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PRESERVATIVE EFECTIVENESS TEST OF DRY WATER EXTRACT OF GAMBIER (*Uncaria gambir Roxb.*) IN CREAM TYPE OF COSMETICS

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ABSTRACT

Gambier is an endemic plant that can grow properly such as in Indonesia it has been empirically used for natural coloring, antiinfection and burns wound healing. Gambier used as antimicrobials because its catechins compound. Catechins mechanism as antimicrobial are break the cell wall or cell membrane of bacteria, other mechanism is to precipitate of protein because the catechins properties is same with phenolic compound. This research aims to know the preservative effectivity of dry water extract of gambier (*Uncaria gambir Roxb.*) in cream type of cosmetics. Research methods are total plate count, yeast and mold count, logs and percentage of preservative effectivity test. The data of the result there are: gambier extract in *Pseudomonas aeruginosa* cream shows the preservative requirement at 2%, 2,5% and 3% concentrations with 100% of reduction and 3 logs reductions on 14th days testing; gambier extract in *Staphylococcus aureus* cream shows the preservative requirement at 3% concentrations with 100% of reduction and 3 logs reductions on 7th days of the test; gambier extract in *Enterobacter aerogenes* cream shows the preservative requirement at 2%, 2,5%, 3% and 3,5% concentrations with 100% of reduction and 3 logs reductions on 28th days of the test; gambier extract in *Candida albicans* cream shows the preservative requirement at 2%, 2,5% and 3% concentrations with 100% of reduction and 3 logs reductions on 7th days of the test; gambier extract in *Aspergillus niger* cream shows the preservative requirement at 2,5% and 3% concentrations with 100% of reduction and 3 logs reductions on 7th days of the test. Dry water extract of gambier has the potential that can be used as a microbial preservative in cream type of cosmetics

Keywords: *Gambier extract, catechins, preservative, antimicrobial, percentage, and logs of reduction*

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INTRODUCTION

Gambier is an endemic plant that can grow properly in Indonesia. Gambier (*Uncaria gambir Roxb.*) is a kind of sap/gum from heater water extract from gambier plant.(1) The main compound of gambier plant is catechin. (2) Catechin is included in phenolic compound, which phenolic compound has function as antimicrobial with its mechanism to break the bacterial cell wall and membrane, so, the permeability of bacterial cell disturbed. Another catechin mechanism is by precipitating microorganism protein compounds (3). From the previous research, founded gambier product extract showed catechin as antibacterial because its ability to again gram-positive bacterial such as Staphylococcus aureus in gambier extract with ethyl acetate solvent is stronger than extract with ethanol as solvent (4).

Cream is defined as a viscous liquid or semi-solid emulsion of either the water-in-oil or oil-in-water type (5). Cream preparations contain parts of water and oil sat they are easily contaminated by microorganisms. To prevent this, preservatives and antiseptics can be added to cosmetic preparations. Preservatives are additives in cosmetics to prevent cosmetic damage caused by microorganisms (6). Preservative selection is an important factor in the safety and stability of pharmaceutical preparations. The use of chemical substances causes adverse effects on health and the environment (7). So we need a preservative that is safe and has good effectiveness. One source of potential antibacterial compounds is plants (8).

Based on this research before, obtained problem formulation and the aim of this research there are to know how the effectiveness of dry water extract gambier as cosmetic cream preparations.

METHODS

Gambir Preparation and Extraction

Gambir chunks come from the gambir plantation of the community in Muaro Paiti, Lima Puluh kota, West Sumatra. The gambier chosen is gambier with a dark brown color with a diameter of ± 4 cm and a thickness of ± 3 cm. Can be seen in Figure 1.



Figure 1. Gambir chunks

Gambir chunks are pollinated. 100 grams of gambier powder in a 2 liter Erlenmeyer, add 500 ml of distilled water. Heat 1 hour and filtered. Let stand until a precipitate forms. After forming 2 phases separated by the filtering process. The precipitate was dried in an oven at 100^0C for 1 hour. The dry precipitate is powdered using a mortar. 100 grams of purified gambier powder was dissolved in 500 ml of ethyl acetate, refluxed for 1 hour at a stirrer speed of 2rpm and a temperature of 65^0C – 76^0C. The filtrate obtained was thickened using a rotary evaporator.

Cream Preparation Procedure

The cream formula of this research is a modification of the vanishing cream formula, an O/W type emulsion. Material optimization was carried out by varying stearic acid and TEA. The manufacturing process begins with heating the mortar and stamper. Mix the stearic acid oil phase and adeps lanae melted together in a water bath at 60^0 -70^0C. Mix the liquid phase of TEA, glycerin and distilled water and then heat it at 60^0 -70^0C. The liquid and oil phases are melted and mixed in the mortar and hot stamper. Stir the mixture of the two phases until a homogeneous creamy mass is formed. Add tocopherol, stir homogeneously. Dissolve gambir dry water extract powder with a few drops of DMSO. Add a solution of dry aqueous extract of Gambir with DMSO in the formed cream base, grind, stir until homogeneous.

Preservative Effectiveness Test

Preservative effectiveness test was carried out on cream extract concentrations F1 2%, F2 2.5%, F3 3%, F4 3.5% against several microorganisms consisting of *Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes, Aspergillus niger*. For *Enterobacter aerogenes* bacteria, a positive control test and a negative control were added. The positive control cream used is a Moisturizer Cream product Marcks' Moisturizer Cream that uses phenoxyethanol and chlorphenesin as preservatives. Negative control cream is a cream made with a cream base formulation only, without gambier extract. Attached is a table of vanishing cream formulations.

Table 1. Vanishing Cream Formula

Material	**F1** %	**F2** %	**F3** %	**F4** %
Gambir Extract	2	2,5	3	3,5
Adeps lanae	5	5	5	5
Tokoferol	0,05	0,05	0,05	0,05
TEA	1	1	1	1
Gliserin	7	7	7	7
Asam stearat	10	10	10	10
Aquadest	Add 100	Add 100	Add 100	Add 100

Dissolve the test cream in chloride buffer solution with ratio 1:1. Heat at temperature 40^0C-45^0C homogenize with vortex mixer. A cream sample added with gambier extract that previously has been dissolved to DMSO. The testing doing in sterile condition like tools and material that have been sterilized before in sterile room. First testing is calculation and determination of total plate count and yeast mold number to determine of which bacterial and fungus suspension that used and inoculated to cream sample to be tested (9).

RESULT AND DISCUSSION

Total Plate Number and Initial Yeast Mold Number on microbes, to determine the bacterial suspension used to be inoculated on the cream sample to be tested.

Table 2. Determination of Bacterial Suspension by Total Plate Number Method

Bacteria	10^{-4} A	10^{-4} B	10^{-5} A	10^{-5} B	10^{-6} A	10^{-6} B	10^{-7} A	10^{-7} B	10^{-8} A	10^{-8} B	10^{-9} A	10^{-9} B	10^{-10} A	10^{-10} B
EB	1	1	1	1	45	36	16	7	2	2	1	1	1	1
PA	41	1	10	3	11	6	10	1	1	1	1	2	1	1
SA	1	1	1	67	1	16	12	1	4	1	1	0	0	0

Notes
EB = *Enterobacter aerogenes*
PA = *Pseudomonas aeruginosa*
SA = *Staphylococcus aureus*
A = 1st Petri Dish
B = 2nd Petri Dish

Table 3. Calculation of Bacterial Total Plate Number

FP / Bacteria	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
EB	$2x10^4$	$2x10^5$	$8{,}1x10^7$	$2{,}3x10^8$	$4x10^8$	$2x10^9$	$2x10^{10}$
PA	$4{,}2x10^5$	$1{,}3x10^6$	$1{,}7x10^7$	$1{,}1x10^8$	$2x10^8$	$3x10^9$	$2x10^{10}$
SA	$2x10^4$	$6{,}8x10^6$	$1{,}7x10^7$	$1{,}3x10^8$	$5x10^8$	$1x10^9$	-

Notes
EB = *Enterobacter aerogenes*
PA = *Pseudomonas aeruginosa*
SA = *Staphylococcus aureus*
FP = Dilution Factor

The condition of the bacterial suspension used is bacterial suspension that containing 10^8 cfu/ml of bacteria (10). So that the 7th dilution suspension was used to be inoculated cream.

Table 4. Determination of Fungus Suspension by Yeast Mold Number

FP	10^{-4} A	10^{-4} B	10^{-5} A	10^{-5} B	10^{-6} A	10^{-6} B	10^{-7} A	10^{-7} B	10^{-8} A	10^{-8} B	10^{-9} A	10^{-9} B	10^{-10} A	10^{-10} B
Yeast CA	300	77	45	300	23	15	1	0	1	0	0	0	0	0
Mold ASP	300	300	300	300	39	18	4	4	2	2	1	0	0	0

Notes
CA = *Candida albicans*
ASP = *Aspergillus niger*
FP = Dilution Factor
A = 1st Petri Dish
B = 2nd Petri Dish

Table 5. Calculation of Fungus Yeast Mold Number

FP	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Yeast CA	$1{,}885x10^{6}$	$1{,}725x10^{7}$	$1{,}9x10^{7}$	$5x10^{6}$	$5x10^{7}$	-	-
Mold ASP	$3x10^{6}$	$3x10^{7}$	$2{,}85x10^{7}$	$4x10^{7}$	$2x10^{8}$	$5x10^{8}$	-

Notes
CA = *Candida albicans*
ASP = *Aspergillus niger*
FP = Dilution Factor

For yeast mold suspension conditions used are those containing 10^7 cfu/ml of yeast mold (10). So that the 5th dilution suspension was used for *Candida albicans,* and 6th dilution suspension was used for *Aspergillus niger* to be inoculated.

Inoculated all cream sample with 1 ml suspension of microbial test. Volume of suspension no more than 1% of sum of cream tested. Homogenized microbial suspension using vortex mixer.

Move the inoculated cream with microbial suspension into different pots for each variant microbial suspension and testing time at 0, 2nd, 7th, 14th and 28th days of testing. Testing doing by 10 multiple dilution series using peptone saline solution that containing 1% polysorbate 80. Cream that not testing yet put in room temperature (20^0C-25^0C) during the test. Sum of microbial determined by spread total plate number method in TSA media for bacteria and pour yeast mold number method in SDA media for yeast and mold (fungus). Do the test by incubating duplicated petri dish. For bacteria incubated in temperature 35 ± 2^0C for 24-48 hours, yeast and mold incubated in temperature 25 ± 2^0C for 3-5 days. Count the sum of alive microbial by logs and percentage reduction formulation. Criteria that extract is effective as antimicrobial is when the result showing at least 2 logs or 99% in 2nd days of testing and 3 logs or 99,9% in 7th days of testing and no more increasing number of microorganisms during the observation in normal data variation.

Result of the test to the cream sample with dry water extract of gambier with interval days in 0, 2nd, 7th, 14th, 28th days showed by the tables below. Positive and negative control tests were carried out only on one of the test microbes for validation purposes.(10) In this study Enterobacter aeruginosa bacteria were used because cosmetic products that

have a high water content such as oil-in-water cream are products that are most likely to have Enterobacter aerogenes microbial contamination.

Table 6. Percentage and Reduction Logs of *Enterobacter aerogenes*

Sample	Interval days	%Reduction		Reduction Logs	
		Result	Criteria	Result	Criteria
2%	T0-T2	4,0%	99%	0 logs	2 logs
	T0-T7	98,0%	99,9%	2 logs	3 logs
	T0-T14	99,6%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
2,5%	T0-T2	71,0%	99%	1 logs	2 logs
	T0-T7	96,0%	99,9%	1 logs	3 logs
	T0-T14	99,5%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3%	T0-T2	83,3%	99%	1 logs	2 logs
	T0-T7	99,7%	99,9%	2 logs	3 logs
	T0-T14	99,7%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3,5%	T0-T2	52,8%	99%	0 logs	2 logs
	T0-T7	99,2%	99,9%	2 logs	3 logs
	T0-T14	99,6%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
Control (-)	T0-T2	58,3%	99%	0 logs	2 logs
	T0-T7	96,7%	99,9%	1 logs	3 logs
	T0-T14	95,8%	99,9%	1 logs	3 logs
	T0-T28	96,7%	99,9%	1 logs	3 logs
Control (+)	T0-T2	80,0%	99%	1 logs	2 logs
	T0-T7	92,0%	99,9%	1 logs	3 logs
	T0-T14	99,0%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs

Table 7. Percentage and Reduction Logs of *Staphylococcus aureus*

Sample	Interval days	%Reduction		Reduction Logs	
		Result	Criteria	Result	Criteria
2%	T0-T2	89,1%	99%	1 logs	2 logs
	T0-T7	99,0%	99,9%	3 logs	3 logs
	T0-T14	99,7%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
2,5%	T0-T2	62,5%	99%	0 logs	2 logs
	T0-T7	93,8%	99,9%	2 logs	3 logs
	T0-T14	100%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	2 logs	3 logs
3%	T0-T2	65,0%	99%	0 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3,5%	T0-T2	96,6%	99%	1 logs	2 logs
	T0-T7	99,6%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs

Table 8. Percentage and Reduction Logs of *Candida albicans*

Sample	Interval days	%Reduction		Reduction Logs	
		Result	Criteria	Result	Criteria
2%	T0-T2	98,4%	99%	2 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
2,5%	T0-T2	94,2%	99%	1 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3%	T0-T2	81,6%	99%	1 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3,5%	T0-T2	96,9%	99%	2 logs	2 logs
	T0-T7	96,9%	99,9%	2 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs

Table 9. Percentage and Reduction Logs of *Aspergillus niger*

Sample	Interval days	%Reduction		Reduction Logs	
		Result	Criteria	Result	Criteria
2%	T0-T2	93,1%	99%	1 logs	2 logs
	T0-T7	96,6%	99,9%	1 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
2,5%	T0-T2	93,1%	99%	1 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3%	T0-T2	90,0%	99%	1 logs	2 logs
	T0-T7	100%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3,5%	T0-T2	91,7%	99%	1 logs	2 logs
	T0-T7	95,8%	99,9%	1 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs

Table 10. Percentage and Reduction Logs of *Pseudomonas aeruginosa*

Sample	Interval days	%Reduction		Reduction Logs	
		Result	Criteria	Result	Criteria
2%	T0-T2	8,6%	99%	0 logs	2 logs
	T0-T7	85,7%	99,9%	3 logs	3 logs
	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
2,5%	T0-T2	72,2%	99%	1 logs	2 logs
	T0-T7	66,7%	99,9%	3 logs	3 logs

3%	T0-T14	100%	99,9%	3 logs	3 logs
	T0-T28	100%	99,9%	3 logs	3 logs
3,5%	T0-T2	75,0%	99%	1 logs	2 logs
	T0-T7	50,0%	99,9%	2 logs	3 logs
	T0-T14	100%	99,9%	2 logs	3 logs
	T0-T28	100%	99,9%	2 logs	3 logs
	T0-T2	80,0%	99%	1 logs	2 logs
	T0-T7	80,0%	99,9%	1 logs	3 logs
	T0-T14	80%	99,9%	1 logs	3 logs
	T0-T28	100%	99,9%	2 logs	3 logs

The test result and calculating of reduction get the reduction percentage and logs of gambier extract in cream with bacterial sample of *Pseudomonas aeruginosa* which eligible are 2%; 2,5% and 3% concentration of gambier extract with reduction number 100% and 3 logs reduction in 14th days of testing. Gambier extract in cream with bacterial sample of *Staphylococcus aureus* which eligible is 3% concentration of gambier extract with reduction number 100% and 3 logs reduction in 7th days of testing.

Table 10. One Way Anova Statistical Test

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Persentase_EA	16	,8765	,26006	,04	1,00
Persentase_SA	16	,9408	,12222	,63	1,00
Persentase_CA	16	,9800	,04690	,82	1,00
Persentase_AN	16	,9752	,03604	,90	1,00
Persentase_PA	16	,8114	,24593	,09	1,00
Sample	16	2,5000	1,15470	1,00	4,00

Gambier extract in cream with *Enterobacter aerogenes* which eligible are all concentration type with reduction number 100% and 3 logs reduction in 28th days of testing. Gambier extract in cream with *Candida albicans* which eligible are 2%; 2,5%; 3% concentration with reduction number 100% and 3 logs reduction in 7th days of testing. Last, gambier extract in cream with *Aspergillus niger* which eligible are 2,5% and 3% concentration with reduction number 100% and 3 logs reduction in 7th days of testing.

Based on the results of the one way ANOVA statistical test, there was no relationship between extract concentration and % reduction in each test bacteria. All Cream

Formulas at gambier extract concentrations of 2%, 2.5%, 3% and 3.5% all have a reduction value of 100% and 3 log reduction on the day of testing. So it can be assumed that gambier extract at a minimum concentration of 2% is effective as a preservative for all tested bacteria.

The result showing dry water extract of gambier has potential as antimicrobial preservative in cream preparate. The things that effect the result test there are because of its extract is has not been doing the quantity test of number/level of catechin in extract. Other factors are extract concentration not optimal yet, inhibiting mechanism, aerobe and anaerobe microbial variant and possibility of microbes more adaptive to inhibitory substance.

Catechin is a phenolic compound that has effect as antimicrobial with mechanism damaging wall cell of bacteria, interfere the metabolism in cytoplasm membrane and enzymes in bacteria (11). Mechanism catechin to against growth inhibition of *Candida albicans* is damaging fungal cell which is resulting leakage (damaging the permeability) of protein and nucleic acid (12). Mechanism catechin to inhibiting growth of *Aspergillus niger* is suppressing the biophile formation so the fungal metabolism is disrupted (13).

The others studies showed that the total microbial mouthwash gambier during storage ranges from 0 to 4 colonies/ml (14). Methanol extract have growth inhibitory activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella sp*. Local inhibition of *Staphylococcus aureus* and *Bacillus cereus* are relatively wider than other microbes (15). The study are confirms the ability of gambier as an antimicrobial is quite good.

CONCLUSION

Referring to the result of the research that has been discussed, the result of effectiveness test of the dry water extract of gambier is almost met the criteria, which is the number of microbes showed the decrease at least 2 logs or 99% in 2^{nd} days of testing and 3 logs or 99,8% in 7^{th} days of testing and no more increase further of microbial growth. So

that the conclusion that dry water extract of gambier that containing catechin compound has potential as preservative in cream type of cosmetics.

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