to determine any significant difference among the different treatments. Continuous extraction is also suggested as the method of extraction to ensure a more complete exhaustion of extractives.

Since separate irritation tests were not performed on the formulated base cream and on the extract alone, it is recommended that their safety be evaluated to determine the possible cause of irritation. In addition to dermal irritation test, ocular irritation test should also be performed to further assess the safety of the hair cream. Histopathology in conjunction with the irritation tests should be conducted to determine microscopic physical and physiological changes in the skin. Additionally, it is recommended that chronic toxicity of the final hair cream be established.

Stability studies for the final hair cream formulation should be done to establish its appropriate storage conditions.

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