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FORMULATION OF AN ANTI-DANDRUFF SHAMPOO FROM HERBAL EXTRACT AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

Khadija Ali[1], Ruqayyah Moiz[2], Auj Zehra[3], Aleena Rauf[4], Said Miraj Ud Din[*5], Khushbakht Asad[6], Muhammad Sayaf[7], Muhammad Adnan Iqbal[*8], Ikram Ullah[9], Mushtaq Ahmad[10]

[1,3,4,*8]*Department of Chemistry, University of Agriculture Faisalabad, 38000, Pakistan*
[2]*Institute of Microbiology, Government College University Faisalabad*
[*5,7,9]*Department of Zoology, University of Shangla, Shangla, Khyber Pakhtunkhwa 19100, Pakistan*
[6]*Department of Zoology, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa 23200, Pakistan*
[10]*Institute of Chemical Sciences, University of Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan*

[1]khadijamajeed110@gmail.com,[2]ruqayyahmoiz92@gmail.com,[3]aujzehra1998@gmail.com, [4]aleenarauf24@gmail.com , [*5]saidmiraj@bs.qau.edu.pk, [6]khushbakht@awkum.edu.pk, [7]sayaf6466@gmail.com , [*8]adnan.iqbal@uaf.edu.pk/adnan.iqbal@gmail.com,[9]ikramicup.edu@gmail.com, [10]mushtaaq342@gmail.com

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Corresponding Author: *
Said Miraj Ud Din
Muhammad Adnan Iqbal

Abstract

Shampoos constitute a significant part of the cosmetic industry due to its importance in hair care. Dandruff is a prevalent scalp disorder affecting people globally of any gender and ethnicity. The aim of the study was to develop and test an herbal anti-dandruff shampoo based on Eucalyptus and Neem extract to treat dandruff, and to evaluate its antibacterial efficacy. Eight different shampoos formulations (F1-F8) with varying compositions of natural active ingredients were prepared. The formulations were tested in terms of their antibacterial activity at concentrations of 30%, 50%, 70%, and 100 % against Staphylococcus epidermidis and physicochemical properties as well as organoleptic properties such as pH, viscosity and foam volume were accessed. The data was statistically analyzed. The findings revealed that Eucalyptus and Neem extract had a high antibacterial efficacy without any observed adverse effects. Among all formulations, F7 has the highest anti-dandruff efficacy with a maximum zone of inhibition of 2.17 cm at 70 % concentration. The results indicate that the herbal shampoo preparations based on Eucalyptus and Neem can be effective and safe alternatives to the traditional synthetic anti-dandruff products such as selenium disulfide, zinc pyrithione and salicylic acids.

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Graphical abstract

INTRODUCTION

Hair is a multifunctional and biologically rich structure that experiences constant growth and shedding cycles, even though it is externally inert (Stanic et al., 2015). The follicle is the living part of hair and it is located under the epidermis and produces hair, whereas the part of the hair that is seen is called the shaft and is made up of keratinized cells (Gray, 2017; Grymowicz et al., 2020). The scalp is a very sensitive and specialized region of the skin characterized by a large number of sebaceous glands and hair follicles and therefore most prone to microbial imbalance and inflammatory processes (Martel et al., 2024). Poor scalp hygiene may result in excessive sebum production and moisture retention, which forms the environment in which different scalp disorders are likely to develop (Narshana and Ravikumar, 2018; Sravanthi et al., 2021). Approximately three out of four adults have had at least one type of scalp-related disorder in their lifetime, such as dandruff, seborrhea, psoriasis, folliculitis, alopecia, and fungal or parasitic infections (Dreno et al., 2019; Chang et al., 2020). Of all these, dandruff and seborrheic dermatitis are the most widespread and occur mostly in seborrheic parts of the head (Borda and Wikramanayake, 2015). Dandruff influences about 50 % of the global adult population, and its prevalence is higher among males, especially in the age range of 20-50 years (Manuel and Ranganathan, 2011; Sheth and Dande, 2021). Clinically, dandruff is also known as pityriasis capitis or seborrhea sicca, is a chronic, recurring condition that is both microbial and host-mediated. Traditionally, the pathogenesis of dandruff was associated with the proliferation of the species of the Malassezia, especially with *Malassezia restricta* and *Malassezia globosa* (Park et al., 2017). The occurrence of a higher number of *Staphylococcus epidermidis* and decreasing Cutibacterium acnes have been regularly observed in dandruff-affected scalps and are estimated to facilitate inflammation and aid fungal overgrowth (Wang et al., 2015; Park et al., 2017; Tao et al., 2021). This change in scalp microbiome indicates that the treatment of dandruff should go beyond eliminating fungal colonization to restoring bacterial balance. Standard anti-dandruff treatments are based on singular antifungal, antibacterial, anti-inflammatory, or keratolytic drugs, which include zinc pyrithione, selenium disulfide, and salicylic acid (Thomas and Khasraghi, 2020).

Although such agents are clinically effective while prolonged use is frequently linked to negative effects such as scalp irritation, dryness, and disturbance of the natural microbiota, especially in patients with sensitive skin (Narshana and Ravikumar, 2018).

Frontier in Medical & Health Research

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These restrictions have led to a growing fascination with milder, vegetative alternatives embedded in the shampoo formula, which is the main and most acceptable topical delivery system to treat the scalp (Gubitosa et al., 2019; Malpani et al., 2020). Plant-based bioactive have received significant interest in cosmetic and pharmaceutical research because they exhibit antimicrobial activity, biocompatibility, and have good safety profiles (Azadbakht et al., 2018). Among them, Eucalyptus and neem species are abundant in essential oils and phenolic compounds with known antibacterial and antifungal effects (Ghasemian et al., 2019; Abelan et al., 2022). However, limited studies are available to evaluate their use in shampoo formulations for controlling bacteria associated with dandruff. Thus, the current research was to develop and test herbal anti-dandruff shampoo formulations from Eucalyptus and neem extract in a combination with natural organic components and to access its antibacterial efficacy against *Staphylococcus epidermidis*.

MATERIALS AND METHODS

Plant extracts, essential oils, and selected organic excipients were used to formulate an herbal anti-dandruff shampoo. Formulation was split into three steps; aqueous phase, oil phase and cool-down phase. All ingredients were weighed using a calibrated digital analytical balance for shampoo formulation. The oil and aqueous phases were made separately and subjected to the same temperature (50°C) to allow equivalent melting and solubilization of the components. The oil phase was added into the aqueous phase gradually under constant mechanical stirring at 1500 rpm to ensure a homogeneous and constant emulsion formation before reaching the desired temperature. After the emulsification process, the formulation was left to cool at room temperature, and then the addition of heat-sensitive ingredients, such as antioxidants, active plant extracts, essential oils, and preservatives, was performed with gentle stirring. The pH was adjusted to the required physiological range with the help of an appropriate acid. Plant extracts and essential oils were prepared in house to be used in the shampoo formulations. A solvent extraction of leaves of *Eucalyptus spp*. and *Azadirachta indica* (neem) was conducted using a Soxhlet apparatus, where distilled water and methanol were used as solvents in extraction. Eucalyptus and neem aqueous extracts had yield percentages of 7.54 and 7.03 respectively, whereas the methanolic extract percentages were 9.34 and 9.01 respectively. The necessary oils were extracted through a conventional distillation process and kept under favorable conditions until they were added to the shampoo formulations. Different combinations of extracts and essential oils were incorporated into the shampoo base, and seven anti-dandruff formulations were designed as shown in Table 1 (Malviya and Sharma, 2014).

Table 1. Formulation of shampoo with different active ingredients a (F1= Basic Formulation, F2-F8 = Anti-dandruff formulations)

Ingredients (grams)	F1	F2	F3	F4	F5	F6	F7	F8
Distilled Water	2.5	----	----	----	----	----	----	----
Eucalyptus Extract (Methanol)	----	0.65	1.25	----	2.5	1.25	----	2.5
Eucalyptus Extract (Water)	----	1.25	1.25	2.5	----	1.25	2.5	----
Neem Extract (Methanol)	----	0.65	----	----	----	----	----	----
Surfactants	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Base oil	0.3	----	----	----	----	0.3	0.3	0.3
Eucalyptus Essential oil	----	0.3	0.3	0.3	0.3	----	----	----
Emulsifier	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Stabilizer	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Conditioning Agent	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Preservative	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Frontier in Medical & Health Research

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Characterization and Quality Evaluation of Shampoo Formulations

Shampoo preparations formulated were put under a sequence of quality control and characterization tests as per the standard cosmetic evaluation protocols. They involved organoleptic analysis, physicochemical analyses to stabilize the formulation, performance and acceptability by the consumer. To measure the aesthetics quality and the physical appearance of the shampoo formulations, organoleptic properties of the shampoo formulations such as color, odor, clarity, and texture were assessed (Umar et al., 2020). The physicochemical characterization of the shampoo formulations was conducted with the help of the known protocols and the establishment of the pH, foam volume, viscosity, and the performance parameters. The pH of every shampoo formulation was measured at a digital pH meter which was calibrated. To do this, each of the formulations was diluted with distilled water in 10% (w/v) and the pH was measured in triplicate (Malviya and Sharma, 2014; Revansiddappa et al., 2018).

The foaming capacity was measured by the cylinder shake technique (Klein, 2004). In a few words, 5mL of each shampoo formulation was pooled to 50 mL graduated cylinder and diluted with distilled water. The volume of foam obtained was measured and noted after 5 min of shaking after vigorous shaking of the cylinder during 4 to 5 min (Umar et al., 2020). The viscosity was measured with the help of a Brookfield viscometer (DV-II+ Pro) equipped with a spindle number 61. Measurements were taken at rotational speeds of 3, 5, 12, 30 and 60 rpm to measure flow behavior. All the measurements were obtained in three replicates, and the viscosity value was reported in centipoise (cps) (Revansiddappa et al., 2018; Umar et al., 2020).

Antimicrobial Testing

Agar well diffusion method based on a standard protocol was used to determine the antimicrobial activity of active ingredients and shampoo formulations. The isolate of *Staphylococcus epidermidis* was obtained from the scalp of a dandruff patient as outlined in the Supplementary File. Samples of dandruff were picked aseptically at the vertex and crown areas on sterile cotton swabs, inoculated on mannitol salt agar and allowed to incubate at 37 °C over 48 h. Gram staining, microscopic observation, and biochemical tests, such as catalase, citrate, indole, Voges Proskauer, sugar fermentation, urease, and oxidase tests, were used to determine the isolates (Saxena et al., 2018; Shivamallu et al., 2021). A nutrient agar was made and inoculated with *Staphylococcus epidermidis* and sterilized. Each test sample was placed in 100 µL into wells with a diameter of 1 cm, and ciprofloxacin was used as the positive control. Plates were incubated at 37°C during the period of 24 hours and zones of inhibition (ZOI) were calculated (Umar et al., 2020). The same protocol was used to test seven available formulations of shampoos against *Staphylococcus epidermidis* at 30, 50, 70 and 100% concentration.

Statistical analysis

All the results were calculated in triplicate, and their means ± SDs were calculated. All the statistical analysis was conducted using Microsoft Excel version 16 (Umar et al., 2020).

RESULTS

Characterization of Shampoo

Organoleptic parameters

The physical characteristics of every shampoo formulation were analyzed. The color of formulation F1 was white because no active plant extracts were used in the formulation; the color was largely determined by the stabilizers and emulsifiers contained in the base formulation. Conversely, formulations F2-F8 had minor color differences, which were explained by the addition of various plant extracts. All preparations had a pleasant smell: F2 F8 exhibited a strong eucalyptus order because of the presence of eucalyptus and neem essential oil and extracts. Besides, all the formulations had a smooth and creamy texture with pleasant odor (Table 2).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Physiochemical parameters

pH Measurement

A digital pH meter was used to determine the pH of each of the shampoo formulation. All the formulations had pH in the range of 5-6. Triplicate measurements were done and mean and standard deviations were calculated (Table 2).

Table 2. Organic parameters of shampoo F1-F8

S. No.	Biochemical test	Result of isolated bacteria	Results in literature (Langlois et al., 1990; Sah et al., 2018)
1	Catalase test	+	+
2	Citrate test	-	+
3	Indole test	-	-
4	VP test	+	-
5	Sugar fermentation test	+	+
6	Oxidase test	-	-
7	Urease test	+	+

Viscosity Measurement

It was determined that the viscosity of the formulated shampoos was varies at various spindle speeds. The values of viscosity were between 4015.4 cP and 4160.3 cP. Although, the viscosity differences between the test with the basic formulation (F1) and the ones with the active ingredients (F2-F8) was minimal.

Foam Volume

The cylinder shake was used to measure foam volume. The foam volumes of all the formulations were 25-27 mL, which was measured after 1, 5, and 10 minutes. The level of the foam did not change significantly with time, which was an indication of the foam being formed consistently.

Testing antimicrobial activity

The bacterial isolate of the dandruff-infected scalp samples grew creamy greyish-white colonies following incubation. Gram staining identified the bacteria as Gram-positive cocci forming grape-like clusters. Table 3 showed the result of the biochemical tests and confirmed that the isolate was *Staphylococcus epidermidis*.

Tables 3. Biochemical Characterization of Shampoo Various Formulation (F1-F8).

S. No.	Shampoo Formulations	Color	Odor	Texture	pH
1	**F1**	White	Pleasant	Creamy	5.7±0.0
2	**F2**	Greenish Brown	Pleasant	Creamy	5.6±0.0
3	**F3**	Light Brown	Pleasant	Creamy	5.5±0.1
4	**F4**	Dark Brown	Pleasant	Creamy	5.6±0.0
5	**F5**	Light Brown	Pleasant	Creamy	5.5±0.2
6	**F6**	Greenish Brown	Pleasant	Creamy	5.6±0.0
7	**F7**	Greenish Brown	Pleasant	Creamy	5.6±0.0
8	**F8**	Light Brown	Pleasant	Creamy	5.6±0.0

Volume 4, Issue 1, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Active Ingredients

Necessary oils and plant extracts have been tested against *Staphylococcus epidermidis* by agar-well diffusion method. Essential Oils i.e., Eucalyptus oils (a commercial extract of the oil) were tested in a concentration of 2 percent, 3 percent, and 5 percent. Inhibition zones were measured and findings are summarized in Figure 1.

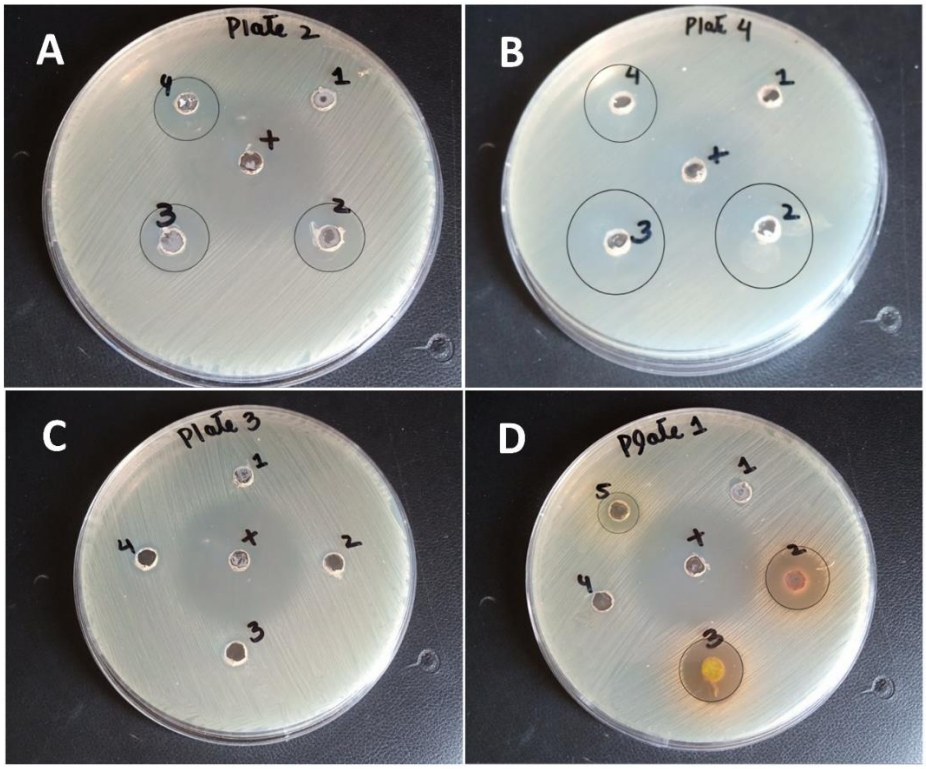

Figure 1. Plate showing inhibition zones against *Staphylococcus epidermidis* (A) & (B), neem oil (C), and herbal extracts (D).

Neem essential oil was inactive at the 2% and 3%, whereas a small diameter of inhibition (0.1 cm) was detected by using the 5% concentration (Figure 1C). Eucalyptus and neem leaf extracts were made using water and methanol and tested against *Staphylococcus epidermidis*. There was a higher area of inhibition in Eucalyptus extracts than neem extracts. Neem aqueous extract exhibit insignificant antibacterial activity (Figure 2 & Figure 3).

Evaluation of Anti-dandruff Activity

Eight shampoo formulations (F1–F8) were subjected to testing in 30, 50, 70, and 100 percent concentration to *Staphylococcus epidermidis*. Measurement of zones of inhibition was done and the result is given in Figure 4. All preparations exhibited antibacterial activity to differing degrees. Neem oil did not show any activity against *Staphylococcus epidermidis* at 2% and 3% concentrations as shown in Figure 1(C) and Figure 2. It demonstrates a zone of inhibition of 0.1 cm at 5% against *Staphylococcus epidermidis*. After interpreting these results, eucalyptus essential oil (E1) was selected as an active ingredient in shampoo because it showed sufficient activity against bacteria even at lower concentration. Eucalyptus extract showed a maximum zone of inhibition of 2.67 cm and 2.33 cm against *Staphylococcus epidermidis* in comparison to neem extract as shown in Figure 1(D) and Figure 3. The zone of inhibition for neem extract in methanol was 1.5 cm.

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

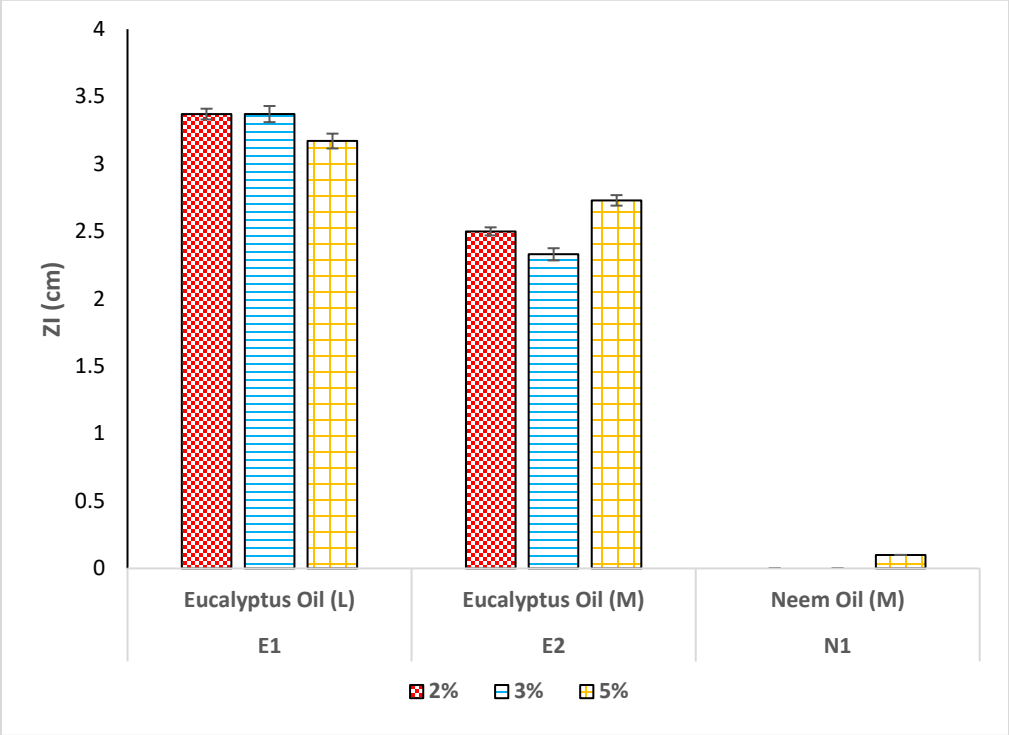

Figure 2. Graphical representation of anti-bacterial activity of essential oils. L= lab extracted, M= Market oil.

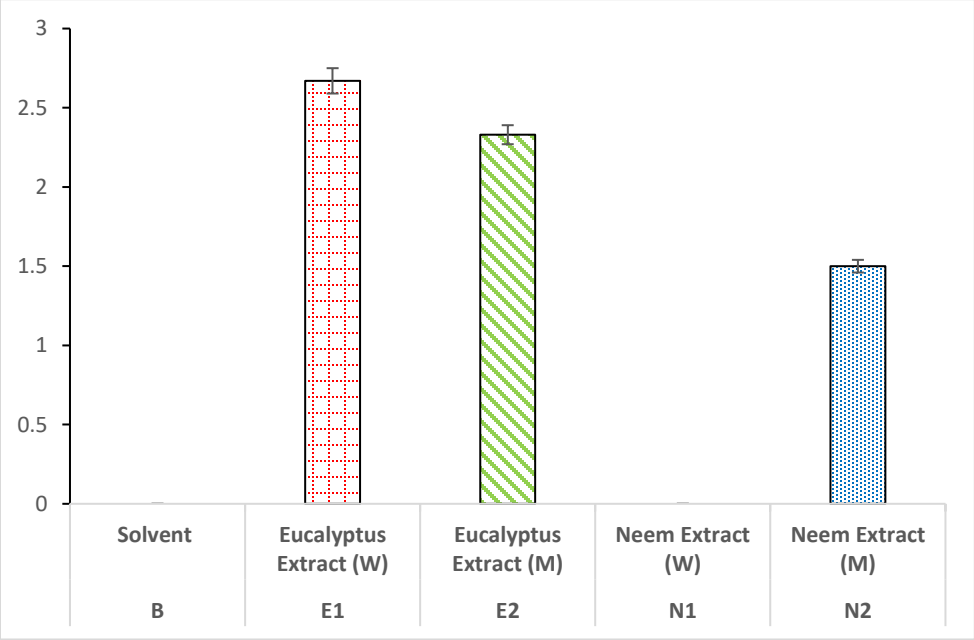

Figure 3. Graphical representation of anti-bacterial activity of eucalyptus and neem extracts.

Anti-dandruff activity of Shampoo

Eight shampoos formulations (F1-F8) were tested on the antibacterial activity using *Staphylococcus epidermidis* and titrations of 30, 50, 70 and 100 percent. Basic formulation F1 exhibited the inhibition zones of 1.3, 1.67, 1.43 and 1.53 cm respectively. The inhibition zones of Formulation F2 were of 1.9, 1.87, 1.87, and 1.87 cm and F3 1.57,

Frontier in Medical & Health Research

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1.8, 2.07, 2.03 cm, F4 1.47, 1.57, 1.93, and 1.63 cm, and F5 1.73, 2.03, 2.13, 2.0 cm respectively. Formulation F6 exhibited an inhibition behavior of 1.63, 1.83, 1.8, and 1.93 cm, Formulation F7 exhibited an inhibition behavior of 1.83, 2.17, 2.07, and 2.03 cm. Formulations F3 (2.07 cm), F5 (2.13 cm), and F7 (2.17 cm) showed the highest antibacterial activity at 70% concentration (Figure 4; Table 4)

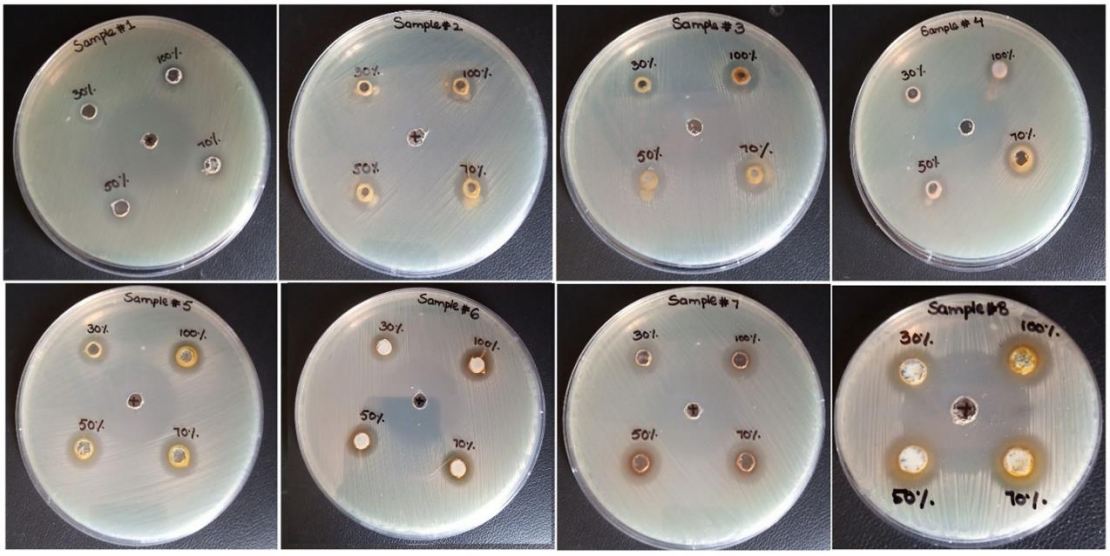

Figure 4. Plates (F1-F8) showing inhibition zones of various formulations of shampoo *against Staphylococcus epidermidis*.

Table 4. Activity of different formulations of shampoo against *Staphylococcus epidermidis*

Formulation of Positive Control Shampoo	(cm)	30% (cm)	50% (cm)	70% (cm)	100% (cm)
F1	3.3 ± 0	1.3 ± 0	1.67 ± 0.12	1.43 ± 0.06	1.53 ± 0.06
F2	3.3 ± 0	1.9 ± 0.1	1.87 ± 0.06	1.87 ± 0.21	1.87 ± 0.21
F3	3.3 ± 0	1.57 ± 0.06	1.8 ± 0	2.07 ± 0.06	2.03 ± 0.06
F4	3.3 ± 0	1.47 ± 0.06	1.57 ± 0.06	1.93 ± 0.06	1.63 ± 0.06
F5	3.3 ± 0	1.73 ± 0.12	2.03 ± 0.06	2.13 ± 0.06	2.0 ± 0
F6	3.3 ± 0	1.63 ± 0.06	1.83 ± 0.06	1.8 ± 0.1	1.93 ± 0.06
F7	3.3 ± 0	1.83 ± 0.06	2.17 ± 0.06	2.07 ± 0.06	2.03 ± 0.06
F8	3.3 ± 0	1.57 ± 0.06	1.57 ± 0.06	1.93 ± 0.06	1.97 ± 0.06

DISCUSSION

The present study presents the use of herbal extract in shampoo formulation and evaluating its antibacterial activities. Antibacterial activities against *Staphylococcus epidermidis*, physicochemical parameters of various shampoo formulations were accessed. The color differences between the shampoo formulations that contain plant extracts was explained by the existence of phenolic compounds and other bioactive constituents that were extracted by the polar solvents, including water and methanol (Arisandi et al., 2019). The presence of terpenoids

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

and flavonoids, which are recognized phytochemical constituents of *Azadirachta indica* (neem) leaves, caused the greenish coloration of the formulations of the neem-based product (Ujah et al., 2021). Eucalyptus essential oil and formulation excipients, such as emulsifiers and thickeners, affected the pleasant odor and smooth consistency of all the formulations, as they may help decrease the product stability and sensory characteristics (Sharmeen et al., 2021; Sravanthi et al., 2021). All of the formulations had pH values within the range of 5 to 6, which is believed to be the best range to keep the scalp healthy and hair intact. Slight acidic shampoos can be used to maintain the natural scalp barrier and to avoid cuticle injury in hair, but alkaline compositions can cause hair frizz and irritation (Kumar & Mali, 2010; Manisha et al., 2013).

The pH was measured as normal within the physiological range of pH of the scalp, which ranges around 5 (AlQuadeib et al., 2018). There were slight differences in viscosity between the baseline formulation and the formulations added with essential oils. These variations are due to the incorporation of essential oils, which have been known to affect rheological behavior of shampoo formulations when applied within a reasonable concentration of essential oils (Umar et al., 2020). The volume of foam generated by all formulations was consistent, which demonstrates that surfactants were the key contributors to the formation of the foam, whereas antimicrobial activity was not affected by the lathering capacity (Rolls, 2020). Morphological characteristics of the isolated microorganism were characterized as *Staphylococcus epidermidis* through colon morphology, gram staining, and biochemical test. These results aligned with previously known features of *Staphylococcus epidermidis*, which is a Gram-positive bacterium that is usually linked to dandruff and scalp diseases (Langlois et al., 1990; Sah et al., 2018). The efficacy of the Eucalyptus essential oil as an antibacterial agent was also better than the efficacy of neem oil and this can be explained by the high level of bioactive monoterpenes which include 1,8-cineole. These are substances that are known to interfere with the cell membrane of bacteria and prevent the growth of microbes (Ghasemian et al., 2019; Abelan et al., 2022). The low antibacterial activity at high level of oil in question can be explained by decreasing the diffusion of oil components in the agar medium.

Neem oil was only observed to be carrying out antibacterial activity at higher concentrations, as previously reported (Kang et al., 2021). Eucalyptus leaf extracts demonstrated better antibacterial activity than neem extracts among plant extracts. The increase in activity can be explained by the presence of phenolic compounds and essential oil residues in the leaves of the Eucalyptus (Yaseen, 2016; Elansary et al., 2017; Devi et al., 2019). The lack of antibacterial effect of Neem aqueous extract can be attributed to the inefficient extraction of active constituents, changes in the phytochemical profile or environmental factors, including climate and harvesting period (Arora and Kaur, 1999; Gul and Bakht, 2015; Prakash et al., 2007). The simple shampoo formulation (F1) showed antimicrobial activity because of the existence of surfactants like coco glucoside, which has been known to have intrinsic antimicrobial activities (Revansiddappa et al., 2018; Boonprakobsak and Rachtanapun, 2022).

The anti-bacterial activity of the formulations that included Eucalyptus essential oil and extract was superior, which showed that bioactive molecules, including 1,8-cineole, limonene, and pinene, work synergistically (Dhakad et al., 2018; Ghasemian et al., 2019). F3, F5, and F7 were the most active formulations as they showed the greatest antibacterial properties, especially when used in a 70% concentration. The enhanced activity of these formulations may be explained by the synergistic or individual actions of eucalyptus essential oil and extracts that have an effective effect in inhibiting the growth of *Staphylococcus epidermidis*. Notably, the highest zone of inhibition was observed with formulation F7 and this indicates that the aqueous extract of eucalyptus is highly effective in the control of the bacteria that cause dandruff.

Conclusion and recommendations

The present study demonstrated plant-based formulation of shampoo and its acti-bacterial activities against *Staphylococcus epidermidis* using eucalyptus and neem extracts. The formulated shampoo (F3, F5 and F7) exhibited significant anti-bacterial activities, supporting the use of eucalyptus

Frontier in Medical & Health Research

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for managing scalp related microbial infections. The highest inhibitory anti-bacterial efficacy was observed at F7 formulation with the highest concentration of 70% (2.17 cm zone of inhibition). The study highlights the use of eucalyptus extract in developing safe, eco-friendly, anti-dandruff products having acti-bacterial efficacy. The finding suggest plant based herbal shampoo can serve as natural and effective alternative to synthetic products. Further, research should be conducted to optimize plant-based act-dandruff shampoo to enhance its antibacterial activities. Dermatological safety assessments such as skin irritation, and sensitization tests should be conducted to ensure long term use and safety. Volunteers based researches are suggested with following standard ethical consideration.

Authors contributions

The research study was designed by K.AL, RM, and MAI. Laboratory work was conducted by K.Al, RM, AZ, AR. Images were captured and graphs were created by KAl. Manuscript was written by KA SMUD, KAS, MS and IU. Manuscript was edited by MA. The research supervisor was MAI. SMUD also played a role in manuscript formatting and continuance guidance to the authors.

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Conflict of interest

The authors declare no conflict of interest.

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Frontier in Medical & Health Research

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