Available online at https://journal.rescollacomm.com/index.php/ijeer/index

International Journal of Ethno-Sciences and Education Research

e-ISSN 2776-7590

Vol. 3, No. 1, pp. 14-20, 2023

Learning About Evaluation and Antifungal Activity of Simpur Leaf Extract Shampoo (*Dillenia Indica L*) Towards Candida Albicans Causes Dandruck

Fahmi Sidiq[1*], Hery Fauzi[2]

[1,2] *Pharmacy Study Program, Faculty of Health Sciences, Universitas Perjuangan Tasikmalaya, Indonesia*

**Corresponding author email: fahmisidiq78@gmail.com*

Abstract

Simpur leaf (*Dillenia indica L*) is a plant that contains polyphenolic compounds, flavonoids, steroids, terpenoids, tannins and saponins that function as antifungal agents. Candida albicans is a fungus that can cause skin candidiasis. The purpose of this study was to formulate simpur leaf extract into a shampoo dosage form by testing its antifungal activity. The shampoo preparations were made in 3 formulas with varying concentrations of 5%, 10% and 20% then an evaluation was carried out which showed the shampoo preparations had met the requirements such as organoleptic, homogeneity, pH, viscosity, foam height, stability and hedonic tests. The antifungal activity test used the well method with negative control without the addition of extract and 2% ketoconazole was used as a positive control. Tests of simpur leaf extract against the fungus Candida albicans showed a concentration of 20% had the largest inhibitory diameter of 11.55 mm compared to concentrations of 5% (6.398 mm) and 10% (8.375 mm). While the shampoo preparation test showed that F3 with a concentration of 20% had the largest inhibition zone diameter of 12.008 mm compared to F1 and F2 with a concentration of 10% (9.775%) and 5% (6.725 mm). The negative control had an inhibition zone of 2.13 mm and the positive control had an inhibition zone of 13.78 mm.

Keywords: Anti-Fungal, Candida albicans, Leaf Simpur (*Dillenia indica L*), Shampoo Preparation

1. Introduction

Hair is one part of the body that can provide beauty and protection to the scalp. There is a skin disease on the head, one of which is caused by the condition of the skin that is moist or sweats easily. Uncontrolled self-care and a lack of understanding about maintaining health are the main causes of growing fungal diseases (Ebrahim & El Gaali, 2015).

Dandruff is caused by excessive exfoliation of scalp cells because the keratinization process is not perfect, so the symptoms are white scales and can cause the scalp to become dirty and smell bad and can even cause hair loss (Rajput, 2015). As for biological factors that can cause a person to get dandruff, namely genetic factors, fatigue, geographical location in Indonesia with a tropical climate, stress and fast skin growth (Al Badi & Khan, 2014). Meanwhile, according to (Narshana & Ravikumar, 2018) dandruff is caused by excessive sweat gland secretion or the role of microorganisms that make a metabolite that can induce the formation of dandruff. The fungus Candida albicans is one of the causes of dandruff (Tao & Wang, 2021). Candida albicans is a species of pathogenic fungus from the ascomycota group. This species of fungus can cause opportunistic infections or candidiasis of the skin, mucosa and internal organs of humans.

One medicinal plant that has the potential to be used as an alternative to anti-dandruff shampoo preparations is simpur leaf extract (*Dillenia indica L*). The simpur plant or the Latin name *Dillenia indica L* is a plant that is commonly found in Indonesia, such as the islands of Java, Kalimantan and Sumatra. *Dillenia indica* belongs to the *Dilleniaceae* family, has sixty *Dillenia* genera with the widest distribution area (Gholamreza et al., 2011).

Simpur is a plant that has very dense leaves and large and quite a lot of fruit. Traditionally, people usually use various kinds of simpur such as wood for fuel, fruit and leaves used as anti-dandruff shampoo. According to (Gholamreza et al., 2011) simpur leaves contain secondary metabolite compounds, namely alkaloids, polyphenols, flavonoids, steroids, terpenoids, tannins and saponins.

Saponins and flavonoids are included in the phenol group which states that phenol and phenolic compounds are strong antifungal elements at concentrations commonly used (1-2% water solution). Phenols and their derivatives can cause protein denaturation, saponins are known to have antifungal properties while flavonoids can damage cell membranes (Halith et al., 2009). Terpenoids including triterpenoids are bioactive compounds that function as

antifungals. These terpenoids can inhibit fungal growth, both through the cytoplasmic membrane and interfere with the growth and development of fungal spores (Yadav & Mohite, 2020).

Based on research conducted by (Alam et al., 2011) that simpur tree bark at concentrations of 5%, 10% and 20% was able to inhibit the growth of the fungus Candida albicans with an inhibition zone of 13.13 mm. Several studies have also been conducted, simpur leaves have pharmacological effects as anti-oxidants and have cytotoxic activity (Gholamreza et al., 2011). Other research states that the simpur plant (*Dillenia indica L*) has anti-oxidant and anti-bacterial properties. Simpur leaf plants have also been studied to provide anti-fungal (anti-fungal) effects against C. Albicans, Aspergillus niger, and Saccharomyces cerevisiae as well as anti-inflammatory effects (Wibawa & Lugrayasa, 2021).

Shampoo is a cosmetic preparation in the form of liquid, emulsion, gel or aerosol which contains surfactants and has humectant detergent properties that produce foam. Shampoo aims to clean the hair and scalp to make it clean, as soft, manageable and shiny as possible (Yadav & Mohite, 2020).

In addition to the active ingredients of Simpur leaves, the shampoo ingredients that play an important role are surfactants, the reason being that surfactants are substances that can clean hair, because their molecular structure which consists of hydrophilic and lipophilic parts, has the ability to lower the surface tension between water and dirt so that it is suspended into the aqueous phase. (Wibawa & Lugrayasa, 2021).

Sodium lauryl sulfate and cocamide DEA were used as surfactants in this study. Sodium lauryl sulfate is an anionic surfactant which is often used in the manufacture of shampoos which has cleansing power and creates lather. At a concentration of 10%, sodium lauryl sulfate has excellent foaming power, but the disadvantage is that it can irritate the skin, so it must be added with a combination of nonionic secondary surfactants. One of the nonionic secondary surfactants that is suitable for use with sodium lauryl sulfate is cocamide DEA (diethanolamine), which can increase viscosity and form finer foam and form a dissolving process without irritating the skin. The advantages of cocamide DEA are that it can increase the stability of the preparation, is not toxic, has good compatibility with the skin and mucous membranes so that it can be used on sensitive skin (Wibawa & Lugrayasa, 2021).

2. Materials and Methods

2.1. Tools and materials

The tools used in this study included a rotary evaporator, mortar, stamper, water bath, stir bar, 500 ml beaker glass (pyrex), test tube (pyrex), blender (Omega), pan, filter, spatula, analytical balance, parchment, watch glass, dropping pipette, petri dish (NORMAX), porcelain cup, pH meter (ATC), shampoo bottle (30 ml carex bottle), aluminum foil, 500 ml measuring cup (pyrex), glass jar , scissors, Laminar air flow (LAF), spirit lamp, paying paper, magnetic stirrer (IKA C-MAG HS 7), Mattress thread, wire loops, autoclave (All American), incubator.

The ingredients used include simpur leaf extract (*Dillenia indica L*), 96% ethanol (DPH), sodium lauryl sulfate (DPH), cocamide DEA (DPH), CMC (DPH), methyl paraben (DPH), propylene glycol (DPH). , citric acid (DPH), menthol (DPH) and distilled water, dragendroff reagent, mayer reagent, $FeCl_3$, H_2SO_4 concentrated, magnesium powder, PDA medium (potato dextrose agar) ammonia, ketoconazole shampoo, Mg powder, 2 N hydrochloric acid, concentrated HCl , reagent, nutrient agar, chloroform, fungus Candida albicans.

The simpur plant was obtained from Sukasuka Village, Tanjungjaya District, Tasikmalaya Regency, West Java. Plant determination was carried out at the Herbarium for the School of Life Technology (SITH) Bandung Institute of Technology number 775/ITI.C11.2/TA.00/2022. This study used the Candida albicans fungus with ATCC 11537 obtained from the Parasitology Laboratory, Faculty of Medicine, University of Indonesia.

2.2. Research Path

1. The sample used was simpur leaves (*Dillenia indica L*) which were obtained from Bebedahan Village, Sukasuka Village, Tanjungjaya District, Tasikmalaya Regency.
2. Plant determination aims to test the correctness of plants specifically by identifying parts of the plant to determine more distinctive characteristics. Plant determination was carried out in the Laboratory at the Herbarium of the College of Life Sciences (SITH) ITB.
3. The sample used is simpur leaves (*Dillenia indica L*) as much as 5 kg of leaves then wet sorting, washing, drying in the sun, dry sorting, mashed using a blender then sieved using a 60 mesh.
4. 500 g of Simplisia simpur leaves were extracted using 5 liters of 96% ethanol or 1: 5 using the maceration method. Then let it stand and stir for 8 hours and the solvent is replaced every 24 hours for 3 days. The results of the macerate obtained were then concentrated using a rotary evaporator and then evaporated using a porcelain cup over a water bath until a thick mass was obtained. Furthermore, the extract results obtained were weighed and the yield value was calculated, then a phytochemical screening test was carried out for the powder and the extract (Kaur & Arora, 2009)
5. Listed in Table 1 is the design of ingredients and formulations of shampoo preparations:

Table 1: Shampoo formula.

Ingredients	Formula 0	Formul 1	Formula 2	Formula 3
Simpur leaf extract	-	5%	10%	20%
Sodium lauryl sulfat	8%	8%	8%	8%
Cocamide DEA	4%	4%	4%	4%
CMC	3%	3%	3%	3%
Metil Parben	0.15%	0.15%	0.15%	0.15%
Menthol	0.5%	0.5%	0.5%	0.5%
Citric Acid	O.3%	0.3%	0.3%	0.3%
Propylene glycol	10%	10%	10%	10%
Aquadest ad	ad 30 ml	ad 30 ml	ad 30 ml	ad 30 ml

6. All ingredients are weighed beforehand according to the formulation. CMC was first developed in hot water in a mortar (M1). Then dissolve the methyl paraben with a few drops of ethanol until it dissolves (M2). Then the distilled water is heated on a hot plate at 600C and add sodium lauryl sulfate, stir until homogeneous. Cocamide DEA is added into it while continuing to stir until the liquid thickens (M3). The distilled water fraction of Simpur leaves was mixed into M3 and stirred until homogeneous. Then citric acid is added to the shampoo solution which was previously dissolved using a few drops of ethanol and then stirred. Then the shampoo solution is cooled and menthol is added which has been dissolved with a few drops of ethanol and stirred. Diluted with distilled water up to 30 ml and stirred until homogeneous (Alam et al., 2011).
7. Evaluation of shampoo preparations includes organoleptic test, homogeneity test, pH test, viscosity test, foam height test and hedonic test.
8. Antifungal Activity Test
   a. Preparation of PDA media (Potato Dextrose Agar)
      PDA media was weighed in the amount of 9.75 grams of PDA media then put into 250 ml Erlenmeyer and dissolved in 250 ml of distilled water then stirred until the resulting suspension was homogeneous, then heated on a hot plate until boiling or the color of the media changed to clear, then sterilized using an autoclave at room temperature. 121°C for 15 minutes (Ethics, 2019).
   b. Preparation of Mushroom Suspension
      Candida albicans is cultured in agar media slanting and suspended with 3 ml of NaCl, then taken as needed and then put in the seeding medium. Then it is mixed and the turbidity is adjusted according to Mc. Farland's solution (Földes et al., 2020).
   c. Preparation of Anti Fungus Testing Samples
      The samples used in this test were preliminary testing of simpur leaf extract on fungal growth with concentrations of F1 (5%), F2 (10%) and F3 (20%), and testing of simpur leaf extract shampoo preparations with concentrations of F1 (5%), F2 (10%) and F3 (20%). Preparation of anti-fungal from simpur leaf extract by means of pure extract results that have been obtained then concentrations (5%), (10%) and (20%) (v/v) in 30 ml preparations. To make a 5% concentration, 1.5 grams is taken in 30 ml and to make a 10% concentration, 3 grams are taken in 30 ml and a 20% concentration is taken 6 grams in 30 ml (Alam et al., 2011).
   d. Antifungal Activity Testing
      Testing the antifungal activity using the well-diffusion method begins by making a 5 mm well in each petri dish. All wells were filled with 0.2 gram of positive control (2% ketoconazole) and anti-dandruff shampoo preparations of simpur leaf extract with a predetermined concentration. Then incubated at 38˚C for 24 hours. Then incubate and measure the diameter of the clear zone (inhibition zone) using a caliper through the center of the well (Földes et al., 2020).
   e. Data analysis
      The data analysis used is using the one way annova test, then proceed with the Tukey test.

3. Results And Discussion

This study aims to make anti-dandruff shampoo preparations from simpur leaves by testing antifungal activity.

3.1. Extraction Results

Extraction results from simpur leaves obtained a viscous black extract with a distinctive simpur odor with an extract weight of 49.018 grams and an extract yield of 9.803%. Then the drying shrinkage inspection value obtained the drying shrinkage percent value of 6.56% ± 1.077. The drying shrinkage value obtained shows that the simplicia drying shrinkage fulfills the standard requirements where the drying shrinkage value is not more than 10%.

3.2. Phytochemical Screening

The next test, namely phytochemical screening, was carried out on powders and extracts. The purpose of phytochemical screening is to prove the presence of secondary metabolite compounds contained in simpur leaf samples.

The results of the phytochemical screening showed positive results for secondary metabolites in the simplicia powder and extracts, the results obtained were the same as for the presence of polyphenols, steroids, terpenoids and saponins. However, the examination results of alkaloids and tannins showed negative results.

Table 2: Results of Simpur Leaf Phytochemical Screening

Secondary Metabolites	Result		Description	
	Extract	Powder	Extract	Powder
Flavonoids	+	+	Orange	Orange
Saponins	+	+	There is foam	There is foam
Tannins	-	-	No white precipitate	No white precipitate
Steroids and terpenoids	+	+	Brown ring and green ring	Brown ring and green ring
Polyphenols	+	+	Purplish blue	Purplish blue

Information :
(+) : Positive, there is a compound content
(-) : Negative, no compound content

The preparation consists of 4 formulations with different concentration variations, namely F1 5%, F2 10% and F3 20% and F0 without the addition of extract from simpur leaves. The shampoo preparations were then subjected to preparation evaluation tests which included organoleptic tests, homogeneity tests, pH measurements, viscosity tests, foam height tests and hedonic tests.

Figure 1: Shampoo preparations

The organoleptic test results for the shampoo preparations showed that F1 and F2 were brownish black in color with a viscous liquid dosage form with a menthol scent and a distinctive simpur odor. Meanwhile, the F3 shampoo is black in color with a thick liquid form and has a menthol and simpur odor. This shows that the addition of extract concentration causes the color of the preparation to become darker because the color of the extract is black.

The homogeneity test showed good results, namely homogeneity in the tests that had been carried out on the four simpur extract shampoo formulation formulas.

Testing the pH of the 5% F1 shampoo preparation was 5.4 ± 0.0173 and F2 showed a result of 5.61 ± 0.115 while at a concentration of 20% F3 pH 6.01 ± 0.13. These results indicate that the pH test meets the requirements because according to SNI standards No.06-2692-1992 good shampoo preparations range from pH 5-9 because it is the normal pH of the scalp and does not irritate the scalp so it is safe to use.

Table 3: pH test results

pH testing	Formula		
	F1	F2	F3
Experiment I	5.41	5.72	6.09
Experiment II	5.41	5.49	5.86
Trial III	5.38	5.63	6.08
Average ± SD	5.4 ± 0.0173	5.61 ± 0.115	6.01 ± 0.13

Information:
F1: Formula extract concentration 5
F2: extract concentration formula 10%
F3: extract concentration formula 20%

In the viscosity test, the results obtained were F1 (1128 Cp), F2 (1450 Cp) and F3 (2104 Cp) and it can be said that they have met the requirements because they are in the appropriate range for the viscosity test standard for shampoo preparations, namely between 400-4000 Cp (Alam et al., 2011).

Foam height test on simpur leaf extract shampoo with concentrations of F1 5 cm, F2 5.5 cm and F3 6 cm. These results show a number that is in accordance with the standard requirements for foam height for shampoo preparations, which range from 1.3-22 cm (Földes et al., 2020). Can be seen in Table 4.

Table 4: Viscosity test results.

Formula	Viscosity Value (Centiposie)	Test Standard	Description
F1	1128 Cp		Qualify
F2	1450 Cp	400-4000	Qualify
F3	2104 Cp		Qualify

Information:
F1: Formula extract concentration 5
F2: extract concentration formula 10%
F3: extract concentration formula 20%

The hedonic test of 10 respondents showed the highest results in liking the shape and also the smell of the shampoo preparation where the form of the shampoo is a viscous liquid that is easy to pour then the aroma of the shampoo is interesting which is produced from the addition of menthol ingredients and has a distinctive simpur smell.

The next test was the shampoo preparation stability test which included organoleptic test, pH test and viscosity test at 4°C for 24 hours, then continued to be stored at a higher temperature, namely 40°C for 24 hours for 12 cycles. The results obtained were that the shampoo preparations at each concentration had a black color, the dosage forms were viscous liquid and had the smell of simpur and bauk has simpur. Then the results of testing the stability of pH and viscosity are still in the range that meets the requirements of shampoo preparations.

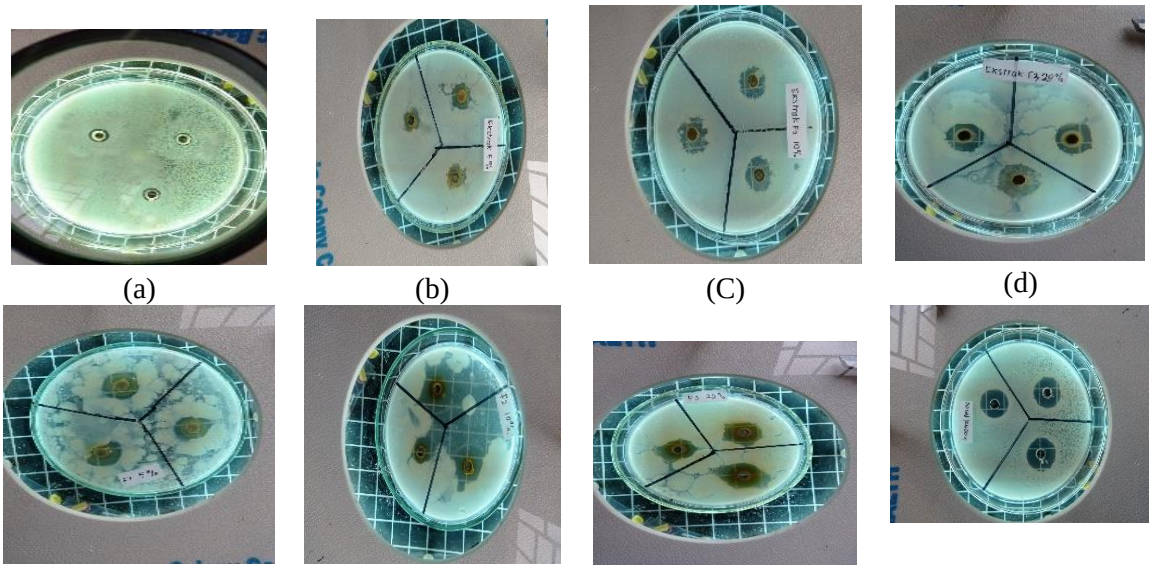
In testing the anti-fungal activity of anti-dandruff shampoo from simpur leaf extract with concentrations of F1 (5%), F2 (10%) and F3 (20%) as well as the negative control and positive control of ketoconazole, it was shown that extracts and preparations can inhibit the growth of candida albicans fungus by indicated by a clear zone around the well.

Table 5: Antifungal Activity Test Results

Formula	Concentration	Inhibition	Inhibition Category
	F0 (Negative Control)	2.13 Mm	Weak
	Ketoconazole (Positive Control)	13.78 Mm	Strong
Extract F1	5%	6.398 Mm	Currently
Extract F2	10%	8.375 Mm	Currently
Extract F3	20%	11.55 Mm	Strong
F1	5%	6.725 Mm	Currently
F2	10%	9.775 Mm	Currently
F3	20%	12.008 Mm	Strong

Description: <5 mm (Weak), 5-10 mm (Medium), 11-20 mm (Strong), >20 mm (Very Strong)

Negative control test results obtained, namely the presence of an inhibition zone of 2.13 mm included in the weak category. Materials that have the potential to inhibit fungal activity are methyl parabens which function as preservatives for shampoo preparations, so they have the ability to inhibit the growth of contaminants in fungi. Apart from methyl paraben, another potential ingredient is propylene glycol as a humectant which has anti-microbial capabilities so that the base can help inhibit the growth of candida albicans (Thakur et al., 2012). In the 5% F1 test the extract and the shampoo preparation the results obtained were not much different where the F1 extract with an inhibition zone of 6.398 mm and the F1 shampoo preparation with a value of 6.725 were included in the medium category for fungal inhibition. The concentration of the extract and 10% F2 shampoo resulted in an average zone of inhibition for the extract was 8.375 mm and the shampoo preparation with an average of 9.775 mm was included in the medium category. Testing the concentration of extracts and shampoo preparations, namely F3 20%, the results obtained were 11.55 inhibition zones for extracts and shampoo preparations of 12.008 mm and could be categorized into a strong inhibition zone. The results of the diameter of the inhibition zone of the extract with the ketoconazole positive control showed no significant difference in results, this indicated that the antifungal activity of simpur leaf extract (*Dillenia indica L*) was not better than synthetic antifungal activity. The diameter of the inhibition zone in the positive control was 13.78 mm in the strong category, which means that it was almost the same as the simpur leaf extract shampoo preparation. The data that has been obtained is then statistically processed using SPSS software version 28.



(a) (b) (C) (d)

(e) (f) (g) (h)

Figure 2: The results of the shampoo inhibition zone

Information:
a = Negative control
b = Extract 5%
c = Extract 10%
d = Extract 20%
e = F1 5%
d = F2 10%
e = F3 20%
g = positive control

From the results of the antifungal test, the simpur leaf extract shampoo preparation increased the inhibition zone formed due to the higher the concentration, the more antifungal compounds that can inhibit the growth of the fungus. The inhibition zone generated in the test is based on the presence of secondary metabolites in plants. The content of secondary metabolites in plants that act as anti-fungal substances in simpur leaves include saponins, flavonoids and terpenoids. The results of the soursop leaf phytochemical screening showed that the leaves contain compounds namely polyphenols, flavonoids, steroids, terpenoids, and saponins.

4. Conclusion

Based on the evaluation results of simpur leaf extract shampoo (Dillenia indica L) with different concentration variations namely F1(5%), F2(10%) and F3(20%) can be formulated into shampoo preparations, simpur leaf extract has Candida antifungal activity albicans with a concentration (5%) had an inhibition zone of 6.398 mm which was included in the medium category, a concentration (10%) had an inhibition zone of 8.375 which was included in the medium category and the concentration (20%) had an inhibition zone of 11.55 which was included in the strong category. In the shampoo formulations F1, F2 and F3 the antifungal activity of Candida albicans with the greatest inhibition of F3 with a concentration of 20% had an inhibition zone of 12.008 mm included in the strong category.

References

Al Badi, K., & Khan, S. A. (2014). Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos. *Beni-Suef University Journal of Basic and Applied Sciences*, 3(4), 301-305.

Alam, M. B., Chowdhury, N. S., Mazumder, M. E. H., & Haque, M. E. (2011). Antimicrobial and toxicity study of different fractions of Dillenia indica Linn. bark extract. *International Journal of Pharmaceutical Sciences and Research*, 2(4), 860.

Ebrahim, A. A., & El Gaali, E. E. (2015). Physiochemical Properties of Mango (Mangifera indica L.) Seed Kernel's Oil. *Sudan Academy of Sciences Journal*, 10, 80-92.

Földes, T., Banhegyi, I., Herpai, Z., Varga, L., & Szigeti, J. (2000). Isolation of Bacillus strains from the rhizosphere of cereals and in vitro screening for antagonism against phytopathogenic, food-borne pathogenic and spoilage micro-organisms. *Journal of applied microbiology*, 89(5), 840-846.

Gholamreza, D. N., Fariba, S., Payam, K., Ehsan, M., & Javad, J. (2011). Formulation of herbal conditioner shampoo by using extract of fenugreek seeds and evaluation of its physicochemical parameters. *African journal of pharmacy and*

pharmacology, 5(22), 2420-2427.

Halith, S. M., Abirami, A., Jayaprakash, S., Karthikeyini, C., Pillai, K. K., & Firthouse, P. M. (2009). Effect of Ocimum sanctum and Azadiracta indica on the formulation of antidandruff herbal shampoo powder. *Pharm Lett*, 1, 68-76.

Kaur, G. J., & Arora, D. S. (2009). Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. *BMC complementary and alternative medicine*, 9(1), 1-10.

Narshana, M., & Ravikumar, P. (2018). An overview of dandruff and novel formulations as a treatment strategy. *Int J Pharm Sci Res*, 9(2), 417-431.

Rajput, R. (2015). Understanding hair loss due to air pollution and the approach to management. *Hair Ther Transplant*, 5(133), 2.

Tao, R., Li, R., & Wang, R. (2021). Skin microbiome alterations in seborrheic dermatitis and dandruff: a systematic review. *Experimental Dermatology*, 30(10), 1546-1553.

Thakur, N. K., Bharti, P., Mahant, S., & Rao, R. (2012). Formulation and characterization of benzoyl peroxide gellified emulsions. *Scientia Pharmaceutica*, 80(4), 1045-1060.

Wibawa, I. P. A. H., & Lugrayasa, I. N. (2021). Potential study of Dillenia serrata Thunb. fruit extract from Bali Botanical Garden's collection. *Berkala Penelitian Hayati Journal of Biological Researches*, 26(2), 79-84.

Yadav, A. R., & Mohite, S. K. (2020). Formulation and evaluation of antidandruff shampoo. *Research Journal of Topical and Cosmetic Sciences*, 11(2), 55-58.