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FORMULATION AND EVALUATION OF LANTANA CAMARA L. LEAF EXTRACT HAIR TONIC AS AN ANTI-DANDRUFF AND HAIR GROWTH AGENT

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ABSTRACT
Hair problems like loss and dandruff (Pityrosporum ovale) are common, fueling the search for natural alternatives. Lantana camara L. leaves, rich in flavonoids, saponins, and tannins, were selected for their antifungal and hair-growth promoting potential. This study formulated and evaluated a Lantana camara L. leaf ethanol extract hair tonic as an anti-dandruff agent (in vitro) and a hair growth promoter (in vivo). The extract was obtained via maceration and phytochemically screened. Hair tonic formulations (15%, 20%, 25%) underwent evaluation for physical stability, pH, viscosity, and microbial contamination, all meeting SNI standards. In vitro anti-dandruff tests against P. ovale used the disc diffusion method, while in vivo hair growth promotion was tested on mice for 30 days. Results confirmed the extract contained alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. All formulas were stable and met SNI. The 25% formula (F3) showed superior microbial safety and strong in vitro antifungal activity (10.7 mm inhibition zone, comparable to positive control). In vivo, F3 significantly increased mouse hair length, comparable to commercial tonic by day 20. Flavonoids and saponins are suspected to contribute to hair growth stimulation. In conclusion, the Lantana camara L. leaf ethanol extract hair tonic 25% (F3) is safe, stable, effective as an anti-dandruff agent in vitro, and a hair growth promoter in vivo, positioning it as a promising natural alternative.

Keywords: anti-dandruff; hair growth; hair tonic; lantana camara L.; pytirosporum ovale

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INTRODUCTION

The extreme heatwave affecting Asia since early 2023, with temperatures consistently above 40°C for weeks, has raised significant health concerns, particularly for scalp and hair health. Excessive ultraviolet (UV) radiation exposure and low air humidity can damage the scalp and hair strands, leading to issues such as dryness, irritation, brittleness, and hair loss (Kim et al., 2021). Furthermore, unhealthy lifestyles, the use of chemical products like hair dyes (Ardhany & Soraya, 2017), and chronic stress (Rajput, 2022) exacerbate these conditions. A survey by Jakpat indicated that 64.7% of respondents reported hair loss, making it the most prevalent hair problem in Indonesia, followed by dandruff (44.3%). (York et al., 2020) highlighted the severe impact of hair loss on an individual's self-esteem, self-image, and overall quality of life. Similarly, dandruff, primarily caused by Pityrosporum ovale fungal infection, can significantly undermine self-confidence (Mahataranti et al., 2012). While hair tonics are a common solution, many commercial products pose potential risks of side effects from synthetic chemicals. Consequently, there is a substantial demand for hair tonic formulations derived from natural sources. Lantana camara L. leaves have emerged as a promising candidate due to their rich content of secondary metabolites, including flavonoids, saponins, and tannins (Sari, 2023). These compounds have demonstrated antibacterial and antifungal activities (Sukandar & Ekawati, 2006), as well as hair growth-promoting potential (Mulyanti et al., 2019). Given the urgency of hair health concerns and the natural efficacy of Lantana camara leaves, this study aimed to formulate and evaluate the effectiveness of an ethanol extract of Lantana camara leaf hair tonic preparation as an anti-dandruff agent in vitro and a hair growth stimulant in vivo.

METHOD

The primary material utilized in this study was fresh Lantana camara L. leaves, sourced from Cot Teungoh, Sigli, Pidie District, Pidie Regency. The plant material was authenticated by the Herbarium Medanense (MEDA) at Universitas Sumatera Utara. The chemical reagents and media used included ethanol (Merck), distilled water (Waterone), propylene glycol (Merck), sodium benzoate (Merck), sodium metabisulfite (Merck), menthol (Merck), NaCl (Merck), DMSO (Merck), and Pityrosporum ovale fungus (obtained from the Microbiology Laboratory of Universitas Sumatera Utara). Potato Dextrose Agar (PDA) (Merck) served as the fungal growth medium. Laboratory equipment comprised a rotary evaporator, analytical balance, various glassware, a pH meter, a viscometer, incubation equipment (oven, incubator), and a colony counter.

Extraction of Lantana camara Leaves

Fresh Lantana camara leaves underwent sorting, thorough washing, and air-drying. The dried leaves were then pulverized into a fine powder. Maceration was employed for extraction using 96% ethanol as the solvent for 7 days at room temperature. The mixture was subsequently filtered until a clear filtrate was obtained. The collected filtrate was concentrated using a rotary evaporator at 40°C to yield a viscous extract (Depkes RI, 1979).

Formulation of Hair Tonic Preparation

Hair tonic preparations were formulated with varying concentrations of Lantana camara leaf ethanol extract as the active ingredient (15%, 20%, and 25%), alongside an extract-free base (0%). The formulation followed a modified method from (Yusuf et al., 2021). The Lantana camara leaf extract was accurately weighed and completely dissolved in distilled water. Propylene glycol was then added and mixed until a homogeneous solution was achieved. Separately, sodium metabisulfite and sodium benzoate were dissolved in distilled water. The dissolved extract solution, along with the dissolved sodium metabisulfite and sodium benzoate solutions, were gradually incorporated and stirred until homogeneous. Finally, menthol was dissolved in ethanol and slowly added to the mixture with continuous stirring to ensure homogeneity. The prepared formulations were evaluated for organoleptic properties (color, odor, form), homogeneity, pH, viscosity, and microbial contamination. All formulations were required to comply with the specifications outlined in SNI 16-4955-1998 for hair tonic preparations.

Table 1.
Lantana camara Leaf Extract Hair Tonic Formula

No.	Hair Tonic Ingredient	Function	Blanko (0%)	Formula I (15%)	Formula II (20%)	Formula III (25%)
			Concentration (%)			
1	Lantana camara leaf extract	Active Ingredient	-	15	20	25
2	Ethanol 70%	Solvent	60	60	60	60
3	Propylene Glycol	Adjuvant	10	10	10	10
4	Sodium Benzoate	Preservative	0.25	0.25	0.25	0.25
5	Menthol	Cooling Sensation	0.1	0.1	0.1	0.1
6	Sodium Metabisulfite	Antioxidant	0.2	0.2	0.2	0.2
7	Distilled Water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

In vitro Anti-dandruff efficacy test

The anti-dandruff activity of the hair tonic preparations was assessed against Pityrosporum ovale using the agar diffusion method with paper discs. The fungus was cultured on PDA medium and incubated at 20-25°C for 2x24 hours (Sriwijaya et al., 2018). The diameter of the zone of fungal growth inhibition was observed and measured after incubation.

In vivo Hair Growth Efficacy Test

The hair growth-promoting effect was evaluated in vivo using 24 male white rats, randomly assigned to 6 groups: normal, F0 (base), positive control (commercial hair tonic), F1 (15%), F2 (20%), and F3 (25%). The animals were housed under standard conditions, adhering to ethical

guidelines for animal research. The dorsal area of each rat was shaved, and the respective hair tonic preparations were applied daily for 30 days. Parameters observed included hair length, hair diameter, and hair weight, measured at specific time intervals. Statistical analysis was performed to determine the significance of the effects (Yoon et al., 2010).

Data Analysis

Hair growth data were statistically analyzed using SPSS software, employing Analysis of Variance (ANOVA) with a significance level of $p < 0.05$ (Nursalam, 2016).

RESULT

Plant Identification and Extract Preparation

The plant sample was identified by Herbarium Medanense (MEDA) at Universitas Sumatera Utara under accession number 839/MEDA/2025, confirming its identity as Lantana camara L., belonging to the Verbenaceae family. This identification was crucial for ensuring the authenticity and correct species of the plant material used in the research. From 1200 grams of dried Lantana camara leaf powder, 148.36 grams of thick extract were successfully obtained, resulting in a yield of 12.36%. This yield indicates the efficiency of the extraction process. Organoleptically, the obtained viscous extract exhibited a blackish-green color, a characteristic odor, and a bitter taste.

Phytochemical Screening

Phytochemical screening of the Lantana camara leaf ethanol extract yielded positive results for all tested compound classes: flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. These findings are highly significant, as these compounds are known to possess diverse pharmacological activities relevant to the objectives of this study. The presence of flavonoids was indicated by the formation of an orange color, consistent with established literature (Harborne, 1987). Alkaloids were detected by the formation of a yellow precipitate in the Mayer test and an orange color in the Dragendorff test, according to (Depkes RI, 1995) guidelines. The saponin test produced a stable foam of 2 cm height after the addition of 2N HCl, confirming the presence of saponins (Depkes RI, 1995). The presence of tannins was demonstrated by a color change to blackish-green upon addition of $FeCl_3$ (Depkes RI, 1995). Meanwhile, steroid compounds were identified by the formation of a green solution (Harborne, 1987), distinguishing them from triterpenoids which typically appear red or purple.

Extract Characterization

Characterization of the extract was performed to ensure its quality and uniformity. The test results indicated: water content of 8%, ash content of 3.5%, acid-insoluble ash content of 10%, water-soluble extractive content of 24.6%, and ethanol-soluble extractive content of 9.3%. These parameters provide vital information regarding the quality, purity, and content of both water-soluble and ethanol-soluble components within the extract, all of which contribute to the stability and efficacy of the final preparation.

Antifungal Activity of Lantana camara Leaf Extract

The preliminary antifungal activity test of the Lantana camara leaf ethanol extract demonstrated varying inhibitory effects against Pityrosporum ovale, with the diameter of the inhibition zone increasing proportionally with extract concentration. At concentrations ranging from 10% to 20%, the inhibition zone diameters fell into the moderate category (9.0 mm – 10.1 mm). For concentrations of 25% to 50%, the inhibition zone diameters were categorized as strong (11.7 mm – 15.1 mm). Based on the criteria by (Baiti et al., 2018), an inhibition zone of 5-10 mm is classified as moderate, while 11-20 mm is strong (Baiti et al., 2018). These results served as a crucial basis for selecting the extract concentrations (15%, 20%, 25%) for the hair tonic formulation, aiming to achieve optimal antifungal effectiveness in the final product.

Evaluation of Lantana camara Leaf Extract Hair Tonic Preparation

Organoleptically, all hair tonic formulations (F0: without extract, F1: 15% extract, F2: 20% extract, F3: 25% extract) were liquid. F0 was clear, while F1, F2, and F3 exhibited a brownish-black color and a characteristic Lantana camara leaf odor. All formulas displayed good physical homogeneity, with no visible coarse particles.

pH Measurement

The pH value of the preparation is critical for its safety and comfort during application to the scalp.

Table 2.
pH Measurement

No.	Formula	pH
1	F0 (base)	6.98 ± 0.08
2	F1 (15%)	5.32 ± 0.01
3	F2 (20%)	5.28 ± 0.01
4	F3 (25%)	5.13 ± 0.01

All hair tonic formulas, including the base and those containing the extract, exhibited pH values within the range of 5.13-6.98. This range complies with the requirements of SNI 16-4955-1998 (pH 3.0-7.0), indicating that the preparations are safe and unlikely to cause scalp irritation due to excessively acidic or basic pH. The decrease in pH with increasing extract concentration is likely attributable to the acidic nature of some phytochemical components present in the extract (Febriani et al., 2016).

Viscosity Measurement

Viscosity measurement indicated that all hair tonic formulations had viscosity values within the range of 3.68-4.54 cP.

Table 3.
Viscosity Measurement

No.	Formula	Speed	Viscosity (cP)
1	F0 (base)	30 RPM	3.68 ± 0.01
2	F1 (15%)	30 RPM	4.02 ± 0.02
3	F2 (20%)	30 RPM	4.53 ± 0.03
4	F3 (25%)	30 RPM	4.54 ± 0.02

These viscosity values are below 5 cP, which classifies the preparations as liquid hair tonics and fulfills the quality requirement for good hair tonic viscosity. This liquid consistency facilitates ease of application to the scalp.

Microbial Contamination Test

Microbial contamination testing was conducted to ensure the safety of the preparations from microbial growth.

Total Plate Count (TPC)

Formulas 2 (20%) and 3 (25%) met the TPC requirements (maximum 10^{-5} colonies/gram) with values of 1.6 x 10^{-4} colonies/gram and 2.5 x 10^{-4} colonies/gram, respectively. However, Formula 1 (15%) did not meet the requirement, showing a value of 7 x 10^{-6} colonies/gram.

Identification of Staphylococcus aureus

All three formulas (15%, 20%, 25%) yielded negative results for Staphylococcus aureus, thereby meeting safety requirements.

Identification of Candida albicans

Formula 3 (25%) showed a negative result and met the requirements. Conversely, Formula 1 (15%) and Formula 2 (20%) did not meet the requirements, as Candida albicans was still detected at 181.5 colonies/gram and 49 colonies/gram, respectively.

The results of the microbial contamination tests indicate that the 25% extract concentration (F3) was most effective in controlling microbial growth, including fungi and bacteria, thus conforming to cosmetic safety standards. Lower extract concentrations (15% and 20%) demonstrated susceptibility to Candida albicans contamination, underscoring the importance of adequate extract concentration for effective antimicrobial action.

Preparation Stability (Cycling Test)

Accelerated stability testing (cycling test) was performed to predict the long-term stability of the preparations. After 6 cycles of temperature fluctuations (4°C and 40°C), all formulas (F0, F1, F2, F3) exhibited good stability. No crystal formation, changes in color, or alterations in odor were observed. Although a slight decrease in pH and viscosity occurred across all formulas, these values remained within the permissible range for hair tonic preparations. This indicates that the formulated Lantana camara leaf extract hair tonics possess adequate physical stability against temperature fluctuations (Febriani et al., 2016).

Preparation Irritation Test

An irritation test was conducted on rabbit skin using the highest extract concentration (F3: 25%) to ensure safety. Observations over 72 hours revealed no signs of erythema (redness) or edema (swelling) (Latifah et al., 2016). This indicates that the Lantana camara leaf extract hair tonic preparation, even at its highest concentration, did not cause skin irritation, rendering it safe for topical application. This finding is also applicable to the lower concentrations (15% and 20%).

Effectiveness on Hair growth (In vivo)

The efficacy of hair growth was evaluated in vivo using 24 male white rats, which were divided into 6 groups: normal, F0 (base), positive control (commercial hair tonic), F1 (15%), F2 (20%), and F3 (25%).

Hair Length Growth Results

Measurements of the average hair length of rats from Day 10 to Day 30 exhibited variations among the groups:

Table 4.
Results of average hair length

Group	Average Length (mm) ± SD		
	Day 10	Day 20	Day 30
Normal	6.97±3.31	8.41±2.50	15.74±5.39
F0 (base)	8.57±1.85	12.24±0.33	12.54±0.33
Positive Control (commercial hair tonic)	14.63±0.61	16.80±1.54	17.87±4.75
F1 (15%)	11.34±4.06	13.40±0.96	13.67±0.71
F2 (20%)	13.10±0.37	13.4±0.89	13.77±4.26
F3 (25%)	11.40±7.40	14.45±1.47	14.51±1.42

Hair length data for all treatment groups across Day 10, Day 20, and Day 30 demonstrated normal distribution and homogeneous variances, fulfilling the assumptions for ANOVA analysis. At Day 10, no significant difference in hair length was observed among treatment groups. At Day 20, a statistically highly significant difference in hair length was found among the groups. However, by Day 30, no statistically significant difference was detected. This outcome may indicate that the treatment effects reached a plateau or that data variability increased among group.

Hair Diameter Results

Measurements of rat hair diameter on Day 10, Day 20, and Day 30 also showed varying patterns:

Table 5.
Results of average hair diameter

Group	Average Diameter (mm) ± SD		
	Day 10	Day 20	Day 30
Normal	0.007±0.00	0.010±0.00	0.011±0.00
F0 (base)	0.007±0.00	0.016±0.01	0.016±0.01
Positive Control (commercial hair tonic)	0.007±0.00	0.011±0.00	0.017±0.01
F1 (15%)	0.007±0	0.008±0.00	0.014±0.00
F2 (20%)	0.008±0.01	0.01±0.00	0.01±0.01
F3 (25%)	0.007±0	0.016±0.11	0.02±0.02

The data showed an increase in the mean hair shaft diameter from Day 10 to Day 30 across all treatment groups. However, normality testing indicated that some groups did not follow a normal distribution; therefore, a non-parametric statistical approach was applied. The Kruskal–Wallis test revealed no statistically significant differences in hair diameter among the groups during the observation period. Further pairwise comparisons using the Mann–Whitney test consistently demonstrated no significant differences between any group pairs at each time point measured. Thus, it can be concluded that none of the formulations exhibited a statistically significant effect in increasing hair shaft diameter compared with the control group or with each other throughout the 30-day observation period.

Hair Weight Results

Measurements of the average hair weight of rats on Day 30:

Table 6.
Results of average hair weight of mice on day 30

Group	Average Hair Weight (grams) ± SD
Normal	0.045 ± 0.02
F0 (base)	0.021 ± 0.00
Positive Control (commercial hair tonic)	0.046 ± 0.00
F1 (15%)	0.038 ± 0.02
F2 (20%)	0.046 ± 0.01
F3 (25%)	0.046 ± 0.00

Hair weight data for all treatment groups were normally distributed and exhibited homogeneity of variances, fulfilling the assumptions required for parametric testing. ANOVA results revealed statistically significant differences in the average hair weight among the treatment groups. Post hoc analysis indicated that the normal group had significantly higher hair weight compared to the base and 15% hair tonic groups. In contrast, the commercial hair tonic, 20% hair tonic, and 25% hair tonic groups demonstrated average hair weights comparable to the normal group, with no statistically significant differences detected.

Antifungal Activity of Hair Tonic Against Pytirosporum ovale

Testing of the hair tonic preparations' antifungal activity showed that higher concentrations of Lantana camara leaf extract resulted in larger inhibition zone diameters against Pityrosporum ovale.

Table 7.
Results of hair tonic’s antifungal activity

Concentration (%)	Average Diameter (mm)
F0 (base)	0±0.00
F1 (15%)	8.7±0.17
F2 (20%)	9.5±0.20
F3 (25%)	10.7±0.17
Positive Control (commercial hair tonic)	11.0±0.00

Analysis of the results showed a highly statistically significant difference in the diameter of the inhibition zone among the treatment groups. This indicates that the type of treatment (formula concentration) was a dominant factor influencing the size of the inhibition zone. The negative control group exhibited no inhibition zone. All tested formula concentrations (F1, F2, F3) were proven significantly effective in inhibiting fungal growth compared to the negative control. There

was a clear and positive correlation, indicated by an increase in the diameter of the inhibition zone. It can be concluded that the 25% hair tonic (F3) demonstrated comparable effectiveness to the Positive Control. This is supported by the Games-Howell test results (p=0.270), which showed no statistically significant difference between these two groups. This indicates that the hair tonic with a 25% concentration possesses strong antifungal potential, with efficacy equivalent to the positive control used. The inhibitory power is characterized by the presence of a clear zone and is used to determine the strength level of the tested drug or preparation as an antifungal agent (Sari, 2023).

DISCUSSION

The antifungal assay confirmed that the ethanol extract of Lantana camara leaves possesses notable antifungal activity against Pityrosporum ovale, a yeast commonly associated with dandruff. The dose-dependent increase in inhibition zone suggests that higher extract concentrations enhance antifungal potency, likely due to a greater availability of active compounds such as flavonoids, tannins, and triterpenoids, which are reported to exhibit antifungal mechanisms by disrupting fungal cell membranes and inhibiting spore germination. The classification into moderate and strong inhibition categories validates the extract's potential as an antifungal agent. These findings provided the scientific rationale for selecting extract concentrations of 15%, 20%, and 25% in the formulation of the hair tonic, aiming to balance efficacy with formulation stability and safety.

The significant increase in hair growth observed on Day 20 suggests that Lantana camara extract actively stimulates hair follicle activity during the mid-phase of the treatment period. The lack of significant differences at Day 30 could be explained by the treatment reaching maximum efficacy, where hair growth had plateaued, or by increased inter-individual variability in response to the extract. The observed hair growth activity can be attributed to the bioactive compounds identified in the extract, particularly flavonoids and saponins. Flavonoids, with keratolytic and antioxidant properties, strengthen capillary walls and promote the transition of hair follicles from the telogen (resting) phase to the anagen (growth) phase, leading to denser and healthier hair growth. Saponins contribute by enhancing blood circulation around hair follicles, ensuring adequate nutrient delivery, and maintaining scalp health due to their natural cleansing properties. These compounds likely act synergistically to accelerate anagen activity and support hair elongation, consistent with the findings of (Koralina, 2023).

Although the mean hair shaft diameter showed a gradual increase from Day 10 to Day 30 across all treatment groups, the statistical analysis using non-parametric methods did not confirm any significant differences among the groups. This finding suggests that, while Lantana camara extract contains bioactive compounds with potential to strengthen hair structure, its effect on enhancing hair shaft thickness within a 30-day observation period could not be statistically demonstrated. One possible explanation is that the study duration was relatively short, as changes in hair diameter generally require longer periods to become measurable due to the extended nature of the hair growth cycle. In addition, biological variability among the experimental animals may have contributed to increased data dispersion, thereby reducing the likelihood of achieving statistical significance. It is also plausible that the main mechanisms of the active compounds, particularly flavonoids and saponins, are more closely associated with stimulating hair follicle activity and promoting elongation during the anagen phase rather than producing rapid changes in hair shaft thickness. Taken together, these factors indicate that while there is an observable positive trend, further studies with longer treatment durations and possibly adjusted formulation concentrations are required to validate the potential of Lantana camara extract in promoting hair shaft diameter.

The results indicate that hair weight was significantly reduced in the base and 15% hair tonic groups compared with the normal group, suggesting that a minimum concentration threshold of Lantana camara extract is required to exert a measurable effect on maintaining or promoting hair mass. Conversely, the 20% and 25% extract formulations, as well as the commercial hair tonic, were able to sustain hair weight at levels comparable to the normal group, demonstrating their relative effectiveness in preventing hair loss. These findings are consistent with the proposed mechanisms

of flavonoids and saponins, which enhance microcirculation around hair follicles, stimulate the anagen phase, and improve nutrient supply to developing hair shafts. The ability of higher concentrations of Lantana camara extract to maintain hair weight close to normal suggests a dose-dependent effect, further supporting its potential as an active ingredient in hair tonic formulations.

The presence of flavonoids, saponins, and tannins in the Lantana camara leaf extract, as identified in the phytochemical screening, can explain its hair growth-promoting activity. Flavonoids are known as keratolytic agents that can strengthen capillary walls and stimulate the transition of hair follicles from the telogen (resting) phase to the anagen (growth) phase, thereby initiating the growth of new, denser hair. Flavonoids also act as antioxidants, protecting hair follicles from damage and preventing hair loss. Saponins are believed to increase blood flow to hair follicles, which is essential for nutrient supply and preventing hair loss, and also act as natural cleansing agents that maintain scalp health. Meanwhile, tannins can protect hair proteins crucial for healthy hair growth. The synergistic combination of these compounds likely contributed to the observed increase in hair growth effects (Koralina, 2023).

CONCLUSION

In conclusion, this study successfully formulated a stable and safe Lantana camara L. ethanol extract hair tonic preparation that complies with SNI 16-4955-1998 standards. The 25% extract concentration (F3) demonstrated statistically comparable antifungal efficacy to the positive control. In in vivo testing, this preparation showed potential for stimulating hair length growth. This effect was most evident on Day 20, where the 25% hair tonic exhibited efficacy comparable to the commercial positive control product. The presence of flavonoids, saponins, and tannins in the extract is hypothesized to play a synergistic role in stimulating hair follicles, prolonging the anagen phase, and enhancing scalp blood circulation. By Day 30, the differences in hair length among groups tended to equalize. Overall, the Lantana camara leaf ethanol extract hair tonic, particularly at 25% concentration, presents a promising, safe, and effective natural alternative for hair care, possessing both anti-dandruff and hair growth-promoting capabilities. However, further research is needed to specifically isolate and identify which secondary metabolite compounds contribute most to the anti-dandruff and hair growth promoting activities. This will enable the development of more standardized formulas and a more focused hair tonic potential.

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