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Formulation and evaluation of propolis extracts based shampoo on dandruff causing bacteria

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ABSTRACT

Aim: Dandruff is characterized by the white flakes in the hairs and can be caused by dry skin and mainly by fungal growth of Malassezia yeasts. To treat this condition mainly synthetic type of active ingredient shampoos are used which gave severe adverse effect toward users like hair fall and weakening of the hair roots. In this study, we formulate a shampoo containing an active ingredient "Propolis" an antimicrobial agent which is also known as bee glue. Formed by the combination of bee wax and flower exudate collected from the flower bud.
Methodology and results: In this study, propolis extracts have been used as the antimicrobial agent in the shampoo formulation for treating dandruff-causing bacteria, *Staphylococcus aureus.* Interestingly, the developed propolis shampoo showed is 10-fold more effective against *S. aureus* compared to the propolis extracts alone. This is due to the presence of Tween 80 as the surfactant used in the formulation which adds to this antibacterial effect. The formulated shampoo was also compared with the commercially available shampoo (Safi Shayla brand) for physicochemical properties. Overall evaluation of the shampoo with propolis found to have pH (6-7), good foaming ability, less wetting time, a good percentage of solid content and viscosity. Also, the formulated shampoo has greater stability under accelerated room temperature and accelerated the ageing condition.
Conclusion, significance and impact of study: This study demonstrated the propolis extracted can be used as a potential antimicrobial agent. As it came from the natural resource the acceptance will high among the consumers.

Keywords: Propolis, herbal shampoo, antimicrobial, physico-chemical properties

INTRODUCTION

Dandruff is the most common scalp disorder that mostly affects adults (Borda and Wikramanayake, 2015). It can be said that dandruff is a condition on the scalp where white flakes can be seen on the hair which causes itchiness (Chaijan *et al.*, 2018). Dandruff is a group of corneocytes, where they retained a large cohesion between them and which become unattached from the stratum corneum surface. The main causes of dandruff on the human scalp are due to the presence of oils and dirt in the surrounding air and also the growth of bacteria such *as Staphylococcus aureus*, *Propionibacterium* sp. and fungi, *Malassezia* sp. (Honnavar *et al*., 2017). There are various types of anti-dandruff shampoos are available in the market where mostly employed chemicals as active ingredients to treat the bacteria or yeast on the scalp. Some of the chemical ingredients used in the synthetic antidandruff shampoo resulting in baldness, dryness of hairs, irritation to scalp and eyes (Al Badi and Khan, 2014). To overcome this problem, herbal medicine was chosen as an active ingredient in shampoo formulation to treat dandruff and protect the scalp. There are large numbers of herbal drugs extracted from plants are reported to have medical benefits. Among them, propolis which is also known as bee glue is a resinous substance that is synthesized by honeybees from substance collected from tree buds, saps, mucilage's, lattices and other plant sources. In the ancient time, propolis has been used in the traditional medicine due to its biological properties that rich in phenolic acids, flavonoids, aromatic acids and diterpenoid acids (Elnakady *et al*., 2017). Propolis had shown an antifungicidal activity for *Malassezia pachydermatis* and antibacterial activities for *S. aureus* and *S. intermedius* (Cardoso *et al*., 2010).

There are many applications of propolis mostly in the biomedical due to a variety of biological properties. This is because propolis has been claimed to have antiviral,

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antibacterial, anti-inflammatory and antifungal activities. However, scientific researches on propolis are limited especially studies of propolis for the use in a topical application, lotion for healing septic wounds (Othman *et al*., 2016) and also the use in creams or ointments for curing cold sores (Sosmowski, 1983).

There is much research that shown that propolis exhibits antibacterial activity on some bacteria such as *Candida albicans*, *S. aureus*, and *Escherichia coli* (Chen *et al*., 2018). Propolis extracted using ethanol had shown high antibacterial property against a type of Gram-positive bacteria compared to Gram-negative bacteria and yeast (Silici and Kutluca, 2005). Even though extracted raw propolis has shown significant killing effects on the bacteria, there is a need to identify the effectiveness of propolis based shampoo in combat dandruff problem. At the outset of this study, only a handful of publications have been reported on the possible use of propolis as an antidandruff agent in shampoo. Thus, this study is to further explore on propolis usage as an active ingredient in antidandruff shampoo.

In this paper, we have formulated propolis based shampoo to evaluate and compare the physicochemical properties, stability, cleaning performances with commercial synthetic shampoo. We also evaluated the antibacterial effect of the formulated shampoo on Gram-positive bacteria, *S. aureus* which is one of the dandruff-causing bacteria in humans.

MATERIALS AND METHODS

Materials

Raw propolis was directly collected from hives of stingless bees located in Kuantan, Malaysia. Ethylenediaminetetraacetic acid (EDTA), Tween 80, sodium carboxymethyl cellulose, sodium benzoate, citric acid, DMSO, ethanol, hexane, paraffin wax and nutrient agar were purchased from Sigma-Aldrich (USA). Coconut oil and SAFI Shayla - antidandruff shampoo was bought from the market. *Staphylococcus aureus* strain was obtained from the Centre Laboratory of Universiti Malaysia Pahang.

Preparation of propolis extract

Crude propolis pieces were grounded into a fine powder by utilizing a hammer. The crude propolis was kept under -20 °C. Then, the raw propolis powder was dried using the freeze-drying method before it was used for further processes. Ten gram of propolis powder was mixed with 100 mL of 70% ethanol solvent in a conical flask for 3 min at room temperature. The propolis with ethanol solvent was left overnight at room temperature in an incubator with a speed of 120 rpm.

Purification of propolis extract

The suspension prepared in previous paragraph was filtered through Whatman No. 1 filter paper. The filtrate solution after the filtration process was kept on a dark and airtight sealed bottle. This filtered solution was then concentrated using a rotary evaporator with a rotation speed of 80 rpm and a temperature of 40 °C. Finally, the propolis extract was freeze-dried under 72 mT pressure at –98.4 °C using VirTis Benchtop Pro freeze dryer (SP Scientific, New York, United States).

Formulation of the herbal shampoo

Table 1 shows formulations for an anti-dandruff shampoo with and without propolis extract, SP and SWP respectively. The shampoos were formulated by mixing the ingredients according to the proportions as stated in Table 1. The propolis powder was dissolved with Tween 80 in a beaker. In another beaker, the carboxymethyl cellulose which is the thickener was added drop by drop into a beaker that contains required water for the formulation in order to avoid clumping. Both of these mixtures were combined then sodium benzoate and EDTA were added together. The combinations were mixed and stirred continuously by using a homogenizer until the homogenous mixture was formed. The pH of the mixture was modified by adding a sufficient quantity of citric acid solution until the pH value lies between pH 5 to pH 7. Developed shampoos (SP and SWP) were stored in a suitable container and used for further evaluations. In this study, commercial antidandruff shampoo SAFI Shayla (SAFI) was used as a comparison.

Evaluation of shampoo formulation

Organoleptic studies

The formulated antidandruff shampoo from propolis extract (SP) was evaluated for the colour, odour and texture. These characteristics of formulated antidandruff shampoo from propolis extract were compared with the commercial shampoo (SAFI) and with the formulated anti-dandruff shampoo without containing propolis extract (SWP).

(i) Determination of pH

Ten percent (v/v) shampoo solution was prepared by mixing 10 mL of formulated anti-dandruff shampoo from propolis extract (SP) into 90 mL of distilled water in a beaker. The 10% v/v of shampoo solution in distilled water was tested for pH value by using pH meter at room temperature. The reading of the pH meter was observed and recorded. These steps were repeated for commercial shampoo SAFI Shayla (SAFI) and formulated anti-dandruff shampoo without propolis extract (SWP).

(ii) Determination of the percentage of solid content

The clean and dry evaporating dish was weighed. Four gram of SP shampoo was placed on the previous evaporating dish and weighed. The shampoo with the

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evaporating dish was weighed again to confirm the exact weight of the shampoo. The shampoo on the evaporating dish was placed on the hot plate at 100 °C and was evaporated until the liquid portion of shampoo evaporated. The weight and the percentage of the solid content of shampoo after complete drying were calculated. These steps were repeated for SWP and SAFI. Percentage of solid content was calculated by using equation below:

solid content (%) = [(Weight of dried shampoo/ Initial weight of shampoo) × 100]

(iii) Test to evaluate foaming ability and foaming stability

A 50 mL of the 1% SP sample was prepared by adding 0.5 mL of shampoo with 49.5 mL of distilled water. Fifty millilitre of 1% shampoo solution was put into a 250 mL graduated cylinder. Cylinder shake method was used for determining foaming ability. The graduated cylinder was covered with hand and was shaken for 10 times. The total volume of foam contents after 1 min was recorded. The volume of foam at 1 min intervals for 4 min was observed and recorded. These steps were repeated for SAFI and SWP.

(iv) Viscosity test

The viscometer (Brookfield) with different spindle speeds from 1 rpm to 100 rpm was used to determine the viscosity of the shampoo. Spindle CCT 25 was used to measure the viscosity of the shampoo. The temperature and size of the container were kept constant throughout the study. These steps were repeated for SWP and SAFI.

(v) Wetting time test

One percent (1% v/v) SP sample solution was prepared by mixing 1 mL of the SP sample into 99 mL of distilled water in a beaker. The filter paper was cut into 1-inch diameter discs. The surface of the disc was placed on the surface of 1% SP shampoo solution and the stopwatch was started to record the wetting time. The time taken for the disc to start sinking was observed and noted as the wetting time. These steps were repeated for SWP and SAFI.

(vi) Cleaning ability test

The cleaning test was done on 5 g wool yarn. The wool yarn was soaked in water for 24 h, dried and weighed (W1). It was then dipped in simulated dirt (1 g coconut oil and 1 g paraffin wax dissolved in 100 mL hexane). It was removed, dried and weighed (W2). The wool yarn was then placed in the shampoo (SP, SWP and SAFI) and shaken. It was taken out, rinsed in clean water, dried and weighed (W3). The cleaning was calculated as below:

C (%) = [(W2-W3)/(W2-W1) × 100]

Stability testing

The shampoo formulations were subjected to different storage conditions which is accelerated ageing (45 °C) and room temperature (25 °C) for 4 weeks. Before and after the storage, the shampoo formulations were inspected for their organoleptic characteristics (colour, smell, phase separation), viscosity and their pH value was determined (Mahmood *et al*., 2018).

Antibacterial evaluation

The active ingredient propolis extracts were subjected to *in vitro* testing using the disc diffusion method to evaluate its anti-bacterial activity against *S. aureus*.

(i) Preparation of solution of active ingredient

In 2.5 g of EEP powder was weighed and transferred to a 10 mL beaker of containing 10 mL of absolute DMSO. Then, the mixture was stirred quickly. Then, serial ten-fold dilution was made to make a stock solution of propolis with the concentration of 2500 µg/mL in 1% DMSO. Then, this stock solution was further diluted using ten-fold dilutions to make a concentration of propolis extract solution within the range of 0.25-2500 µg/mL.

(ii) Determination of MIC

The broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of ethanol extract of propolis solution and shampoo formulation as recommended by the National Committee for Clinical Laboratory Standard (NCCLS) (Klančnik *et al*., 2010). The concentrations of propolis extracts solution (PE) that has been tested on the growth of *S. aureus* are ranging from 2500-2.5 µg/mL. The 96-wellplate were filled with 10 µL of overnight growing culture at turbidity of McFarland standard in the test well and added with 190 µL of different concentration of propolis extract separately. Test wells were prepared in triplicate. Well for sterility control was prepared by filling only sterile broth medium in the well. The absorbance of each well was determined using an automatic tray reader adjusted at 630 nm. The well plate was incubated for 24 h at 37 °C with a rotation speed of 120 rpm. Then, the absorbance value in each test well was read again after incubation. These absorbance values were subtracted from those obtained before incubation. The MIC was defined as the minimum concentration of the solution required to inhibit the growth of bacteria.

RESULTS AND DISCUSSION

Formulation of antidandruff shampoo from propolis extract

Nowadays consumers expect for shampoos with unique functions which are far beyond the general functions. However, the majority of ingredients in synthetic shampoo

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have been under severe attack as it gives potential risk with its usage, like hair fall, allergic reactions to skin, weakening of the hair roots (Trüeb, 2007). Hence, people more prefer to use a shampoo with safer natural and naturally derived ingredients. In this study, we prepared a herbal antidandruff shampoo from propolis extract was introduced. This antidandruff shampoo was formulated by ingredients in a definite amount as shown in Table 1. Tween 80, the surfactant in this formulation is a natural component that emulsifies the formulation and resists separation of the shampoo. It possesses good detergency and foaming properties. Propolis was used as an antidandruff agent in this shampoo as it contains bioflavonoid content in it which shows an antibacterial effect towards *S. aureus* bacteria (dandruff causing bacteria). A shampoo should have adequate viscosity to facilitate removal from the bottle but prevent drip down from hair during use (Patidar, 2018). Sodium carboxymethyl cellulose is a non-toxic ingredient that was used as a viscosity builder and stabilizer for the shampoo. Citric acid was added to adjust the pH to the desired level within the range. The formulated shampoo was further preserved with sodium benzoate in a small quantity which is safe to be used.

The yield of propolis extracts is higher by using maceration by ethanol where 58.16% yield obtained which is more than half of the initial weight of propolis can be obtained by this method, maceration by ethanol which uses ethanol at 70% of concentration. As the percentage of yield is more, it means that active compounds or amount of waxes that have been extracted also in a large amount (Trusheva *et al*., 2007).

Organoleptic studies

A shampoo like any other cosmetic preparation should have a good appealing physical appearance. The colour, odour and texture of the formulated shampoo with propolis extract (SP) were found to be acceptable (Al Badi and Khan, 2014). The formulated antidandruff shampoo gives a clear texture, herbal smell and yellowish orange colour. The sample SWP gave an unpleasant odour because it doesn't have any fragrance ingredient or propolis content in the formulation.

Determination of pH

The pH value of shampoo is really vital for improving and enhancing the quality of the hair. Inappropriate pH value of shampoo can influence the tolerance at the skin and eye level. Table 2 shows that all the three shampoo's pH value fall between the range of 5 to 7 which is near to the skin pH. The pH value for commercial shampoo found to be nearly neutral (6.96±0.03). The sample SP (6.12±0.01) is slightly acidic than the sample SWP (6.37±0.02), this is due to the presence of propolis extract. High pH of shampoo which is too alkaline can cause dehydrative effect, irritability and can leave the hair dry and brittle.

Determination of the percentage of solid content

Percentage of the solid content of a shampoo determines the solid mass of material in the shampoo. Good quality of shampoo usually has 20% to 30% solid content (Al Badi and Khan, 2014). The percentage of solid content for all the tested shampoo was found to be within the range of 22% to 24%. Since the values are within the expected range, it is proven that all the shampoos have the right amount of solid content where the shampoos are easy to be applied and rinsed out from the hair. If the shampoo contains a high content of solid, it will be hard to apply into the hair or difficult to wash out. The percentage of solid content found after the shampoo was dried for the SP sample is 22.63±0.08. The percentage of solid content gained for the SWP sample and SAFI sample is 22.74±1.16 and 23.23±0.71, respectively (Table 2). The percentage of solid content in SP and SWP are almost equal due to the same formulation.

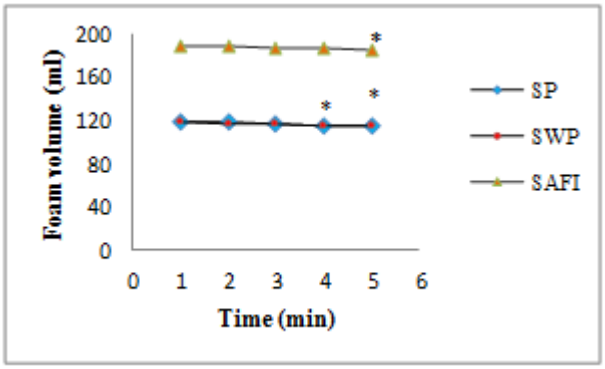

Figure 1: Foam retention profiles of shampoos (SP, SWP and SAFI), n=3, **p*<0.05 was taken as significantly different at 1 min on their respective samples SP, SWP and SAFI.

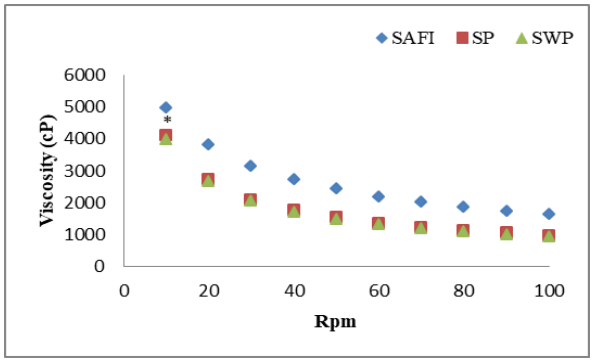

Figure 2: Result for the viscosity test for samples at different rpm (10-100rpm), **p*<0.05 was taken as significantly different from commercial shampoo (SAFI) at 10 rpm.

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Table 1: The compositions of formulation for anti-dandruff shampoo from propolis extract per bottle (100 mL).

Purpose	Material	Percentage required		
Diluent	Distilled water	Qs to 100	Qs to 100	
		SP	SWP	SAFI SHAMPOO
Antibacterial	Propolis	0.5	Nil	Zinc pyrithione, Nigella sativa
Surfactant	Tween 80	20.0	20.0	Sodium lauryl sulphate
Thickener	Sodium carboxymethyl cellulose	1.3	1.3	Hydroxypropyl methylcellulose
Stabilizer	Ethylnediaminetetraacetic acid (EDTA)	0.05	0.05	Polyethelene glycon (PEG)
Preservative	Sodium benzoate	0.10	0.10	Phenoxyethanol
pH stabilizer	Citric acid	As needed	As needed	Sodium chloride

Table 2: Physico-chemical evaluation of formulated and commercial shampoo.

	SP	SWP	SAFI SHAMPOO
Colour	Yellowish orange	Colourless	Light blue
Odour	Herbal smell	Chemical smell	Floral smell
Texture	Clear	Clear	Milky opaque
pH (10% solution)	6.12 ± 0.01*	6.37 ± 0.02*	6.96 ± 0.03
% solid contents	22.63 ± 0.08	22.74 ± 1.16	23.23 ± 0.71
Foam volume (mL)	119 ± 1.73	118 ± 2.00	190 ± 2.00
Wetting time (sec)	46.10 ± 4.17	46.26 ± 3.00	44.66 ± 2.32

Table 3: Stability studies of shampoo at room temperature and 45 °C for 4 weeks.

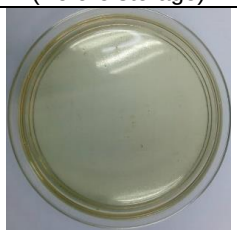
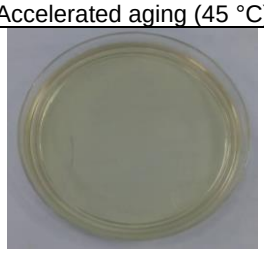
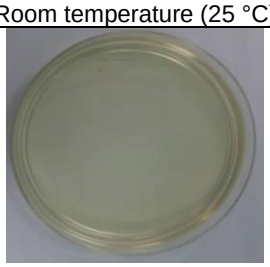
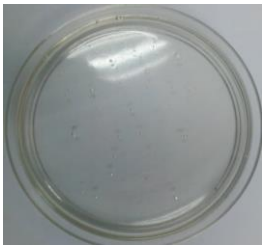
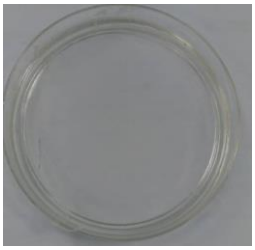
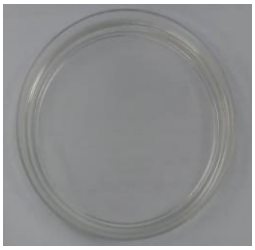
Sample	Storage condition		
	Fresh samples (Before storage)	Stability after 4 weeks	
		Accelerated aging (45 °C)	Room temperature (25 °C)
SP			
Observation	• The colour is yellowish • The odour is good • pH : 6.47±0.01 • Viscosity : 3502.87 cP	• No change in colour • The odour is good • pH : 6.76±0.125 • Viscosity : 5758.96 cP	• No change in colour • The odour is good • pH : 6.9±0.045 • Viscosity : 3317.43 cP
SWP			
Observation	• The colour is clear • The odour is not good • pH : 6.43±0.10 • Viscosity : 3478.06 cP	• No change in colour • The odour is not good • pH : 6.76±0.112 • Viscosity : 4643.61 cP	• No change in colour • The odour is not good • pH : 6.88±0.085 • Viscosity : 3264.59 cP

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Table 4: Measurement of inhibition area caused by active on the growth of *S. aureus.*

Dilution factor	The concentration of active in the sample			Inhibition area (mm)		
	PE (µg/mL)	SP (µg/mL)	SWP (g/mL)	PE	SP	SWP
	0.00	0.00	0.00	0.00±0.00	0.00±0.00	0.00±0.00
10^{-4}	0.25	0.25	0.001	0.00±0.00	0.00±0.00	0.00±0.00
10^{-3}	2.50	2.50	0.010	0.00±0.00	0.00±0.00	0.00±0.00
10^{-2}	25	25	0.100	0.00±0.00	8.83±0.29*	0.00±0.00
10^{-1}	250	250	1.000	9.83±0.57*	10.33±0.58*	0.00±0.00
10^{0}	2500	2500	10.00	12.00±0.50*	12.67±0.29*	7.50±0.50*

Each point is mean area, n=3, *p<0.05 was taken as significantly different from the untreated group by one-way ANOVA.

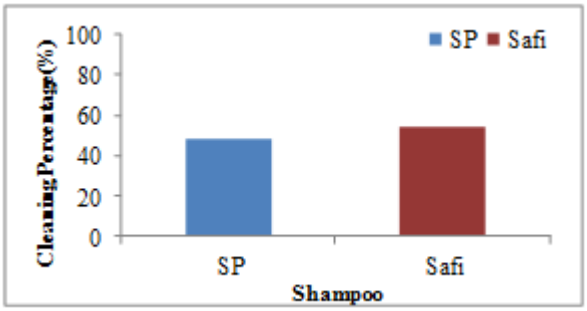

Figure 3: Cleaning ability of shampoo (SP and SAFI), n=3, *p<0.05 was taken as significantly after soaked with simulated dirt on their respective samples SP and SAFI.

Foaming ability and foaming stability test

Foams or lathers are coarse dispersion of gas in a relatively small quantity of liquid generally containing a surfactant. The consumers make a judgement to the performance of the shampoo by looking at its foaming ability. As shown in Table 2, SP and SWP formulation have similar foaming ability whereas the commercial shampoo (SAFI) results in higher foaming ability. SAFI has higher foaming ability due to the presence of sodium lauryl sulphate (SLS) in its formulation which is an effective foaming agent. The foam volume for SAFI which reached up to 190±2.00 mL compared to foam volume of shampoo SP and SWP, which reached up to foam volume of 119±1.73 mL and 118±2.00 mL, respectively. A lot of people have this misunderstanding that if the shampoo does not produce enough lather, it will not effectively clean up the hair. However, foaming doesn't have a great influence on the cleansing property of the product. All the tested shampoos retained their foam volume for 5 min showing that the shampoos have excellent foam stability. The foam retention ability of shampoo samples is shown in Figure 1. There is a significant difference in the values shown by the sample SP and SWP towards the SAFI sample.

Viscosity test

The viscosity of tested shampoos changes gradually as the rpm increases from 10 to 100 rpm. As the rpm increases, the viscosity of the samples decreases. This shows that the formulations are pseudoplastic in nature which is a desirable attribute for a shampoo formulation. The viscosity differences between the samples were observed and compared at a low rotation speed (10rpm) because the shear rates applicable to the flow of shampoo from the bottle are about 5-10 rpm. The viscosity value for all the samples at 10 rpm is within the range of 3000-9000 cP (Moldovan and Părăuan, 2012). A shampoo should have good consistency, where the viscosity should be low enough to ensure a facile removal of shampoo from the package at the same time should be high enough to prevent them from reaching the eyes. Figure 2 shows the viscosity profile for each shampoo.

Wetting time test

Wetting test plays a vital role in the removal of the soil, oil and dye. Wetting phenomena are complicated and depend upon several factors such as diffusion, surface tension and the nature or characteristic of the surface being wetted. The main role of the wetting agent is to reduce surface tension. The results obtained for the wetting time test can be seen in Table 2. The wetting time of three shampoos was found in the order 44.22< 46.10 <46.26 s for SAFI, SP and SWP respectively. The disc in sample SAFI took the least time to sink followed by shampoo SWP and SP; this proves that the detergency level of shampoo SAFI is higher compared to shampoo SWP and SP. The commercial shampoo (SAFI) is made up of many chemical ingredients and surfactant which increased the detergency level, this causes the filter disc to take the least time to sink. The lower the time taken for the filter disc to sink showed the higher the detergency level of the shampoo. (AlQuadeib *et al*., 2018). High detergency level in shampoo enables it to remove dirt and sebum efficiently from the hair.

Cleaning ability test

The cleaning percentage determines the efficacy of the shampoo to remove the dirt from the hair (Pradhan and Bhattacharyya, 2014). The formulate shampoo, SP were compared with SAFI shampoo. The SP has a cleaning percentage of 48.42% and SAFI has a cleaning percentage of 53.94%. SAFI gives better cleaning performance than SP because SP doesn't have

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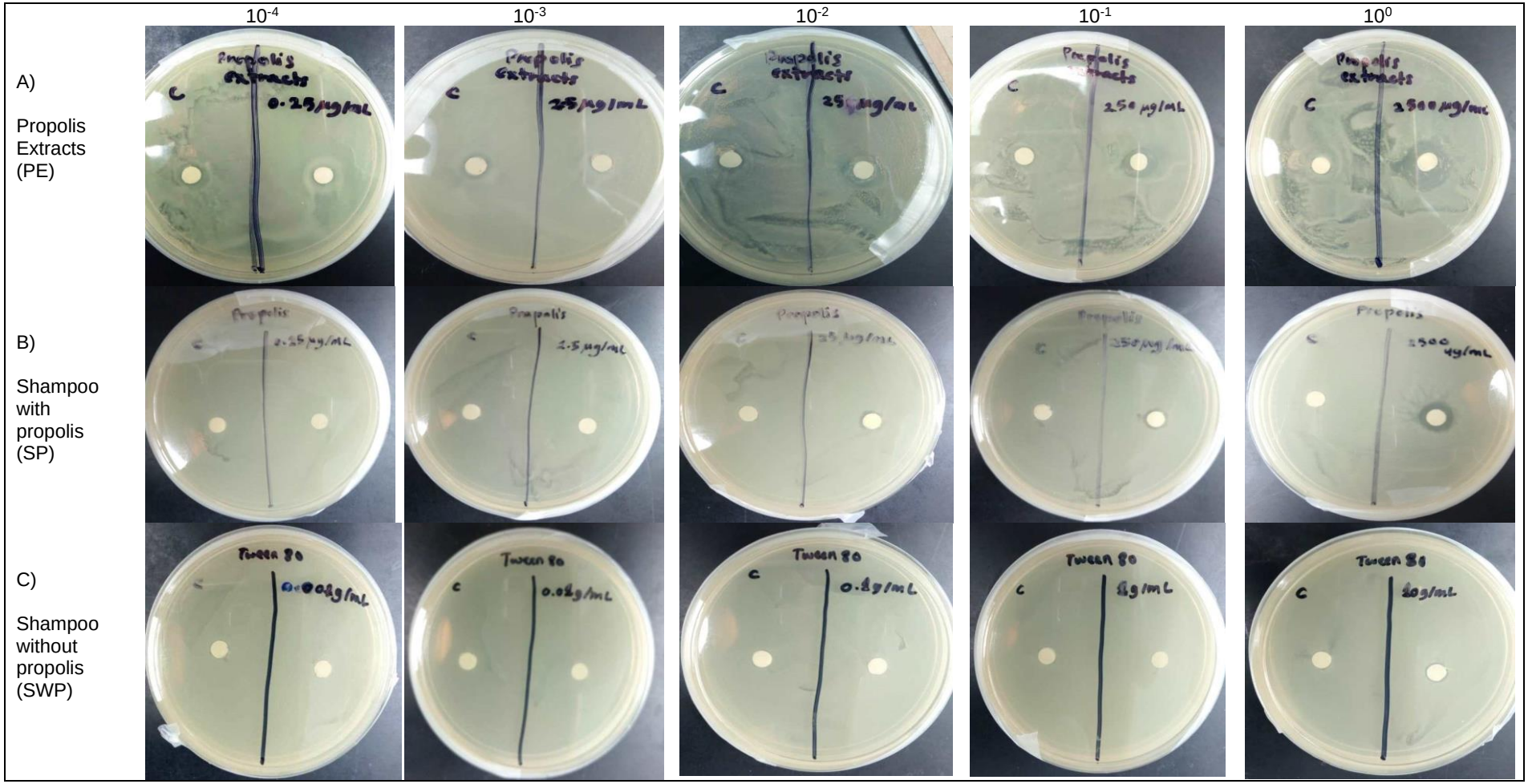

Figure. 4: Zones of inhibition of active against the growth of *S. aureus* at different concentration by ten-fold dilution. A) Propolis extracts (PE) B) Shampoo with propolis extracts (SP) C) Shampoo without propolis extracts (SWP) with dilution factor of: (i) 10^{-4} (ii) 10^{-3} (iii) 10^{-2} (iv) 10^{-1} (v) 10^{0}

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anionic surfactant in its formulation. SP sample could have an even better cleaning effect with the addition of cleaning surfactant in its formulation. The results for cleaning ability are shown in Figure 3.

Stability testing

The stability of shampoo was studied for 4 weeks at two different conditions which is at room temperature (25 °C) and accelerated ageing condition (45 °C). The result of the stability study is shown in Table 3. The organoleptic characteristic of the samples no phase, colour remain same and the odour of the samples also remain unchanged throughout the storage after were tested under severe condition (45 °C and 25 °C) for both SP and SWP shampoos. Thus, the sample can be said to be stable. The colour of the sample, SP which is yellowish orange from day 1 to day 15 remain unchanged. The result from pH testing shows no large variation of the shampoo. The shampoo remains at 5-7 pH which is suitable for this formulation. The viscosity value for all the samples at 10 rpm is still within the acceptable range for a shampoo which is 3000-9000cP (Moldovan and Părăuan, 2012). This indicated that the formulated shampoo remains stable and meet the requirements.

Zone of Inhibition by the disc diffusion method

The stock solution was prepared at 2500 µg/mL and diluted using ten-fold serial dilution method to give a different concentration of propolis extract (PE) solution, 0.25, 2.5, 25, 250, and 2500 µg/mL. The shampoo formulation with propolis extracts (SP) was also been diluted by ten-fold serial dilution to make the concentration to be at the same concentration as the propolis extracts. Table 4 shows the measurement of the inhibition area caused by different formulations. The results show that for PE and SP starts inhibiting the growth of *S. aureus* at 250 µg/mL and 25 µg/mL respectively. This shows that the developed SP shampoo is 10-fold more effective against dandruff causing bacteria compared to the propolis extracts alone. For both sample, PE and SP, activity against, *S. aureus*, the bacteria that causes dandruff, was also markedly higher at higher shampoo concentrations and no inhibition area detected at low concentration. This, therefore, implies that over-dilution of the shampoo below the concentration of 25 µg/mL is likely to reduce its efficacy during application in the process of washing the scalp and hair. As the concentration of propolis extracts solution increases, the zone of inhibition caused by that specific concentration on the growth of *S. aureus* also increases as shown in Figure 4. The zone of inhibition area was observed for SP is 8 mm at its lowest concentration 25 µg/mL compared to PE which needs 250 µg/mL with the range of 12-13 mm. This can prove that propolis extracts exhibit antibacterial properties against *S. aureus* (de Lima *et al*., 2016). It is a desirable phenomenon as the dandruff problems in human is mainly caused by the growth of *S. aureus.* The reason for increases in antibacterial effect for SP is due to the presence of Tween 80 as a surfactant in the shampoo formulations. According to the data in Table 4, it is evident that Tween 80 also exhibits antibacterial properties at the concentration of 10 g/mL and above. The measurement on the inhibition area caused by different sample at different concentration can be seen more clearly in Figure 5. This was also further supported by earlier studies where Tween 80 exhibits antibacterial properties beyond the concentration of 4% (Rebello *et al*., 2014). Without any expectation at the beginning of this study, surprisingly, the developed formulation shows a dual effect of antibacterial against *S. aureus* due to the addition of Tween 80 as a surfactant. This will be a desirable condition for consumers as it will work more effectively in treating dandruff problem in human.

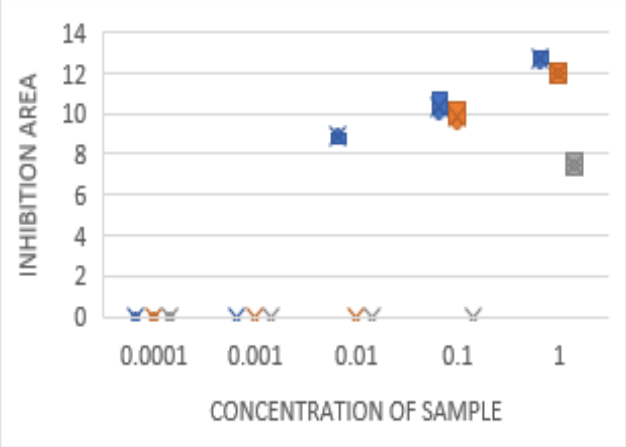

Figure 5: Measurement of inhibition area caused by different sample, SP, PE and SWP at different concentration by 10-fold dilutions.

Determination of MIC by broth microdilution

Broth microdilution method was used to determine the MIC of propolis extracts solution and shampoo formulation for more accurate results. The MIC is the lowest concentration of propolis extracts solution and shampoo formulation that needed to inhibit the growth of dandruff causing bacteria. Comparing to the disc diffusion method which uses ten-fold dilution of PE solution and SP, MIC value that obtained is 250 µg/mL and 25 µg/mL, respectively. However, the broth microdilution method gives a lower MIC value for both samples compared to the disc diffusion method. According to the broth microdilution method, the MIC value for PE and SP is 39.063 µg/mL and 19.53 µg/mL respectively. This shows that the lowest concentration that needed by developed shampoo to inhibit the growth of dandruff causing bacteria, *S. aureus* is lower than the lowest concentration needed by propolis extracts. This is another 44 evidence that the developed shampoo works better in treating against dandruff-causing bacteria, *S. aureus* compared to the propolis extracts alone.

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CONCLUSION

The main aim behind this research was to formulate a stable and functionally effective antidandruff herbal shampoo by excluding synthetic active ingredients which are normally incorporated in such formulation for the antidandruff agent and make use of natural sources, that is propolis extracts as the antibacterial agent. This work has shown that the development of this shampoo is able to treat the most common bacteria *S. aureus* which causes dandruff problem on the human scalp. The propolis extracts show the antibacterial effect at lower concentration towards the growth of *S. aureus*. Interestingly, the developed shampoo with the propolis extracts shows a much 10-fold better antibacterial effect compared to the antibacterial effect of the propolis extracts itself. This is due to the presence of Tween 80 as the surfactant used in the formulation which adds to this antibacterial effect. This means that the developed shampoo shows a dual effect on antibacterial activity, therefore it is evident that the developed shampoo is able to treat dandruff in human more effectively. However, further studies need to be conducted on different dandruff causing bacteria and fungus in order to prove the safety and efficacy of this formulation to combat dandruff problems.

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