

Development of Anti-Dandruff Shampoo from Kaffir Lime which is the By-Product of Food Industry

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ABSTRACT

Kaffir lime juice is a by-product of food industry. It could be used as an ingredient to make kaffir lime shampoo. The crude extract of kaffir lime oil from its peel could inhibit the growth of *Candida albicans* which is a micro-organism associated with the dandruff of the scalp. The minimum inhibition concentration (MIC) of *C. albicans* by the oil was 1% (v/v). The kaffir lime juice has acid properties, helps cleaning hair, removes dirt and gives shampoo color. The optimum formula was kaffir lime juice, kaffir lime peel oil, sodium lauryl ether sulfate, coconut diethanolamide, 5-bromo-5-nitro-1,3-dioxane (Bronidox) and water at 15.0, 1.0, 50.0, 30.0, 0.1 and 30.0 % (w/w), respectively. A test with one hundred consumers showed that most of them were quite satisfied with this product especially in reducing dandruff of the scalp. The kaffir lime shampoo has color in L, a and b values of 4.6, -0.67 and 1.0, respectively. Its viscosity was 1,811 centipoise.

Key words: kaffir lime, oil, shampoo, anti-dandruff

INTRODUCTION

Kaffir lime is a well-known herb used in cosmetic industry because its aromatic oil in the peel can be used for anti-dandruff caused by micro-organism (Suksringam, 1984; Jarikpakorn, 2001). It helps reducing itching of the head skin and helps lubricating and darkening the hair (Manosroy and Leelapornpisit, 1992). The kaffir lime juice is naturally acid and good for protecting the skin of the head as well as cleaning the residue of the soap and shampoo (Singhabutra, 1999; Endradjo, 2000) and reducing the damage and splitting of the hair (Suksringam, 1984; Jarikpakorn, 2001). Thus it is preferred to add kaffir lime juice as an ingredient in preparing shampoo (Lawrence *et al.*, 1971). In food industry, only the peel of kaffir lime fruit is used as an ingredient in processing chili paste and

curry and the rest is discarded as waste. This research is aimed at using kaffir lime juice which is a by-product to make good kaffir lime shampoo at low cost .

MATERIALS AND METHODS

Raw materials and micro-organisms

Kaffir lime without peel which is a by-product was obtained from Maesri Food Plant Company. Fresh kaffir lime was bought from Talad Thai Market, Patumtani province. *Candida albicans* which is a micro-organism associated with the dandruff of the scalp was obtained from the Institute of Scientific Research of Thailand.

Growth of yeast *C. albicans*

C. albicans was cultured in liquid media

sabourand dextrose broth in 50 ml flask and rotated at 200 rpm at 37°C for 24 h then the light absorption was measured at 530 nm. The yeast concentration was diluted until 0.05 absorption value, recorded the dilution ratio and used this ratio for the next test (Costa *et al.*, 2000).

The yeast was adjusted for turbidity and swabbed on sabourand dextrose agar. A Whatman no. 1 was cut into sterile disk about 6 mm diameter and placed on the media plate (Chatdamrong, 2001). About 0.45 microliter of filtered kaffir lime oil was dropped on to the disk at different concentrations as follows : 100, 50, 10, 5 and 1% by volume. The oil was previously diluted with Tween 80 (Mann and Markham, 1998). The samples were incubated at 37°C for 24 h. The inhibition zone and MIC were recorded and compared with the control which used Tween 80 without yeast culture and ketoconazole. The kaffir lime juice with aromatic oil that could inhibit the growth of *C. albicans* was further tested using statistical design as 2 × 3 factorial. The first factor was the amount of foam producing agent or coconut diethanolamide at 2 levels, 3 and 5%. The second factor was the amount of the kaffir lime juice at 3 levels; 10, 15 and 20%. The amount of aromatic kaffir lime oil was fixed at 1% (v/v) which was the minimum concentration that could inhibit the growth of *C. albicans* according to the preliminary test. The kaffir lime juice was centrifuged at 5,000 g and the clear juice was collected. The juice was prepared at 10, 15 and 20% by weight and warmed to 75-80°C and kept for one minute. Sodium lauryl ether sulfate 50% by weight and 1% (v/v) of the kaffir lime oil were added and mixed well then the pH was adjusted with sodium hydroxide to pH 5.5. Antimicrobial chemical or 1% Bronidox by weight was added to the mixture plus clean water to get 100% volume. The mixture was left overnight to allow the foam collapsed before filling into clear plastic bottles. The shampoo was tested for sensory and quality by a group of test panel of 30 persons.

Quality analysis

Analysis of physical quality

The viscosity of shampoo was measured with a Brookfield Viscometer model DVIII and the color was measured with a Minolta model CM 3500d. The amount of foam and stability of the shampoo were measured according to Ross Mile Test Method by Harris *et al.* (1975).

Sensory analysis

Home use test was selected as sensory analysis of the shampoo quality. The test panel or consumers used the product at home for 1-2 weeks and gave quality scores from 1 to 9 (1 = extremely dislike and 9 = extremely like). The quality scores of the shampoo tested were viscosity, color, odour, foam formation, the softness of hair after using shampoo and overall preference score. The resulting scores were analyzed for statistical variation and the average difference was measured using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Effects of the kaffir lime juice and its aromatic oil on inhibitions of the growth of *C. albicans*

It was found that kaffir lime juice could not inhibit the growth of *C. albicans* but it was useful due to its acidic property to give nice shampoo color (Singhabutra, 1999; Endardjo, 2000). The kaffir aromatic oil at 1% (v/v) could inhibit the growth of *C. albicans*. The inhibition effect is shown in Figure 1 and Table 1.

Optimum formula of kaffir lime shampoo

The purpose in developing the new formula of kaffir lime shampoo was to obtain natural or close to natural herb shampoo (Standard of industrial shampoo, Ministry of Industry no. 162. 1998). The good shampoo should soften human hair and be effective against dandruff causing by certain groups of yeasts. Six formulae of kaffir lime shampoo with different amount of coconut diethanolamide as well as kaffir lime juice.



Figure 1 Inhibition effect of kaffir aromatic oil 1% (v/v) diluted with Tween 80 on the growth of *C. albicans* as shown by clear zone around the paper dipped in the kaffir oil and placed on the culture media.

The effects of coconut diethanolamide and the kaffir lime juice on the viscosity of the shampoo are shown in Table 2. Usually clear liquid shampoo will have viscosity between 500-1500 centipoise (Leelapornpisit, 2000).

Most of the shampoo formula tested gave high viscosity values. The shampoo formulae 4, 5 and 6 were too viscous and could not be used for consumer test. Diethanolamide is a foaming agent but also helps increasing viscosity of the shampoo. Increasing the kaffir juice in the shampoo formula will reduce the amount of the make up water thus may increase the viscosity of the shampoo. Usually viscous solution is not preferred by the consumer in the clear shampoo because it is difficult to pour out of the bottle. Formulae 1, 2 and 3 were selected for further sensory test by the test panel of 30 persons. The color values were measured as L for brightness, a⁻ for green, a⁺ for red, b⁺ for blue and b⁻ for yellow. The results are shown in Table 3.

Table 1 Effects of the kaffir juice and its oil on inhibition of the of *C. albicans*.

Liquid or mixture used	Average diameter of clear zone (cm ± S.D.)						
	% kaffir lime oil diluted with kaffir juice or Tween 80						
	100	50	10	5	4	1	0.5
Kaffir juice	-	n	n	n	n	n	n
Kaffir oil : Tween 80	2.7±0.30	1.78±0.10	1.35±0.07	1.07±0.09	0.87±0.06	0.83±0.12	-
Tween 80 ^{L/}	-	n	n	n	n	n	n

^{L/} = control

- = no inhibition effect

n = no test

S.D. = Standard Deviation

Table 2 Effects of coconut diethanolamide and the kaffir juice on the viscosity of the kaffir lime shampoo.

Formula (w/w)	% of coconut diethanolamide (w/w)	% kaffir juice (centipoise)	Viscosity
1	3	10	627
2	3	15	1,811
3	3	20	2,725
4	5	10	6,847
5	5	15	8,900
6	5	20	9,732

The brightness of the shampoo increases with the amount of the kaffir juice and coconut diethanolamide added.

Sensory test

Most of the kaffir lime shampoo available in the market are not added with kaffir lime oil so they are not effective in anti-dandruff. The shampoo formulae 1, 2 and 3 were tested by a group of 30 consumers test panel for sensory analysis, visual viscosity, color, odour, amount of foam, actual use, softness of hair after using the product and overall quality. The results are shown in Table 4. It was found that the mean color score of the formula 1 was significantly different from formula 3. There was no difference in color between formula 2 and 3 ($P < 0.005$). There were no statistical differences ($P < 0.05$) among all 3 shampoo formulae (1, 2 and 3) for odour, amount of foam, viscosity and softness of hair after rinsing off the shampoo. The test panel gave moderate

scores for the odour, the amount of foam, viscosity of the shampoo and the softness of the hair after rinsing off the shampoo for all 3 shampoo formulae with no statistical differences ($p < 0.05$). The shampoo formula 2 received the highest score for overall quality which was statistically different from formula 1 but not different from formula 3 ($p < 0.05$), thus formula 2 was selected for further testing.

The shampoo formula 2 was tested for growth inhibition of the yeast *C. albicans* which caused dandruff according to the method developed by (Kajornkiatpanich, and Sukkurdkitpibul, 1990). A commercial kaffir lime shampoo and a synthetic shampoo with 2% ketoconazole were used as control. These commercial shampoo were specially used for anti-dandruff. The results are shown in Table 5. It was found that the kaffir lime shampoo added with 1% (v/v) aromatic kaffir oil could inhibit the growth of *C. albicans* and gave a clear zone of 1.56 cm in diameter compared to the commercial kaffir lime shampoo and anti-dandruff shampoo of 1.27 and 3.48 cm, respectively.

This showed that the experimental shampoo was more effective than commercial kaffir lime shampoo for anti-dandruff but the product was less effective than ketoconazole because it used only 1% oil (v/v) while ketoconazole use 2% chemical active ingredient which was more expensive.

Table 3 Effects of coconut diethanolamide and kaffir juice on the color values of the kaffir shampoo.

Formula	L value	a value	b value
1	3.40 ^f	-0.39 ^e	0.36 ^f
2	4.16 ^e	-0.67 ^d	1.00 ^e
3	5.18 ^b	-1.10 ^b	1.08 ^c
4	4.33 ^d	-0.50 ^c	1.41 ^d
5	4.89 ^c	-1.05 ^b	2.03 ^b
6	6.40 ^a	-1.67 ^a	2.92 ^a

⁽¹⁾ In a column, means followed by the same letter are not significantly different by DMRT at $p \leq 0.05$.

Table 4 Effects of coconut diethanolamide and the kaffir lime juice on sensory quality of the kaffir lime shampoo.

Formula	Visual color	Odour	Viscosity	Foam formation	Softness of hair	Overall likeness
1	5.90 ^b	6.37 ^{ns}	6.05 ^{ns}	6.21 ^{ns}	6.58 ^{ns}	6.37 ^b
2	6.37 ^{ab}	6.53 ^{ns}	6.32 ^{ns}	6.58 ^{ns}	6.58 ^{ns}	6.95 ^a
3	6.48 ^a	6.42 ^{ns}	6.32 ^{ns}	6.37 ^{ns}	6.21 ^{ns}	6.42 ^{ab}

ns = not significant

Table 5 Comparison of developed kaffir lime shampoo added with 1% (v/v) kaffir aromatic oil to the commercial kaffir lime shampoo and anti-dandruff shampoo (2% ketoconazole) for inhibition of *C. albicans* growth.

Shampoo product	Average diameter of inhibition clear zone (cm \pm S.D.)
Developed shampoo with 1% kaffir oil	1.56 \pm 0.32
Commercial kaffir lime shampoo	1.27 \pm 0.13
Anti-dandruff shampoo (2% keto conazole)	3.48 \pm 0.29

CONCLUSION

It was found that kaffir lime juice, a by-product of food industry, could be used to make kaffir lime shampoo. Adding 1% (v/v) kaffir lime oil could inhibit the growth of *C. albicans* which caused dandruff of the scalp. The optimum formula for producing anti-dandruff kaffir lime shampoo consisted of kaffir lime juice, kaffir aromatic oil, sodium lauryl ether sulfate, coconut diethanolamide, preservative (Bronidox L) and water at 15.0, 1.0, 50.0, 3.0, 0.1 and 30.9%, respectively. For consumer test the average scores for color, odour, viscosity, foam formation, softness of the hair and the overall preference were acceptable.

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