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Original Article

EVALUATION OF AN ANTI-DANDRUFF SHAMPOO INCORPORATING ETHANOL EXTRACT FROM CORN SILK (*ZEA MAYS* L.) AGAINST *CANDIDA ALBICANS* FUNGUS: FORMULATION AND ACTIVITY ASSESSMENT

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ABSTRACT

Objective: The aim of this study was to investigate the effectiveness of corn silk (*Zea mays* L.) extract as an antifungal ingredient in shampoo formulation for treating dandruff caused by *Candida albicans.*

Methods: Plant identification, sampling, preparation of simplisia, phytochemical screening, simplisia characterization, ethanol extraction of corn silk, and the formulation of shampoos with extract concentrations of 5%, 10%, and 15%, alongside blank and positive controls. The formulations underwent physical evaluation, irritation testing, and antifungal activity testing using the disc diffusion method.

Results: The corn silk shampoo formulations were stable during storage, non-irritating, was in thick liquid form with yellowish to brown colour, corn scent, had pH between 5.0-5.8, foaming capacity at 10.3-13.0 cm, with the viscocity ranging 1967-2224 cPs, means it met the required standards for shampoo formulation and characterization. Antifungal testing revealed inhibition zone diameters of 0 mm for the blank (F0), 7.87 mm for F1 (5%), 9.46 mm for F2 (10%), 15.89 mm for F3 (15%), and 18.71 mm for the positive control (C+), with a one-way ANOVA test indicating a significant difference compared to the negative control.

Conclusion: Corn silk extract could be effectively formulated into an antifungal shampoo, with the 15% concentration being the most effective against *Candida albicans*, highlighting its potential as a natural ingredient for anti-dandruff shampoos.

Keywords: Anti-dandruff, Candida albicans fungus, Corn silk (Zea mays L.), Shampoo

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INTRODUCTION

Corn ranks among the world's most crucial food crops, standing alongside wheat and rice. Acting as a primary source of carbohydrates in Central and South America, corn also functions as an alternative food staple in the United States. In several countries in Asia, corn serves as the second staple food after rice. Corn plants offer significant benefits for both human and animal consumption. Globally, corn holds the third position among staple crops, following wheat and rice [1, 2].

Corn plants offer a wide range of utility, from its roots to its leaves and fruits. The roots of corn plants have been utilized extensively for medicinal purposes, while the leaves are commonly used as animal feed [3]. Cornfruits, on the other hand, find applications in various culinary uses such as vegetable dishes, popcorn, corn flour, and more. Sweet corn, in particular, is highly favored when boiled or grilled. Generally, people tend to disregard corn silk and consider it as waste [4]. However, corn silk contains bioactive compounds such as flavonoids, saponins, tannins, phenolics, alkaloids, glycosides, and beta-sitosterol, which are beneficial for health [5].

Corn silk refers to a collection of fine, soft, thread-like stigmas that appear yellowish in color. It originates from the female flowers of the corn plant [6]. Initially, corn silk typically exhibits a light green hue, eventually transitioning to red, yellow, or light brown, depending on the variety. The primary function of corn silk is to capture pollen grains for pollination purposes [7]. The hair that adorns the human head is a matter of aesthetic importance, prompting individuals to invest significant time in its care and styling. Scalp issues such as sensitivity, oiliness, and dandruff, which disrupt normal hair growth, are commonly encountered. Among these issues, dandruff remains a significant cause of diminished self-confidence during daily activities [8].

Dandruff is a condition of the scalp characterized by excessive shedding of the top layer of skin, forming fine flakes on the scalp. Common symptoms include the appearance of white flakes on the scalp, itchiness, and sometimes hair loss [9]. The causes of dandruff can include excessive secretion of sweat glands or the involvement of microorganisms on the scalp producing metabolites that induce the formation of dandruff. One of the fungi responsible for dandruff issues on the scalp is the *Candida albicans*fungus [10, 11]. *Candida albicans* is a species of pathogenic fungus belonging to the Ascomycota group [12]. This fungal species is a causative agent of opportunistic infections known as candidiasis, affecting the skin, mucous membranes, and internal organs in humans. Macroscopically, *Candida albicans* appears as round, oval, or elongated cells [13-15].

Shampoo is a cosmetic preparation available in liquid, gel, emulsion, or aerosol forms containing surfactants, thus possessing detergent and humectant properties and capable of producing lather [16]. Shampoo is a cosmetic product used for cleansing the hair, ensuring that both the hair and scalp are clean and as soft, manageable, and shiny as possible [17-19]. Shampoo is utilized to remove unwanted particles such as oil and other debris, while also enhancing the appearance of hair without stripping away excessive sebum, which can make the hair more difficult to manage [20, 21]. Plants contain secondary metabolites that are safer to use compared to synthetic ingredients, making them highly beneficial for formulating shampoos from natural sources [22, 23].

The processing of corn silk is still underutilized by society despite its significant potential benefits. Considering its efficacy, there is a great need for the processing and development of corn silk to create various products [24-26]. One of the traditional medicinal plants that has been widely utilized is corn (*Zea mays* L). A commonly used part of the plant is corn silk, which is a by-product of the food industry. Corn silk is often employed in traditional medicine to lower cholesterol levels. Additionally, the presence of flavonoids and glycosides in corn silk extract can inhibit the growth of bacteria such as *Escherichia coli* and fungi such as *Candida albicans* [27]. The aim of this study was to explore the effectiveness of corn silk (*Zea mays*

L.) extract as an antifungal ingredient in shampoo formulations designed to treat dandruff caused by *Candida albicans***.** Therefore, developing the formulation of shampoo containing extract of corn silk is an innovation using waste and showing an efficacy in inhibiting the growth of microorganisms.

MATERIALS AND METHODS

Study preparation

The study employs purposive sampling methodology to select samples, with the population consisting of *Candida albicans* fungus isolates sourced from the Microbiology Laboratory at the Faculty of Pharmacy, North Sumatera University (Indonesia). The research samples comprise corn silk (*Zea mays* L.) collected from Selotong Village, Secanggang District, Langkat Regency, North Sumatera with specimen number 093/EXT/DKN/7FK/IKH/V/2023 (Medanese Herbarium, North Sumatera University, Indonesia).

Materials

The materials utilized in this research encompass corn silk powder (*Zea mays* L.), 70% ethanol, sodium lauryl sulfate, cocamide DEA, Na-CMC (sodium carboxymethylcellulose), methylparaben, citric acid, menthol, perfume, distilled water, *Candida albicans* fungus, Potato Dextrose Agar (PDA) medium, chloroform, magnesium powder, methanol, 2N HCl (hydrochloric acid), Mayer's reagent, Wagner's reagent, Dragendorff's reagent, chloral hydrate, concentrated H_2SO_4 (sulfuric acid), concentrated HCl (hydrochloric acid), 1% HCl solution, 1% FeCl (ferric chloride), 1% H_2SO_4(sulfuric acid), Liebermann Burchard reagent, 1% $BaCl_2$ (barium chloride), and 0.9% NaCl (sodium chloride).

Plant determination

The determination of corn silk (*Zea mays* L.) was conducted at the Herbarium Medanese (MEDA) in North Sumatera to ascertain its taxonomic identity (No: 922/MEDA/2023).

Sample preparation

Corn silk was collected and separated from impurities, sorted while wet, washed thoroughly with running water until clean, drained, and chopped. Subsequently, the fresh weight of corn silk amounted to 7 kg was measured then dried in a drying cabinet. Corn silk was considered dry when it became brittle (crushed easily). It was then sorted when dry to remove foreign objects, such as unwanted parts and other remaining impurities. The sample was ground using a blender until fine to obtain dry corn silk powder, then sieved using a No. 60 mesh sieve. Finally, it was stored in a dry, tightly sealed container [26, 28].

Phytochemical screening

In the course of this study, we have thoroughly conducted a phytochemical screening on the corn silk sample. This comprehensive analysis allowed us to identify and confirm the presence of various bioactive compounds within the sample, providing valuable insights into its potential therapeutic properties [7, 29, 30]. By meticulously examining the corn silk, we aimed to uncover the different phytochemical constituents, such as alkaloids, flavonoids, tannins, saponins, and terpenoids, which are known to contribute to the medicinal value of plants. This screening process is a critical step in understanding the chemical composition of the corn silk and its possible applications in developing natural remedies or health supplements. The findings from this phytochemical evaluation form the foundation for further research into the bioactive properties of corn silk and its potential benefits in various health-related applications [31, 32].

Evaluations and characterizations

Macroscopic examination and microscopic examination

Macroscopic examination of corn silk involved observing its shape, odor, taste, and color. Microscopic examination was performed on the corn silk powder. The powdered sample was sprinkled onto a microscope slide that had been moistened with chloral hydrate solution and covered with a cover slip. The slide was then observed under a microscope [33].

Determination of water content

The powder was weighed at 2 g in a pre-weighed dish. It was then dried in an oven at a temperature of 100-105 °C for 3-5 h. After drying, it was cooled down and weighed again. The dish containing the sample was reheated in the oven for an additional 30 min, cooled down, and weighed once more. The reduction in weight indicated the amount of water present in the material [34].

Determination of soluble essence content in water

Take 5 g of powder and macerate it for 24 h with 100 ml of chloroform water using a stoppered flask while shaking it repeatedly for the first 6 h and then leave it for 18 h. Filtered, evaporated 20 ml of the filtrate until dry in a tared evaporator cup, heated the remainder at 105 °C until the weight remained constant [34].

Determination of soluble essence content in ethanol

A quantity of 5 g of the powder was subjected to maceration for 24 h with 100 ml of 70% ethanol in a stoppered flask, with intermittent shaking for the first 6 h and subsequently left undisturbed for the remaining 18 h. The mixture was then filtered, and 20 ml of the filtrate was evaporated to dryness in a tared evaporating dish. The remaining residue was heated at 105 °C until a constant weight was achieved [35].

Determination of total ash content

Approximately 2-3 g of the powdered crude drug was placed into a pre-heated porcelain crucible and weighed. It was then spread evenly in the crucible and slowly heated until charred completely. After cooling, it was weighed again. The remaining residue from filtration and filter paper were also placed in the same crucible. The filtrate was poured into the crucible, evaporated, heated until a constant weight was achieved, and weighed [26, 35].

Determination of acid-insoluble ash

The ash obtained from the total ash determination is boiled in 25 ml of 2 N hydrochloric acid solution for 5 min. The portion of ash insoluble in acid is collected after filtration through filter paper. It is then heated until a constant weight is achieved, cooled, and weighed. The content of ash insoluble in acid is calculated relative to the dried material [1, 26].

Extracts preparation

The preparation of corn silk extract is carried out by maceration with a ratio of 1:10 using 70% ethanol solvent. Specifically, 700 g of corn silk is placed into a glass container and immersed in 75 parts of 70% ethanol solvent, totaling 5.250 ml. The container is then covered with aluminum foil and left for 5 d, protected from sunlight, while occasionally stirred. Afterward, the mixture is filtered through filter paper to obtain a filtrate and residue. The residue is then immersed again in 25 parts of 70% ethanol solvent, amounting to 1.750 ml. The container is covered with aluminum foil and left for an additional 2 d, with occasional stirring. After 2 d, the sample is filtered to obtain a second filtrate and residue. The first and second filtrates are combined and then evaporated using a rotary evaporator at a temperature of 40 °C until a thick extract is obtained [1] (table 1).

Procedure for making corn silk ethanol extracts shampoo

Equipment and materials are prepared. Na-CMC, which has been weighed, is added to hot water. It is left for several minutes to swell and stirred slowly (mass 1). Meanwhile, 20 ml of water heated to 60-70 °C is poured into a glass beaker, and sodium lauryl sulfate is added. It is stirred until dissolved (mass 2). Menthol is dissolved in an appropriate amount of distilled water stirred until dissolved, then methylparaben and citric acid are added and stirred until homogeneous, forming solution 3. The solution of sodium lauryl sulphate (mass 2) is slowly added to mass 1 while stirring slowly until homogeneous. Cocamide DEA is gradually added and mixed until homogeneous. Solution 3 is then slowly added to the mixture and stirred until homogeneous. The corn silk extract is added, and the mixture is homogenized before being transferred into 100 ml bottles [1, 31].

Table 1: Shampoo formulation of corn silk extract (*Zea mays* L.)

Ingredients	F0	F1	F2	F3
Corn silk extract	0	5 g	10 g	15 g
Sodium lauryl sulphate	10 g	10 g	10 g	10 g
Cocamide DEA	4 g	4 g	4 g	4 g
Na-CMC	3 g	3 g	3 g	3 g
Menthol	0.25 g	0.25 g	0.25 g	0.25 g
Methyl paraben	0.2 g	0.2 g	0.2 g	0.2 g
Parfum	q. s	q. s	q. s	q. s
Citric acid	0.25 g	0.25 g	0.25 g	0.25 g
Aquadest add	100 ml	100 ml	100 ml	100 ml

Note: F0: Negative control (blank), F1: shampoo containing 5% of corn silk extract, F2: shampoo containing 10% of corn silk extract, F3: shampoo containing 15% of corn silk extract

Characteristic of shampoo formulation

Organoleptic test

Observation is conducted directly, utilizing the human senses as the primary tool to assess the quality of the tested preparation. Organoleptic evaluation includes assessing the form, color, and odor of the shampoo formulation. Other characterizations are homogeinity, pH value, irritation test, test foam height, viscosity and stability test [36].

Antifungal activity test

Sterilization of tools and materials

The testing equipment for antimicrobial activity must be sterilized beforehand to minimize the potential for human error. Glassware such as beakers, measuring cylinders, Erlenmeyer flasks, petri dishes, test tubes, and glass funnels are wrapped in parchment paper or brown paper and sterilized in an oven at a temperature of 160-170 °C for 1-2 h. Rubber-based equipment and test media are sterilized using an autoclave at 121 °C for 15 min. Needles are sterilized by passing them through a Bunsen burner flame [37, 38].

Potato dextrose agar (PDA) media preparation

A total of 5.85 g of Potato Dextrose Agar (PDA) powder is added to an Erlenmeyer flask then dissolved in 150 ml of distilled water. The mixture is heated on a hot plate until completely dissolved. The Erlenmeyer flask is covered with cotton and paper, then sterilized in an autoclave at 121 °C for 15 min. After sterilization, the agar medium is allowed to cool to approximately 45 °C. Subsequently, the medium is poured into petri dishes, approximately 15-20 ml per dish, and left to solidify [38].

Rejuvenation of fungal isolates and preparation of solution turbidity standards (Mc. Farland Solution)

Pure cultures of *Candida albicans* from Department of Microbiology, Faculty of Pharmacy, University of North Sumatra were carefully inoculated onto Potato Dextrose Agar (PDA) plates, ensuring even distribution of the fungal cells. These inoculated plates are then incubated at a controlled temperature of 37 °C for a duration of 48 h, allowing sufficient time for the growth and proliferation of *Candida albicans* colonies. Throughout the incubation period, the plates are periodically monitored to observe the development of distinct fungal colonies characteristic of *Candida albicans* [39].

In parallel, the preparation of the McFarland standard solution is meticulously conducted. This involves precisely mixing 9.5 ml of a 1% H_2SO_4 solution with 0.5 ml of a 1% $BaCl_2$ solution to achieve a total volume of 10 ml. The resulting solution is gently shaken until complete homogeneity is achieved. It is imperative to ensure thorough mixing of the components to obtain a standardized solution with consistent optical density. This McFarland standard solution serves as a reference for the turbidity of microbial suspensions. Prior to each use, the standard solution must be vigorously shaken to maintain uniformity. It is employed as a comparative tool to assess the density of the yeast suspension, aiding in the determination of microbial concentration and facilitating accurate interpretations of experimental results [39, 40].

Preparation of fungal suspension

The *Candida albicans* fungal strain is retrieved from the culture stock using a sterilized inoculation loop, previously sterilized using a Bunsen burner flame. It is then suspended in a test tube containing 10 ml of 0.9% NaCl solution. The suspension is gently agitated until it achieves turbidity equivalent to the 0.5 McFarland standard. This standard turbidity level serves as a reference for the concentration of the fungal suspension, ensuring consistency and accuracy in subsequent experimental procedures.

***Candida albicans* activity testing**

The antifungal activity of the shampoo formulation containing ethanol extract of corn silk is tested using the disc diffusion method. Firstly, 20 ml of Potato Dextrose Agar (PDA) medium is poured into sterilized petri dishes and allowed to solidify. Once solidified, 1 ml of fungal suspension is evenly spread across the surface of the agar medium. Sterilized filter paper discs with a diameter of 6 mm are immersed into the test solution contained in vials with various concentrations and left to absorb the test solution for 5 min. Subsequently, they are placed onto the surface of the agar medium. Each disc is labeled accordingly, and this process is repeated three times [41].

The petri dishes are then incubated at 37 °C for 48 h (2 d). After the incubation period, the diameter of the inhibition zones around the discs is measured using a vernier caliper. This measurement allows for the assessment of the antifungal activity of the shampoo formulation against the *Candida albicans* fungus [37, 42].

Data analysis

The analysis of antifungal activity data involves measuring the diameter of the inhibition zones using a vernier caliper at each concentration. Subsequently, statistical analysis is conducted using ANOVA (Analysis of Variance) in the SPSS program.

ANOVA is employed to assess whether there are statistically significant differences in the mean diameter of inhibition zones among the different concentrations of the shampoo formulation containing corn silk ethanol extract. This analysis helps determine if there is a significant effect of concentration on the antifungal activity. The output from ANOVA provides valuable insights into the effectiveness of the shampoo formulation at various concentrations against the *Candida albicans* fungus. Additionally, post-hoc tests, such as Tukey's HSD (Honestly Significant Difference) test, may be performed to identify specific differences between concentration groups if significant differences are detected by ANOVA.

RESULTS AND DISCUSSION

Result of plant identification

Based on the results of the corn silk identification conducted at the Medanense Herbarium (MEDA) of the University of North Sumatra, it is confirmed that it is indeed corn silk from *Zea mays* L.

Results of phytochemical screening

Phytochemical screening is a crucial step in exploring the potential of medicinal plant resources based on their therapeutic properties.

Phytochemical screening conducted on corn silk aims to identify various classes of metabolites that may be present in the extract, such as alkaloids, flavonoids, tannins, saponins, and others. This step provides a deeper understanding of the chemical composition of corn silk and its potential pharmacological effects. Secondary metabolites found in plants play a crucial role in their medicinal properties. The identification results on corn silk show the positive results in presence of alkaloids, tannins, flavonoids, terpenoids/steroids, and saponins. These compounds possess natural surfactant properties that contribute to their antimicrobial (antifungal) effects.

Table 2: Requirement of medical materials for corn silk (*Zea mays* L.)

No	Amount determination	Result	Characteristic
1	Moisture content	6%	<10%
2	Water-Soluble Extract Content	17%	>7%
3	Ethanol-Soluble Extract Content	20.6%	>0.5%
4	Total Ash Content	4.6%	<5%
5	Acid-insoluble ash	0.6%	<1%

Characterization of corn silk (*Zea mays* L.) simplisia involves both macroscopic and microscopic examinations (table 2). Macroscopic examination entails observing the shape, color, odor, and taste of corn silk to determine its distinctive characteristics directly. Microscopic examination of corn silk powder reveals the presence of pollen grains, epidermis, and vascular bundles with ladder-type thickening, as well as epidermis and parenchyma. Microscopic testing aims to identify diagnostic features of the plant. Determination of the moisture content in simplisia is conducted to ascertain the amount of water contained within it. The moisture content obtained is 6%, which meets the requirements specifying that simplisia's moisture content should generally not exceed 10%. Moisture determination is also associated with the purity of the powder. Lower moisture content reduces the likelihood of microbial, fungal, or insect contamination and preserves the integrity of the active ingredients. Analysis of the water-soluble extract content indicates the amount of chemical compounds dissolved in water within the simplisia. The water-soluble extract content obtained is 17%, meeting the shampoo formulation and characterization requirements stipulating that the water-soluble extract content of simplisia should not be less than 7%. Determining the water-soluble extract content aims to assess the quantity of polar chemical compounds present in the simplisia [43, 44].

The examination results of the characteristics of corn silk powder simplisia revealed a content of ethanol-soluble extract of 20.6%. Corn silk simplisia meets the requirements of the shampoo formulation and characterization, which specifies that the ethanol-soluble extract content of simplisia should generally not be less than 0.5%. Determining the ethanol-soluble extract content helps identify the level of compounds soluble in ethanol, including both polar and non-polar compounds [32]. This indicates that the amount of polar compounds dissolved in water is smaller than the amount of non-polar compounds dissolved in ethanol, serving as an indicator of the presence of beneficial substances soluble in both water and ethanol solvents. The examination results of the characteristics of corn silk powder simplisia yielded a total ash content of 4.6%. Corn silk simplisia meets the requirements of the shampoo formulation and characterization, which states that the total ash content should generally not exceed 5%. Determining the total ash content helps identify the internal mineral content originating from the plant tissue itself and external residues such as sand and soil present in the sample [45].

The examination results of the characteristics of corn silk powder simplisia revealed an acid-insoluble ash content of 0.6%. Corn silk simplisia meets the requirements of the shampoo formulation and characterization, which specifies that the acid-insoluble ash content should generally not exceed 1%. The acid-insoluble ash content indicates the presence of silicates, particularly organic compounds such as metal oxides of Mg, Ca, Pb, and Zn in the simplisia, achieved by dissolving the total ash in hydrochloric acid [45, 46].

Result of shampoo formulation

The shampoo preparations were formulated using a standard formula. This standard formula was modified by incorporating ethanol extract of corn silk (*Zea mays* L.) as an active anti-dandruff agent for the scalp. The concentrations of ethanol extract of corn silk (*Zea mays* L.) used were 5%, 10%, and 15%. The preparation with 0% concentration (blank) had a thick texture and a clear white color, whereas the preparations with concentrations of 5%, 10%, and 15% exhibited a thick texture with a yellowish-brown color (typical of corn silk).

Physical evaluation of shampoo

Organoleptic test

Organoleptic testing of the shampoo preparations containing corn silk extract (*Zea mays* L.) was conducted over 6 cycles to assess the physical appearance of the preparations by observing their shape, color, and odor (table 3). Based on the organoleptic test results of the shampoo preparations containing corn silk extract (*Zea mays* L.), for F0 or the preparation without extract (blank), it was found that the preparation had a clear white color, thick texture and no aroma. For F1 or the preparation with a concentration of 5%, it was found that the preparation had a yellowish-brown color, thick texture, and a distinctive aroma. Similarly, for F2 or the preparation with a concentration of 10%, it was found that the preparation had a bright yellow-brown color, thick texture, and a distinctive aroma. Finally, for F3 or the preparation with a concentration of 15%, it was found that the preparation had a distinctive brown color, thick texture, and a characteristic aroma due to the addition of Popcorn fragrance to the shampoo preparation [47, 48].

Table 3: Organoleptic test during stability test of corn silk (*Zea mays* L.) ethanol extract shampoo

Test	Formula	Cycle of stability					
		1	2	3	4	5	6
Consistency	F0	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	F1	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	F2	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	F3	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
Color	F0	Transparant	Transparant	Transparant	Transparant	Transparant	Transparant
	F1	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown
	F2	Brownish-yellow	Brownish-yellow	Brownish-yellow	Brownish-yellow	Brownish-yellow	Brownish-yellow
	F3	Brown	Brown	Brown	Brown	Brown	Brown
Odor	F0	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless
	F1	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent
	F2	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent
	F3	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent	Corn scent

Notes: F0: Negative control (blank), F1: Shampoo containing 5% of corn silk extract, F2: Shampoo containing 10% of corn silk extract, F3: Shampoo containing 15% of corn silk extract

Homogeneity test

Homogeneity testing aims to determine whether all ingredients are evenly mixed and whether there are no coarse particles present, ensuring that the shampoo can be evenly distributed on the scalp during use. Based on the results of the homogeneity test of F0 (negative control shampoo), F1 (Shampoo containing 5% of corn silk extract), F2 (Shampoo containing 10% of corn silk extract) and F3 (Shampoo containing 15% of corn silk extract) over 6 cycles show that the preparations meet the requirements, being homogeneous with no visible coarse particles [49].

Table 4: The results of pH test of corn silk (*Zea mays* L.) ethanol extract shampoo before and during cycling test

Formula	pH value (+SD)						
	Before	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F0	5.8±0.02	5.8±0.03	5.8±0.02	5.7±0.10	5.7±0.01	5.8±0.02	5.7±0.04
F1	5.4±0.30	5.4±0.04	5.3±0.04	5.3±0.11	5.2±0.03	5.2±0.01	5.2±0.07
F2	5.2±0.02	5.2±0.05	5.1±0.06	5.2±0.07	5.2±0.12	5.1±0.03	5.1±0.03
F3	5.0±0.05	5.0±0.06	5.0±0.03	5.1±0.06	5.0±0.04	5.0±0.06	5.0±0.06

Note: Results presented as mean+SD (Sn=3), F0: Negative control (blank), F1: Shampoo containing 5% of corn silk extract, F2: Shampoo containing 10% of corn silk extract, F3: Shampoo containing 15% of corn silk extract

pH measurement is conducted to determine whether the shampoo preparation complies with the pH standard for shampoos according to the shampo formulation and characterization, which is between 5.0-9.0 (table 4). The pH value should not be too acidic as it can cause skin irritation, nor should it be too alkaline as it can lead to dry and dull hair [50]. A slightly acidic pH helps prevent dandruff-causing factors like fungi on the scalp. pH examination aims to assess the acidity level of the shampoo preparation. The pH testing of the shampoo preparations was conducted over 6 cycles (12 d) using the cycling test method. The obtained pH values for preparations F0 (blank): 5.8, F1: 5.4, F2: 5.2, F3: 5.0. Based on these pH measurement results, it is observed that the higher the concentration of ethanol extract of corn silk (*Zea mays* L.) added to the preparation, the lower the pH of the preparation [51, 52]. This is due to the presence of acidic compounds in the corn silk extract. The pH values of all four shampoo preparations meet the shampo formulation and characterization [20].

Table 5: The results of the foaming capacity test of the corn silk (*Zea mays* L.) ethanol extract shampoo

Formula	Foaming capacity							
	Before cycling test				After cycling test			
	Replication			Average (±SD)	Replication			Average (±SD)
	1	2	3		1	2	3	
F0	10.5	10.6	10.9	10.7±0.21	10.8	10.2	10.0	10.3±0.42
F1	11.2	11.5	11.8	11.5±0.30	11.5	11.0	11.5	11.3±0.29
F2	12.3	12.0	12.0	12.1±0.17	11.8	11.9	12.0	11.9±0.10
F3	13.0	12.9	13.2	13.0±0.15	13.0	12.8	13.0	12.9±0.12

Notes: Results presented as mean+SD (Sn=3), F0: Negative control (blank), F1: Shampoo containing 5% of corn silk extract, F2: Shampoo containing 10% of corn silk extract, F3: Shampoo containing 15% of corn silk extract

Foam capacity test results

The observation of foam height is conducted to demonstrate the surfactant's ability to form the foam. Foam in shampoo is crucial as it helps the shampoo adhere to the hair while effectively cleansing it from dirt, dust, dandruff, and maintaining the hair strands to prevent dullness. Foam height testing is performed both before and after the cycling test. The typical requirement for foam height is between 1.3-22 cm. The results of foam height observation indicate an increase in foaming capacity with the addition of ethanol extract of corn silk (*Zea mays* L.) to the shampoo compared to the preparation without the extract (blank) (table 5). This is because the ethanol extract of corn silk (*Zea mays* L.) contains saponins, which act as natural surfactants or foaming agents [15, 53].

Irritation test

Iritation test is conducted to determine the irritation effects of the preparation after usage on the hair and scalp, thereby assessing the safety level of the product. Irritation test in this research has obtained approval from the Health Research Ethics Commission of the nursing faculty, Universitas Sumatera Utara, with certificate number 2818/VII/SP/2023. This test involved 12 volunteers whom are given the shampoo preparation on the back of their ears and observed for 24 h. Parameters observed include redness, itchiness, and swelling. Based on the results of the irritation test, there was no side effects such as redness, itchiness and swelling on the volunteers' skin. Therefore, it can be concluded that the shampoo preparation containing ethanol extract of corn hair (*Zea mays* L.) is safe to used. This is because the ingredients in the formulation do not contain substances that can irritate the skin [5, 53].

Stability test

The stability testing of the shampoo preparation is conducted organoleptically, assessing its form, color, and scent in each cycle. Throughout the storage period, every 6 cycles, there were no observed changes in the form, color, and aroma of the four shampoo preparations. This stability is attributed to storing the shampoo preparations in well-sealed containers, protecting them from direct sunlight [54].

Table 6: Results of viscosity test on the corn silk (*Zea mays* L.) ethanol extract shampoo

Formula	Viscocity (cPs)			Average (±SD)
	I	II	III	
F0	2224	2209	2240	2224±15.50
F1	2163	2177	2123	2154±28.02
F2	2087	2040	2010	2045±38.81
F3	1995	1952	1955	1967±24.01

Notes: Results presented as mean+SD (Sn=3), F0: Negative control (blank), F1: Shampoo containing 5% of corn silk extract, F2: Shampoo containing 10% of corn silk extract, F3: Shampoo containing 15% of corn silk extract

The viscosity measurement (table 6) of the shampoo formulation aims to determine the thickness level of the shampoo preparation. Viscosity testing of the shampoo formulation is conducted after storage by pouring 50 ml of shampoo into a beaker glass and measuring its viscosity using a Brookfield viscometer. Based on the viscosity test results of the shampoo formulations F0, F1, F2, and F3, the average viscosity values obtained were 2224 cPs, 2154 cPs, 2045 cPs, and 1967 cPs, respectively. All four formulations meet the requirements of the shampo formulation and characterization, which specifies a viscosity range of 400-4000 cPs. The viscosity observations indicate that the higher the extract concentration, the lower the viscosity. This phenomenon is attributed to the water content of a material with active ingredients, causing the formulation to become thinner and its viscosity to decrease. Despite experiencing a decreation, the shampoo formulations still meet the shampo formulation and characterization requirements, where good shampoo viscosity falls within the range of 400-4000 cPs and meets the specification of being easy to pour into the palm of the hand without spilling easily [54, 55].

Antifungal activity test

The antifungal activity test was conducted with three repetition of test by measuring the diameter of inhibition from vertical and horizontal length then subtracting the disc diameter, which is 6 mm. The measurement from F3 (Shampoo containing 15% of corn silk extract) inhibiton zone can be seen in the picture below.

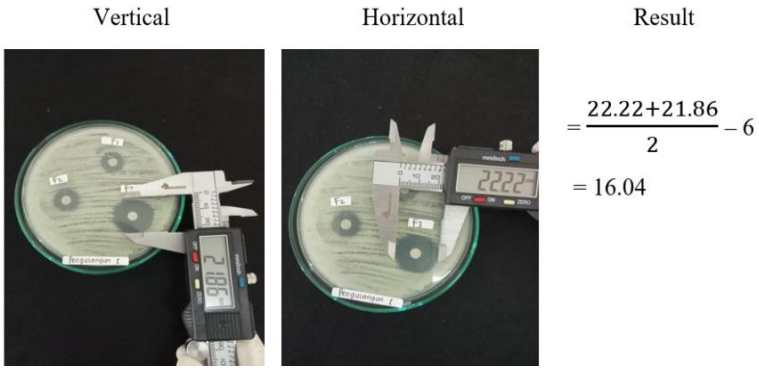

Fig. 1: First measurement of F3 Inhibition zone

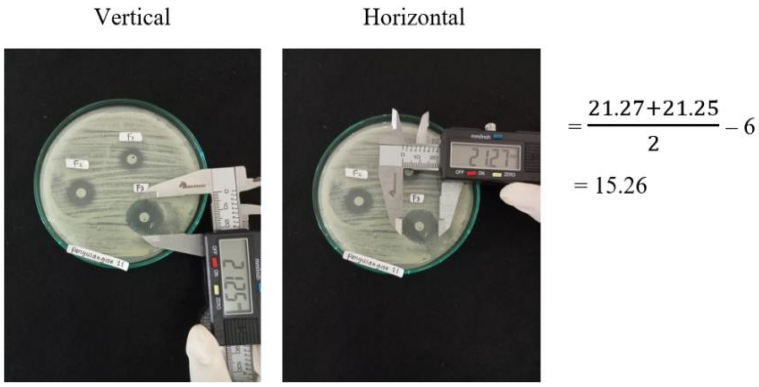

Fig. 2: Second measurement of F3 Inhibition zone

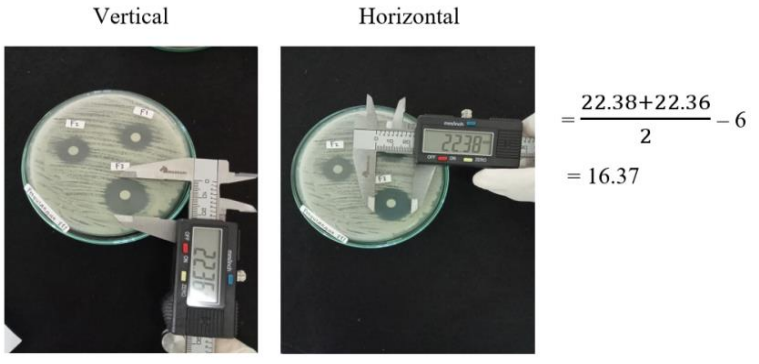

Fig. 3: Third measurement of F3 Inhibition zone

Results from the first, second and third measurement were then being averaged and defined to its category of inhibition toward *Candida albicans.* The same testing is also repeated for F0, F1, F2, and the positive control (fig. 1-3).

Table 7: The results of the antifungal activity test of the corn silk (*Zea mays* L.) ethanol extract shampooagainst *Candida albicans*

No	Formula	Inhibition zone (mm)			Average (±SD) (mm)	Category	Sig. one way anova
		I	II	III			
1	F0	0	0	0	0	No activity	0,000
2	F1	7.1	7.16	9.37	7.88±1.29	Moderate	
3	F2	8.52	9.81	10.07	9.47±0.83	Moderate	
4	F3	16.04	15.26	16.37	15.89±0.57	Strong	
5	C+	18.7	18.6	18.85	18.72±0.13	Strong	

Notes: Results presented as mean+SD (Sn=3), F0: Negative control (blank), F1: Shampoo containing 5% of corn silk extract, F2: Shampoo containing 10% of corn silk extract, F3: Shampoo containing 15% of corn silk extract, C+: Positive control (Brand X® anti dandruff shampoo)

In this study, "Brand X" anti-dandruff shampoo was used as a positive control, resulting in an inhibition zone of 18.71 mm, categorized as strong inhibition. The negative control, using a shampoo base without corn silk ethanol extract (*Zea mays* L.), showed no inhibition (0 mm). In F1, with a concentration of 5%, the inhibition zone was 7.88 mm, and in F2 with a concentration of 10%, the inhibition zone was 9.47 mm. Both of these formulas showed moderate inhibition. In F3 with a concentration of 15%, the inhibition zone was 15.89 mm, classified as strong inhibition. This demonstrates that the saponins present in corn silk (*Zea mays* L.) have a high level of toxicity against fungi. Saponins, which are natural surfactants, work by forming a complex compound with the sterols present in the fungal membrane, causing membrane damage (table 7). The inhibition zone against fungal growth will be larger with higher concentrations of added extract [55, 56].

The one-way ANOVA test resulted in a significance value of 0.000, which is<0.05, indicating a significant difference in the average diameter of the inhibition zones among the treatment groups compared to the negative control. The Tukey HSD test revealed a significant difference between the concentration of F3 compared to F1 and F2, and no significant difference between F3 and C+. Therefore, it can be concluded that the formulation of 15% ethanol extract of corn silk (F3) is the most effective compared to F1 and F2. Furthermore, F3 shown the similar effect as product in market/positive control (Brand X® anti-dandruff shampoo).

CONCLUSION

Based on the research findings, it is evident that corn silk ethanol extract (*Zea mays* L.) can be formulated into a stable shampoo preparation. This conclusion is drawn from the results of organoleptic tests, pH tests, homogeneity tests, foam height tests, and viscosity tests conducted over a storage period of 6 cycles using the cycling test method. However, it is essential to adjust the viscosity of the products to improve the shampoo's acceptance and spreadability in the hair. Shampoo formulation containing 5% corn silk ethanol extract (*Zea mays* L.) demonstrates antifungal activity against *Candida albicans* fungus and exhibits significant differences compared to the negative control. Additionally, the shampoo formulation containing 15% corn silk ethanol extract (*Zea mays* L.) showed strong antifungal activity and has similar antifungal activity toward *Candida albicans* as the positive control did. The shampoo formulation with 15% corn silk ethanol extract (*Zea mays* L.) provides good physicochemical properties and effective antifungal capabilities as anti-dandruff.

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Nil

AUTHORS CONTRIBUTIONS

Leny: Conceptualization, methodology, investigation, data curation, writing. Melia Sari: Writing-review and editing, data curation. Mandike Ginting: Methodology, writing-review and editing. Melisa: Conceptualization, methodology, writing-review and editing, data curation. Benni Iskandar: Conceptualization, supervision, writing–review and editing.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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