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Research Article

Peel-off Mask Formulation from Stem of Sempeng (*Nepenthes gracilis*) as Anti Acne Against *Propionibacterium acnes* Bacteria

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ABSTRACT

Introduction: Stem of Sempeng (*Nepenthes gracilis*), which contain saponin and tannin has been known had antibacterial activity against *Propionibacterium acnes* that usually improve severe of acne. Acne is a skin disease characterized by chronic inflammation in the polisebasca that often occurs in adolescence. **Aims and Objective:** The aims of this research are to develop and test a peel-off mask prepared from stem of Sempeng extracts, which has antibacterial activity against *Propionibacterium acnes*. **Materials and Methods:** Stem of Sempeng was extracted with soxhletation method by ethanol 96%. The extract was formulated into peel-off mask with different extract concentration (0, 1, 5, 10, 15) %. Then the inhibition test of the extract and peel-off mask was measured against bacteria *Propionibacterium acnes*. The data analysis technique used in this study was to measure the zone diameter on a petri dish using a caliper with a millimeter (mm) unit and presented in tables and photos, the results of the inhibition zone measurement were compared with the classification of the response of the extract barrier to the growth of Clinical and Laboratory Standards Institute (CSLI) standard bacteria. **Results:** The result showed that the inhibition zone of the peel off mask preparation of sempeng stem extract in F1 and F2 formulations with extract concentrations of 1% and 5% are categorized as resistant, which means that the *Propionibacterium acnes* bacteria has a low level of sensitivity so that a large inhibition zone is not formed. Whereas in the F3 formulation with a concentration of 10% is categorized as an intermediate, which means in this concentration, the mask can inhibit the growth of the inhibition zone *Propionibacterium acnes* bacteria which is formed quite well, but in this category it will require repeated use or with high doses of use. Then in the F4 formulation with a concentration of 15%, it is categorized as susceptible, which means the mask have a good inhibitory resistance zone, this shows that the increase in high concentration, the greater the inhibition zone as well. **Conclusion:** The peel off mask from ethanol extract of Sempeng stem is able to inhibit the growth of *Propionibacterium acnes* bacteria. The peel mask preparation with concentration 1%, 5%, 10% and 15% forms a clear zone around the disc which means that this peel off mask formulation has activity to inhibits *Propionibacterium acnes* bacteria and is included in the category of susceptible interpretation according to the CLSI standard.

Key words: *Nepenthes gracilis* stem extract, Peel-off mask, *Propinibacterium acnes*.

INTRODUCTION

One of the traditional medicinal plants that has potential as anti-acne is Sempeng. This medicinal plant has long been used by people in Katingan Regency, Central Kalimantan as a traditional medicine in maintaining and caring for the skin. The results of previous research conducted by researchers in 2017.[1] showed that the ethanol extract of Sempeng plant stems contains secondary metabolites, namely saponins and tannins. Tannins have antibacterial action by preparing protein. Antibacterial effect of tannins through reactions with cell membranes, inactivation of enzymes and inactivation of the function of genetic material. The mechanism of action of tannins as an antibacterial is to inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot form.[2] Tannins have antibacterial activity related to their ability to activate microbial cell adhesin, activate enzymes and interfere with protein transport in the inner layer of cells.[3]

The mechanism of action of saponins as antibacterial is that they can cause protein and enzyme leakage from within cells.[4] Saponins can be anti-bacterial because their surface active substances are similar to detergents. As a result, saponins will reduce the surface tension of the bacterial cell walls and damage membrane permeability. This damage to cell membranes greatly disrupts the survival of bacteria.[4] Saponins diffuse through the outer membrane and susceptible cell walls and then bind to the cytoplasmic membrane thereby disrupting and reducing cell membrane stability. This causes the cytoplasm to leak out of the cell resulting in cell death. Antimicrobial agents that interfere with the cytoplasmic membrane are bactericidal.[5]

The pharmacological effect as antibacterial possessed by the two secondary metabolites contained in the ethanol extract of Sempeng stem, has the potential to be developed into a preparation that is used as an antibacterial especially as an anti-acne.[1] This is due to the empirical properties that are used to treat skin health so that the potential of these two plants as antiacne is quite large. In previous research, the research team had prepared a peel off mask from this plant extract and a good formulation had been obtained so that it could be made into a peel off mask preparation. In this study, the inhibition power of the peel off mask of the ethanol extract of Sempeng stem was carried out against the bacteria that cause acne, namely *Propionibacterium acnes*. The hope is that from the research it can be seen the anti-acne potential of the peel off mask of the Sempeng stem ethanol extract.

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MATERIALS AND METHODS

Place and time of research

This research was conducted for 6 months at the Laboratory of Pharmacognosy and Microbiology, Faculty of Health, Muhammadiyah University of Palangkaraya.

Materials and tools

The materials used in this study were Sempeng stem extract, Methyl Paraben, Propyl Paraben, Polyvinyl Alcohol (PVA), Propylene Glycol, 96% Ethanol, 1.2% Clindamycin Gel, commercial peel-off mask, NaCl 0.9%, H_2SO_4 1%, $BaCl_2$ 1%, Manitol Salt Agar (MSA), Blood Agar Plate (BAP), Brain Heart Infusion (BHI) and Nutrient Agar (NA).

The tools used in this research are the evaporator, water bath, pH meter, universal pH, digital scales, percolation tools, porcelain plates, mortar and stampers, petri dishes, stirring rods, callipers, spatula, autoclave, paper straw, beaker glass, erlenmeyer, pearl glass, empty disc, round loop, tweezers, sterile cotton, oven, spatula, tissue, bunsen, gloves, test tube, tube rack, aluminium foil, measuring flask, desiccator, ball pipette, volume pipette and cotton stick

Research stages

Sample selection and making

Sempeng plants used in this study are those from Katingan Regency, Central Kalimantan Province. The part used is the fresh stem.

Simplicia making

Based on Materia Medika Indonesia Volume IV (1995), the process of making Sempeng stem simplicia begins with the collection of the raw materials used, then wet sorting is carried out and washed using clean running water, after which the stems are chopped to expand the surface of the raw material. The next stage is the drying process which is carried out by drying it under direct sunlight then after drying the simplicia is sorted again and mashed until it becomes a powder.

Extract making

The making of Sempeng stem extract using the soxhletation method was carried out by:[6]

Sempeng stem simplicia powder was weighed as much as 50 grams and wrapped using filter paper;

Then the wrapped simplicia is inserted into the device;

96% ethanol solvent is added until it reaches 3 cycles then the shock is assembled and water flows into the condenser;

The extraction was carried out until the liquid extract in the cochlea flask was colourless (clear);

The liquid extract is taken and the evaporation process is carried out using a rotary evaporator until the ethanol content is reduced by half. The extract is evaporated on a water bath using a porcelain cup until a thick extract is obtained;

The viscous extract obtained is weighed. The yield of the thickened Sempeng stem extract is calculated using the formula:

$$\text{Yield} = \frac{\text{Weight of thick extract}}{\text{Weight of simplicia}} \times 100\%$$

Inhibition test of sempeng stem ethanol extract against *Propionibacterium acnes* bacteria

Prepare tools and materials to be used.

Adjusting bacterial suspensions made to the McFarland 0.5 standard.

Taking the *Propionibacterium acnes* bacteria from the suspension, streak on NA media using a sterile cotton swab.

Soaking the blank disc in ethanol extract of Sempeng stem with a concentration of 0.5%, 1%, 5%, 10%, 15%, the disc was taken with sterile tweezers and planted on NA media with a distance of 2 cm per disc.

Soaking the blank disc in positive control Clindamycin with a concentration of 0.5%, 1%, 5%, 10%, 15%, the disc was taken with sterile tweezers and implanted on MHA media with a distance of 2 cm for each disc.

Incubated at 37°C for 24 hours.

Measuring the diameter of the clear area formed around the disc using a calliper as the diameter of the inhibition zone of the Sempeng stem ethanol extract against the growth of *Propionibacterium acnes* bacteria.

Peel-Off mask formulation

PVA was developed with water destilat at 90°C until it fully expanded, then homogenized (M1). The Sempeng stem extract was dissolved with 96% ethanol until dissolved, then methyl paraben and propyl paraben were dissolved with 96% ethanol until dissolved (M2). M2 was added to M1 while still stirring with a stirrer, then added glycerin, propylene glycol and finally added 100 mL of water destilat added until a homogeneous gel mass was formed.

Sterilization of tools and materials

The equipment and materials needed to be sterilized first include petri dishes, cotton sticks, pearl glasses, Erlenmeyer, measuring flasks, beaker glasses, by putting them in the oven at 180°C for 1 hour while the ose and tweezers are sterilized using Bunsen fire and sterilized media using an autoclave at a temperature of 121°C for 15 minutes.

Making BHI (Brain Heart Infusion) media

The BHI media was weighed as much as 1.85 grams then put into the erlenmeyer, then 50 mL of aquadest was added and heated with an electric bath until the media dissolved completely. After the media dissolves and is homogeneous, the media is piped into the test tube as much as 5 mL. Then the media is sterilized using an autoclave for 15 minutes at a temperature of 121°C (Pakekong *et al.*, 2016).

Making BAP (Blood Agar Plate) media

The BAP medium was weighed as much as 4 grams and put into the erlenmeyer, then 100 mL of aquadest was added and heated with an electric bath until the media dissolved completely. Then the media was sterilized using an autoclave for 15 minutes at a temperature of 121°C. While the sterilization process for BAP media is taking place, the stage is continued by taking 7 mL of type O blood. Next, blood is put into an erlenmeyer containing sterile pearl glasses, then shaken until the blood is lysed or the fibrin threads are not visible. After the BAP medium is sterile and the blood lysed, the two are mixed and the media is poured into a sterilized petri dish.

Making NA (Nutrient Agar) media

The NA media was weighed as much as 48.8 grams then put into the erlenmeyer, 350 mL of aquadest was added and heated with an electric bath until the media dissolved completely, then the media was sterilized using an autoclave for 15 minutes with an air pressure of 1 atm at a temperature of 121°C. After the sterilization is over, the media is removed and allowed to stand at room temperature until the media is lukewarm and the media is poured into a petri dish (Ramadanti, 2008).

Propionibacterium Acnes planting

Planting the *Propionibacterium acnes* bacteria was carried out by taking one of the *Propionibacterium acnes* bacteria, then implanting it on BHI media and incubated at 37°C for 24 hours.[7]

Preparation of Mac farland standard solution 0.5

Preparation of the Mac Farland Standard solution was carried out by inserting 0.5 mL of 1% $BaCl_2$ into a test tube. Then added 9.95 mL of 1% H_2SO_4 which is proportional to the estimated number of bacteria, then closed tightly so that evaporation does not occur and the solution is shaken every time it is used to compare with the bacterial suspension.[7]

Making bacterial suspension

Sterile 0.9% NaCl is put into a 5 mL test tube. Bacterial colonies from BAP media were prepared and the spirit lamp was turned on. One eye of *Propionibacterium acnes* bacteria was taken from BAP media then put in 0.9% NaCl until turbidity was obtained which was adjusted according to Mac Farland turbidity standard 0.5. If it is less cloudy, colony is added to the suspension, while if it is too cloudy, 0.9% NaCl is added.

Making positive control

The positive controls used in this study are:

Topical antibiotic preparations, namely Clindamycin ointment.

Peel-off mask preparations on the market.

Creating negative controls

The negative control in this study was the peel-off mask formulation which did not contain the ethanol extract of Sempeng stem.

Inhibition test

Prepare tools and materials to be used

Adjusted the bacterial suspension made to the Mac Farland 0.5 standard.

Taking *Propionibacterium acnes* bacteria from the bacterial suspension, streak on NA media using a sterile cotton swab.

Soaking a blank disc in a peef-off mask formulation with a concentration of 1%, 5%, 10%, and 15% of the stems of Sempeng ethanol extract, then the disc was taken with sterile tweezers and planted on NA media, with a distance of 2 cm for each disc.

Soaking the blank disc in positive control for 1.2% clindamycin gel preparation and peel off mask preparation circulating in the market, then the disc is taken with sterile tweezers and implanted on NA media with a distance of 2 cm from each disc.

Incubated at 37°C for 24 hours.

Measuring the diameter of the clear area formed around the disc using a caliper as the diameter of the inhibition zone of the peel-off mask of ethanol extract Sempeng against the growth of *Propionibacterium acnes* bacteria

Data analysis

The data analysis technique used in this study was to measure the zone diameter on a petri dish using a caliper with a millimeter (mm) unit and presented in tables and photos, the results of the inhibition zone measurement were compared with the classification of the response of the extract barrier to the growth of CSLI standard bacteria.

RESULTS AND DISCUSSION

This study aims to see the potential of Sempeng stem ethanol extract as an innovative cosmetic preparation that can be used to inhibit the growth of acne-causing bacteria, namely *Propionibacterium acnes*. Empirically, Sempeng stems have been used by the community in Katingan Regency as traditional medicine in maintaining healthy skin. In the initial research that has been carried out, it was found that the Sempeng stems contain secondary metabolites, namely saponins and tannins. These two secondary metabolites have activity in inhibiting bacterial growth.[1] This study aims to obtain scientific information about Sempeng stems which will later be used for reference in further research.

Table 1: The formulation of the ethanol extract peel-off mask.

Ingredient	% Konsentrasi				
	F0	F1	F2	F3	F4
Ethanol extract of Sempeng Stem	-	1	5	10	15
Polyvinyl alcohol	10	10	10	10	10
Propylene glycol	10	10	10	10	10
Methyl Paraben	0.15	0.15	0.15	0.15	0.15
Propyl Paraben	0.15	0.15	0.15	0.15	0.15
Ethanol 96%	15	15	15	15	15
Water destilat	add to 100ml	add to 100ml	add to 100ml	add to 100ml	add to 100ml

Table 2: CSLI (Clinical and Laboratory Standards Institute) standards.

Antimicrobial Agent	Test Culture Zona Diameters in mm)		
Clindamycin	Resistant	Intermediate	Susceptible
	≤ 14 mm	15 – 20 mm	≥ 21 mm

Table 3: Inhibition zone measurement of sempeng stem ethanol extracts.

Sample	Concentration	Zone of Inhibition (mm)			Growth Inhibition Response
		I	II	III	
Clindamycin (+)	0,5%	38,6	40,7	37,4	Susceptible
	1%	41,1	41,1	43,4	Susceptible
	5%	44,45	44,45	49,5	Susceptible
	10%	46,2	46,2	46,2	Susceptible
	15%	48,4	48,4	48,4	Susceptible
Sempeng Stem Ethanol Extracts	0,5%	9,05	9,05	9,05	Resistant
	1%	14,5	14,5	14,5	Resistant
	5%	16,55	16,55	16,55	Intermediate
	10%	22,1	22,1	22,1	Susceptible
	15%	23,2	23,2	23,2	Susceptible

Table 4: The results of the positive control inhibition test.

Positive Kontrol	Zone of Inhibition (mm)			Mean + SD (mm)	Interpretation
	I	II	III		
Clindamycin Gel 1,2%	51,25	51,25	41,25	47,91 ± 5,77	Susceptible
Masker peel off commercial	54,15	63,25	63,25	60,21 ± 5,25	Susceptible

Note: SD = Standard Deviation

Table 5: The results of the peel off mask inhibition test.

Formulations of peel-off mask	Zone of Inhibition (mm)			Mean ± SD (mm)	Interpretation (CLSI, 2016)
	I	II	III		
FI	8,3	8,3	10,9	9,1±1,5	Resistant
F2	10,4	10,4	11,1	10,6±0,4	Resistant
F3	13,07	13,07	24,08	16,7±6,3	Intermediate
F4	19,03	19,03	26,01	21,3±4	Susceptible

Figure 1: Sempeng stem ethanol extract.

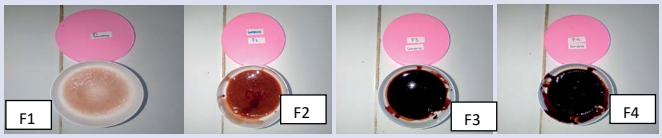

Figure 2: Formulation of the peel-off mask of Sempeng stem ethanol extract.

Note:

F1 = Formulasi masker peel off 1% ekstrak

F2 = Formulasi masker peel off 5% ekstrak

F3 = Formulasi masker peel off 10% ekstrak

F4 = Formulasi masker peel off 15% ekstrak

This research was started by selecting the simplicia of Sempeng stem to be used as the main ingredient in this research. Batang Sempeng is obtained from forests in Katingan Regency. Furthermore, the simplicia is then processed into powder simplicia so that it can be easily carried out for soxhletation extraction.[8] The time or duration of the extraction process determines the content of the compound that comes out of the material, as well as the ratio of the solvent, the amount of extractant involved in the transfer determines the level of the concentration difference which is very important in the diffusion process that affects the compound content.[9] The method used to obtain the stalk of Sempeng extract was the sokhletation method. This method is used because the soxhletation method can extract perfectly because it is done repeatedly, the amount of solvent used is small, and the soxhletation process is fast. The technique in this method uses a solvent that is always new, a simple observation to indicate it is the color of the solvent, where when the solvent is no longer colored (clear) it indicates the metabolite has been found. The solvent used in both extraction processes is 96% ethanol, the use of 96% ethanol because ethanol is universal which can bind all chemical components contained in the simplicia powder, both polar, semi-polar and non-polar.[10]

The average yield of the extract obtained from 1 kg of simplicia of Sempeng stems was 6.1653%. The test for the ethanol extract of Sempeng stems used various concentrations, namely 0.5%, 1%, 5%, 10% and 15%, these concentrations were used because in this test using the determination of MIC (Minimum Inhibitor Concentration) which is the lowest concentration that is still able to inhibit organism growth. The results showed the inhibition of the ethanol extract of Sempeng stem against the growth of *Propionibacterium acnes* bacteria after the incubation process at 37°C using an incubator for 24 hours. The inhibition ability of Sempeng stem ethanol extract against the tested bacteria was indicated by the formation of a clear zone in the disk area.

The results of the inhibition zone measurement were compared with the CLSI Standard (Clinical and Laboratory Standards Institute) category.

Testing of antibiotic potency determination by microbiology as a positive control with the same treatment as Sempeng stem extract using the antibiotic Clindamycin 150 mg with a concentration of 0.5%, 1%, 5%, 10% and 15%. The use of the antibiotic Clindamycin as a positive control is due to the Regulation of the Minister of Health of the Republic of Indonesia regarding General Guidelines for the Use of Antibiotics, Clindamycin inhibits most Gram-positive cocci and most anaerobic bacteria such as *Propionibacterium acnes* which are considered to trigger acne. Based on Antimicrobial Inhibition Based on Inhibition Zone Diameter, the results of the inhibition zone of the Clindamycin antibiotic at concentrations of 0.5%, 1%, 5%, 10% and 15% are interpreted as Susceptible which means they can inhibit pathogenic bacteria, so that they can be used as a guide for treatment right.

The measurement of the zone of inhibition is done by taking a horizontal or horizontal line in the clear area around the disc using a slide. When compared with a positive control that has the same treatment as the extract concentration, the inhibition zone of Sempeng stem extract at a concentration of 0.5% and 1% can be categorized as Resistant, which means that *Propionibacterium acnes* has sensitivity or sensitivity to Sempeng extract, so that the Sempeng extract at a concentration of 0.5 % and 1% are unable to inhibit the growth of *Propionibacterium acnes* bacteria, at a concentration of 5% it can be categorized as Intermediate, which means a state where there is a shift from a sensitive state to a state that is resistant but not completely resistant and is also effectively used at higher doses, or frequencies. more frequent doses, or only effective at certain specific places in the body where it can penetrate to provide a strong concentration, at a concentration of 10% and 15% can be categorized as Susceptible which means that it can inhibit pathogenic bacteria, so that it can be used as a guide for testing the right tan and at this concentration the antibacterial have a good inhibition zone ability, this shows that if the higher the extract concentration, the greater the inhibition zone.

The second stage of this research was to make a peel off mask from the two extracts and then test the inhibition of *Propionibacterium acnes* bacteria. Another ingredient used in this peel off mask formulation is polyvinyl alcohol (PVA) which has a role to provide a film effect that can be peeled off after drying because it has adhesive or adhesive properties.[11] Then Propylene glycol which acts as a humectant will maintain stability, reduce evaporation of water from the preparation and also play a role in maintaining skin moisture, then Methyl Parabens and Propyl Parabens are needed to prevent microbial contamination,[12] the last ingredient is Ethanol 96% which needed to dissolve Methyl Paraben and Propyl Paraben as well as for thick extract from the stems of Sempeng. It is made into an innovative peel off mask because it is easy and practical to use and can help attract blackheads that will cause acne. The result of this physical characteristic test is that there are formulations that meet the requirements and there are formulations that do not meet the requirements. Five peel off mask formulations can be seen in Figure 2.

Based on this first phase of research, the inhibition test was carried out on the peel off mask formulation against the *Propionibacteria acnes* bacteria. This is done because to see the inhibitory activity of the formulation against acne-causing bacteria. In this inhibition test, apart from being carried out on five peel-off mask formulations of the Sempeng stem ethanol extract, it was also carried out on a positive control, namely Clindamycin Gel 1.2% and industrial peel-off mask. The results of the inhibition zone measurement on positive control can be seen in table 4.

CONCLUSION

The research that has been carried out can be concluded as follows:

Peel off mask The ethanol extract of Sempeng stems is able to inhibit the growth of *Propionibacterium acnes* bacteria.

The peel off mask formulation of the Sempeng stem ethanol extract against *Propionibacterium acnes* bacteria can be concluded that the preparation with concentration of 1%, 5%, 10% and 15% forms a clear zone around the disc which means that this peel off mask formulation has activity to inhibits *Propionibacterium acnes* bacteria and is included in the category of susceptible interpretation according to the CLSI standard.

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AUTHOR CONTRIBUTIONS

Rezqi Handayani as conceived, designed the analysis, performed the analysis and wrote the manuscript.

Nurul Qamariah as analyzer, wrote the manuscript and collector of the data.

CONFLICTS OF INTERESTS

Declared none.

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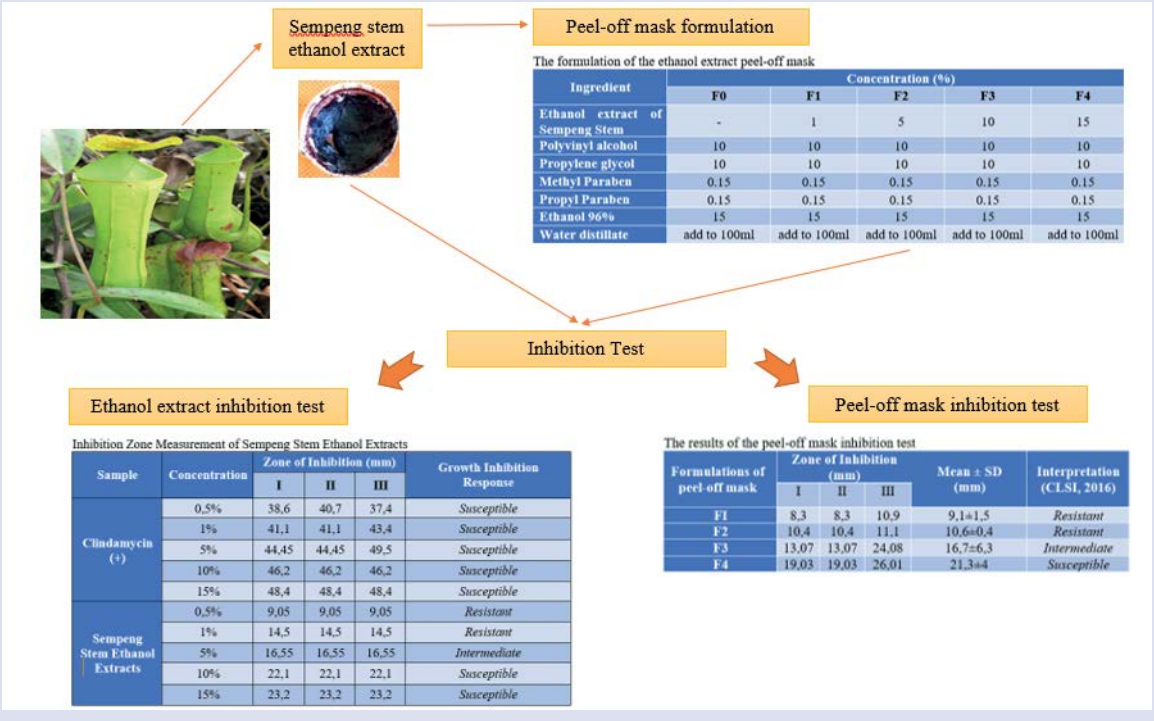
GRAPHICAL ABSTRACT



Ingredient	Concentration (%)				
	F0	F1	F2	F3	F4
Ethanol extract of Sempeng Stem	-	1	5	10	15
Polyvinyi alcohol	10	10	10	10	10
Propylene glycol	10	10	10	10	10
Methyl Paraben	0.15	0.15	0.15	0.15	0.15
Propyl Paraben	0.15	0.15	0.15	0.15	0.15
Ethanol 96%	15	15	15	15	15
Water distillate	add to 100ml	add to 100ml	add to 100ml	add to 100ml	add to 100ml

Sample	Concentration	Zone of Inhibition (mm)			Growth Inhibition Response
		I	II	III	
Clindamycin (+)	0,5%	38,6	40,7	37,4	*Susceptible*
	1%	41,1	41,1	43,4	*Susceptible*
	5%	44,45	44,45	49,5	*Susceptible*
	10%	46,2	46,2	46,2	*Susceptible*
	15%	48,4	48,4	48,4	*Susceptible*
Sempeng Stem Ethanol Extracts	0,5%	9,05	9,05	9,05	*Resistant*
	1%	14,5	14,5	14,5	*Resistant*
	5%	16,55	16,55	16,55	*Intermediate*
	10%	22,1	22,1	22,1	*Susceptible*
	15%	23,2	23,2	23,2	*Susceptible*

Formulations of peel-off mask	Zone of Inhibition (mm)			Mean ± SD (mm)	Interpretation (CLSI, 2016)
	I	II	III		
F1	8,3	8,3	10,9	9,1±1,5	*Resistant*
F2	10,4	10,4	11,1	10,6±0,4	*Resistant*
F3	13,07	13,07	24,08	16,7±6,3	*Intermediate*
F4	19,03	19,03	26,01	21,3±4	*Susceptible*

ABOUT AUTHORS

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