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Siwak (Salvadora persica) extract as a natural anti-halitosis mouth spray

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Abstract. Bad breath or halitosis is a pathologist that often experienced in the community. Bacteria that play a specific role in halitosis are anaerobic. To overcome bad breath due to the development of bacteria in the oral cavity is using Siwak spray. Siwak solution is obtained through maceration. The maceration results went through several stages of testing, namely, organoleptic, pH, and antibacterial testing through Nutrient Agar (NA). Analysis of the quantitative data obtained using the Friedman method. The organoleptic results show that the concentration of 1% that was most accepted by 76.66% of the panellists. The optimal bacterial inhibition was at a concentration of 2%. The pH for the 1% Siwak concentration was the best matches.

1. Introduction

Self-confidence can be referred to several habits from the visual side, such as a person's appearance-behaviour or, and health. One pathologist that is often experienced is bad breath or halitosis [1]. Halitosis is the release of Volatile Sulphur Compound (VSC's) due to the decay of gram-negative bacteria's that causes bad breath. Bacteria that play a specific role in halitosis are anaerobic. All oral bacteria have the potential to release VSC. This is based on ecological factors in the mouth, such as fluctuations in the microbiota composition, nutrient availability, and bacterial metabolism. Ecological factors will influence the amount and quality of halitosis produced [2].

Miswak or Siwak can be used to overcome halitosis due to the development of bacteria in the oral cavity. Siwak, an antimicrobial and antifungal, has many properties to eliminate and reduce the formation of dental plaque [3]. It can also avoid the occurrence of dental cavities (caries) caused by *Streptococcus* mutants [4], curing gum inflammation (gingivitis), preventing inflammation of the mouth (stomatitis) and tonsillitis [5], and bad breath (halitosis) [6].

Siwak has the abundance of natural ingredients, including sodium chloride, potassium chloride, salvadourea, oleic acid, linoleic acid, trimethylamine, thiocyanate, benzyl isothiocyanate, and nitrate, silica, vitamin C, resin, tannin, saponin, N-benzyl-2 phenylacetamide, lignans, flavonoids, fluoride, calcium, sodium bicarbonate and salvadorin [7]. The use of Siwak is more effective as a Siwak spray.

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Siwak extract solution packaged in a sprayer is more environmentally friendly, alcohol-free, and less use of harmful chemicals compared to commercial mouthwash or other halitosis removers.

2. Material and Methods

2.1 Product manufacturing procedure

Siwak stems were thinly sliced and then dried in a laminar dryer at 37°C for 24 hours. The dried samples were blended to form of Siwak's powder. The Siwak powder was filtered using a 60-mesh sieve. A 50 gr Siwak powder was put in a 1000 ml size Erlenmeyer, then macerated using sterile distilled water for 24 hours. The ratio between Siwak powder and sterile distilled water was 1: 5.

The maceration process was carried out in LAF (Laminar Air Flow) to avoid contamination bacteria or fungi. In this maceration process, two layers were formed, the distilled water solution at the top, and the Siwak powder (macerate) at the bottom of the Erlenmeyer.

The maceration solution was then filtered until produce yellow and clear solution. Macerate results were then added by sterile distilled water. Re-maceration solution is poured into a petri dish. Then the Petri dish is wrapped using plastic wrap and oven at 37°C for 24 hours. The results were smooth Siwak powder. Then the soft powder is weighed and dissolved in distilled water to create a spray solution with various formulations. The spray solution was then undergone the antibacterial test, pH test, and organoleptic test.

2.2 Siwak spray formula

Table 1 shows the Siwak's formula.

Materials	Control	Formula 1	Formula 2	Formula 3
Siwak Stem	3%	1%	1,5%	2%
Sodium Metabisulfite	100 ppm	100 ppm	100 ppm	100 ppm
Potassium Sorbate	100 ppm	100 ppm	100 ppm	100 ppm
Mint Flavor	0,15%	0,15%	0,15%	0,15%
Sterile Distilled Water	20 ml	20 ml	20 ml	20 ml

Table 1. The Siwak spray formulation.

2.3 Mouthspray test of Siwak stem extracts

The mouth spray tests are organoleptic and pH test. An organoleptic test on the product examined the colour, smell, and taste [8]. The pH was tested using the pH paper test. The paper test was dipped into the Siwak extract solution for several minutes and contrasted with the pH indicator's colour.

2.4 Antibacterial test of Siwak stem extract solution

The test was divided into five stages: (1) Sterilisation, (2) Preparation of nutrient agar (NA) media, (3) Making tilting medium, (4) Bacterial inoculation on tilting media and (5) Antibacterial activity tests with the diffusion method. In sterilisation, the Petri dishes and separation tools were washed thoroughly using detergent, then dried in an oven. After that, the tools are wrapped in paper and sterilised in an autoclave at 121 °C for 15 minutes [9]. When preparing nutrient agar (NA) media, as much as 3.8 grams of NA media was put into an Erlenmeyer. 100 ml of distilled water was added to dissolve the NA media and then heated on a hot plate. The media was then sterilised in an autoclave at 121 °C for 15 minutes. When making tilting medium, a 5 ml sterilised NA media poured in each of the three sterile test tubes and closed using aluminium foil. The media was sterilised in an autoclave at 121 °C for 15 minutes. After autoclaving, the sterile media was left at room temperature and placed in a tilted position until the media solidifies for bacterial inoculation. The next step is bacterial inoculation on tilting media. Rejuvenated bacteria were taken with sterile needles, then implanted onto the tilted media by scraping the needles. The inoculum was incubated at 37 °C for 24 hours. Finally, antibacterial activity tests with

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the diffusion method was conducted by preparing 5 Petri dishes, pour the NA medium \pm 15 ml into each petri dish, then leave to solidify. A sterile cotton swab was dipped in the bacterial suspension. Bacteria in cotton sticks was rubbed on the surface of the NA medium. Apply the disk that has been soaked in the Siwak extract solution; the first cup was filled with a spray solution of 1% Siwak concentration, second cup with 1.5% spray solution, the third cup with a spray solution of 2% concentration, repeated three times. The petri dish was then incubated for 24 hours at 37 $^{\circ}$ C. Then the inhibition zone (mm) diameter of each concentration was measured.

2.5 Data analysis

The data collected were qualitative data and quantitative data. Qualitative data in the form of evaluation data based on the preference of Siwak spray solution preparations on an organoleptic test can prove the quality of the spray product. Quantitative data were obtained from pH testing and bacterial testing. The data that has been collected will be presented in tabular and graphical form. Quantitative data were in the form of antimicrobial test results and pH tests. The qualitative data was analysed using the Friedman test. Data were processed using Microsoft Excel 2010 and SPSS 20 (Statistical Product and Service Solution) programs. Percentage score to determine the preferred value in a Siwak spray solution using data analysis at different values (intervals).

Based on the calculation of the interval value in the form of a percentage, then the percentage interval value is made based on the preferred value as follows:

Percentage (%)	Favourite Criteria
84 - 100	Strongly like
68 - 83,99	Like
52 – 67,99	Neutral
36 – 51,99	Dislike
20 – 35,99	Strongly dislike

Table 2. The percentage intervals and the preferred score.

The organoleptic test was carried out by using three treatments at different concentrations of 1%, 1.5%, and 2%. The organoleptic testing using non-parametric statistical tests and the quantitative data are calculated in the form of criteria/categories only. The non-parametric test results used were the Friedman test at a significance level of 95%. This quantitative test was carried out using SPSS version 16.0.

3. Results and Discussion

3.1 Organoleptic test

3.1.1 Organoleptic analysis of Siwak spray solutions

Table 3 presents the results of organoleptic analysis based on the colour of the Siwak spray solution used by panellists with an age range between 18-30 years. The results of the colour analysis obtained based on data Table 3. shows the concentration of 1% is preferred than the other concentrations. In contrast, the concentration of 100% obtained the smallest score (48.68%). The Friedman test results on the colour of the spray solution Siwak at different concentrations obtained values p = 0.00. This means that H0 is rejected and H1 is accepted. The analysis result using SPSS was the most favoured by the panellists, namely Siwak solution at a concentration of 1% with the characteristics of a clear solution with a score of 3.37 [8].

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Table 3. Results of organoleptic analysis of Siwak spray solutions.

Concentration of	Score	Strongly	Like (4)	Neutral	Dislike	Strongly	Total
Siwak Spray	(%)	Like (5)	, ,	(3)	(2)	Dislike	
Solution	, ,				, ,	(1)	
1%	Colour	20	45,34	8	2,67	0,67	76,68
	Taste	0	13,34	40	6,67	0	60,01
	Aroma	0	24	28	9,34	0	61,34
1,5%	Colour	6,67	13,34	36	6,67	0	62,68
	Taste	3,34	37,34	18	6,67	0,67	66,02
	Aroma	10	40	16	5,34	0	71,34
2%	Colour	6,67	13,34	22	12	2	56,01
	Taste	33,34	48	2	1,34	0	84,68
	Aroma	0	45,34	22	0	1,34	68,68
100%	Colour	0	13,34	20	12	3,34	48,68
	Taste	0	5,34	4	30,67	2	42,01
	Aroma	0	2,67	2	29,34	4	38,01

Table 4. Organoleptic quality of Siwak spray solutions.

Organoleptic	Sample	Mean Rank	Characteristic	ρ-value
Quality				
	Concentration 1%	3,37	Clear	
Colour	Concentration 1,5%	2,53	Milky White	0,00
	Concentration 2%	2,23	Murky white	
	The concentration of	1,87	Yellowish white	
	100%			
	Concentration 1%	2,32	Clear	
Taste	Concentration 1,5%	2,62	Milky White	0,00
	Concentration 2%	3,68	Murky white	
	The concentration of	1,38	Yellowish white	
	100%			
	Concentration 1%	2,58	Clear	
Aroma	Concentration 1,5%	3,13	Milky White	0,00
	Concentration 2%	3,02	Murky white	
	The concentration of	1,27	Yellowish white	
	100%			

Based on the organoleptic analysis, the taste of the Siwak spray solution in Table 4 obtained the highest score at a concentration of 2%. In contrast, Siwak spray solution concentrations of 1% and 1.5% have almost the same score. At a concentration of 1%, the score was 90 (60.01%), while the 1.5% the score was 99 (66.02%). The lowest score on the organoleptic test of taste in the Siwak spray solution was the 100% concentration, 63 (42.01%). The results obtained based on Friedman's analysis are directly proportional to the results of the organoleptic analysis. At the concentration of 2%, the Siwak spray solution has the highest mean rank value of 3.68, in line with the most preferred Siwak spray solution by a panellist was the 2% Siwak concentration. Based on the ρ -value obtained significance of 0.00, there is a real difference to the treatment given Siwak spray solution concentrations of 1%, 1.5%, 2%, and 100%.

E

A B

C

D

E

Concentration 4

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0,02

3.2 Antibacterial test

The antibacterial test result in the clear zone is presented in Table 5.

Treatment Repetition Clear Zone Picture Average Clear Zone Concentration 1 Α 0,2 В 0,1 \mathbf{C} 0 0.06 D 0 E 0 Concentration 2 A 0 В 0 C 0 0 D 0 Е 0 Concentration 3 A 0,2В 0,1C 0,10,1D 0.1

0

0

0

0

0

0,1

Table 5. Bacteria test results in a clear zone.

Antibacterial activity test was used to find the effectiveness of the Siwak extract to inhibit bacterial growth. The white spot in each petri dish proves the formation of the bacterial zone. The bacterial test can be carried out using the agar diffusion method. This method makes it easy to determine the bacterial growth activity in a sample by observing the formation of inhibitory zones as antibacterial. The wider the inhibitory zone formed, the stronger the antibacterial power [9].

Based on the results of antibacterial tests using Siwak solution samples, it can be seen that the largest inhibition zone formed at a concentration of 2% with an average inhibition zone area of 0.1cm. At 1% concentration, the average inhibition zone was obtained 0.06 cm, and at the administration of a 100% concentration, it only had an average clear zone of 0.02 cm. While at a concentration of 1.5%, the formation of an average inhibition zone is not formed (0). This proves that the Siwak spray solution with a concentration of 1.5% cannot kill bacteria at all. The greater the concentration of extract given, the inhibitory zone area will also be wider [9]. The width of the inhibition zone> 20 mm is very strong, medium if the inhibition zone is 5-10 mm, and weak if <5 mm. It is stated that which has the most optimal bacterial inhibition is Siwak spray solution concentration of 2% [10].

3.3 pH Test

Based on the pH test, the first sample has a pH of 6. The second and third samples have a pH of 5. In contrast, the 4th sample has a pH of 4. The pH range for bacterial growth ranges from 6.5 to 7.5. Meanwhile, a good pH range in the manufacture of mouthwash ranges from 5.71 to 5.98. The pH value in a sample determines the type and amount of bacterial growth production. This shows that the formula used in making Siwak mouth spray outside the pH range of bacterial growth [11].

Siwak, which has been processed in the form of an extract solution, can increase saliva's pH [3]. The content of tannins, which are substances that can make the tissues in the body constrict, has astringent

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properties [12]. These substances can stimulate saliva so that it is more quickly produced by the parotid gland. Besides, the essential oils contained in Siwak produce a stinging taste that can trigger the salivary glands to produce saliva. Alkaloid secondary metabolites such as Salvadorin in the Siwak extract solution are antibacterial to acid-producing bacteria.

4. Conclusions

The organoleptic test showed that the colour of 1% Siwak concentration is clear. However, at a concentration of 2%, the colour was turbid white. The aromas test showed that Formulation 2 (1.5% concentration) has a milky aroma. The best bacteria inhibition was Formulation 3 (concentration of 2%) with an average clear zone area of 0.1 cm.

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