ANTI-ACNE POTENCY OF KEPEL (*STELECHOCARPUS BURAHOL*)'S LEAF AND FRUIT

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Abstract : Kepel leaves were collected from Karang Anyar, Nusa Kambangan, and Cilacap, Central Java, and also from Yogyakarta, Indonesia, while Kepel fruit were collected from Karang Anyar and Yogyakarta, Indonesia. Kepel leaves were extracted with methanol and fractionated with ethyl acetate and water, while the fruits were extracted with hexane and the residue was extracted with ethyl acetic and water. The anti-acne potency of leaves and fruits extract of Kepel was evaluated based on the anti-oxidant activity, *Propionibacterium acnes* lipase inhibitory activity and also antibacterial activity against *P. acnes*. The results showed that all extracts had mild anti-oxidant activity and no lipase inhibitory activity.

Keywords: *Stelechocarpus burahol,* leaves, fruits, anti-oxidant, antibacterial against *Propionibacterium acnes*, lipase inhibitor

INTRODUCTION

Acne is a common skin disease characterized by pimples on the face, chest, and back. It occurs when the pores of the skin become clogged with oil, dead skin cells, and bacteria. Acne is not a simple disease because it may sometimes lead to social phobia, lowered self-image, and depression (Koo and Smith, 1991).

There are at least four major factors that can contribute to the formation of cutaneous acne lesions: increased sebum production, follicular hypercornification, bacterial colonization, and an inflammation response (Nourin and Ballard, 2006). The prevalent bacterium implicated in the clinical course of acne is *Propionibacterium acnes*, a gram positive anaerobe that normally inhabits the skin and is implicated in the inflammatory phase of acne (Strauss et al, 2007). *P. acnes* plays a central role in current concept of acne pathogenesis (Zane, 2005). It appears to be the target of oral and topical antibiotic usage and its population reduction is a just parameter of therapeutic effectiveness of an antibiotic (Burkhart et al 1999). Besides the population of *P. acnes*, lipase activity is an important factor in the pathogenesis of acne. Free fatty acids formed as a result of the effect of *P. acnes* lipase activity on sebaceous triglycerides induce severe inflammation (Higaki, 2003).

Compounds targeting acne therefore should be able to inhibit *P. acnes* population and inhibit *P. acnes* lipase activity, as a result reduce pro-inflammatory lipids in sebum as well as reduce post-acne scar formation. The materials that have antioxidant activity may be useful for relieving hypertrophic scars and keloid formation on the skin (Furakawa et al, 1995). In other words, compounds or materials claiming good for acne control should possess anti-bacterial, anti-lipase, anti-inflammatory, and antioxidant activities.

Stelechorcapus burahol, one of fruit tree originally found in Indonesia, traditionally used as deodorant. In Indonesia, this tree famous with name Kepel or Burahol. To search other potency from this tree, we search the potency of Kepel leave and fruit as anti acne agent. The strategies that we used were using its potency as antimicrob against *Propionibacterium acnes, P. acnes* lipase inhibition and antioxidant activities.

MATERIALS AND METHODS

Collecting and identification of plants

Kepel (*Stelechocarpus burahol*) leaves and fruits used in this study were collected from Cilacap, Nusakambangan, Yogyakarta, and Karang Anyar in Java Island, Indonesia. The leaves collected from all collecting place, while the fruits only collected from Cilacap, Yogyakarta, and Karang Anyar. The identification and voucher specimens were deposited in the Bogor Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia.

Extraction and fractionation

All samples were dried and ground before being submitted to methanol for the leaves and to n-hexane for the fruits parts. The dried plant materials were extracted with solvents (ratio 1 g sample: 10 ml solvent) for 12 hours three times. The extracts were filtered using Whatman filter paper (no. 2) and concentrated in *vacuo* at 30°C using a rotary evaporator. The extract yields were then calculated. The leaves extract then fractionated with ethyl acetate and water. The fruits parts were extracted with hexane and the residue were extracted with ethyl acetic and water.

Bioassay methods

Antimicrobial analysis (Batubara et al 2009)

The test organism used in this study was *Propionibacterium acnes* ATCC 6919. The media consisted of GAM Broth Nissui 0.5%, glucose 1.0% (Wako Pure Chemical Industries, Ltd., Japan), yeast extract 0.3% (Difco Laboratories, France), Nutrient Broth 0.5% (Difco Laboratories, France), and 0.2% Tween-80 (MP Biomedical, Japan). A 95 μl sterilized medium, 100 μl sample (serial concentration, diluted in DMSO 20%) or control, and 5 μl inoculum were added to each well of a 96-well plate (Chomnawang et al., 2005). The inoculum was prepared at the concentration of 10^{-2} CFU/ml. *P. acnes* was incubated in the media for 72 hours under anaerobic conditions. Extract concentration at which there was no visually detectable bacterial growth was described as the MIC (Minimum Inhibitory Concentration).

Ten μl of each media with no visually detectable bacterial growth were inoculated in new 100 μl media. The concentration at which there was no bacterial growth after second inoculation was described as the MBC (Minimum Bactericidal Concentration). The negative control used was DMSO while the positive controls were chloramphenicol (Wako Pure Chemical Industries, Ltd., Japan), tetracycline (MP Biomedical, Japan), and isopropyl methyl phenol (IPMP) (TCI, Japan). Antibacterial assay was conducted at the minimum of three times at different times (each duplo test).

Lipase inhibitory analysis (Batubara et al 2009)

P. acnes was cultured in media (the same as the media in antibacterial assay). The cell suspension was centrifuged at 900 *g* for 10 min and the precipitate was diluted in PBS (phosphate buffer saline) at pH 6.98. The bacteria on this solution was destroyed by micro destruction (TOMY Micro Smash™ MS-100) at 4000 rpm for 30 seconds and centrifuged at 5000 *g* for 60 seconds. The filtrate was collected and placed in a dialysis tube for 6 days. The dialyzate was dried by a freeze drier and was used for successive experiments.

Lipase inhibitory activity assay was conducted using the dimercapto propanol tributyrate (BALB) method (Furukawa et al., 1982). The reagents were 390 μl of 5,5'-dithiobis(2-nitro benzoic acid) (DTNB) in Tris buffer (coloring agent), 10 μl of phenyl methyl sulphonyl fluoride (PMSF, esterase inhibitor), 25 μl of lipase, and 25 μl of sample or solvent (DMSO). All reagents (Dainippon Sumitomo Pharma Co, Ltd, Japan) were added to two tubes (1 and 2) and were both incubated at 30°C for 5 min. Fifty μl of BALB solution (substrate) was added to tube 1 and the solutions (in tubes 1 and 2) were mixed well and incubated at 30°C for 30 min. After 30 min, the reaction in the tubes was stopped by adding 500 μl of stopping reagent. After addition of stopping reagent, a 50 μl of BALB solution was added in tube 2 after which the solutions in both tubes were

mixed well and centrifuged to remove the insoluble materials. The absorbance of each tube was measured at 414 nm. Each experiment was performed in triplicate.
Lipase inhibition was calculated as:
% inhibition = [(A solvent – A sample)/A solvent] x 100%
where A solvent was the difference between absorbance of tube 1 and tube 2 in solvent (DMSO) and A sample was the difference between absorbance of tube 1 and tube 2 of sample. Chloramphenicol, isopropyl methyl phenol, and tetracycline were used as the positive controls.

Antioxidant analysis
The antioxidant assay used in this study adopted a free radical-scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Samples were diluted in ethanol to make a final concentration of 1.67, 3.33, 6.67, 10.00, 13.33, 16.67, 33.33, 66.67, 100.00, 133.33, and 166.67 μg/ml. A hundred μl of sample, 100μl of MES (2(N-morpholino(ethane sulfonic acid) buffer pH 7.4, and 100μl of DPPH (11.8 mg DPPH in 100 ml ethanol) were added to each well of a 96-well plate. After 30 minutes, the absorbance of the mixture was measured at 514 nm. The reaction in DPPH is shown in Fig 5. The positive control was (+)-catechin (Tokyo Chemical Industry, Japan) while ethanol was used as the blank. The inhibitory activity was calculated according to the following equation:
% inhibition = 1- [(A sample – A control) / (A blank- A control)] x 100%
where A sample was the absorbance of sample, A control was the absorbance of (+) catechin and A blank was the absorbance of ethanol. Each sample concentration and positive control was tested in triplicate.

RESULTS AND DISCCUSION

The number of sample collected from Central Java and Yogyakarta are vary based on the number of tree which we can found. From Central Java, we collect samples from Cilacap, Nusa Kambangan, and Karang Anyar. In Cilacap we found only about 2 trees, so we only can collect small amout of samples. In Nusa Kambangan, there are more number of Kepel tree, but we could not found the fruits. Karang Anyar is more suitable place to find Kepel either the leave and also the fruits. In Yogyakarta, we found the tree inside of Yogyakarta Palace. We can found the leave and also the fruits of Kepel there.The amount of samples which we collected is shown in Table 1.

Table 1. The amount of sample which collected from various place in Java Island

No	Part of plant	Collection place	Weight (g)	
			before dried	After dried
1	leaves	Cilacap, Central Java	4000	500
2	leaves	Nusa Kambangan, Central Java	3000	425
3	leaves	Karang Anyar, Central Java	7600	900
4	leaves	Yogyakarta	700	100
5	fruits	Cilacap, Central Java	500	40
6	fruits	Karang Anyar, Central Java	5000	350
7	fruits	Yogyakarta	1300	140

After collected the samples, we separated the fruit become peel, meat and seed part. Each part were dried before extraction. The dry weight of all the samples were shown in Table 1.

Phytochemical screening also performed for all dried samples. The results shown in Table 2. From phytochemical screening, we found that all samples have no alkaloid, triterpenoids, and saponin, but consist of tanin. Most of samples consist of flavonoid, but the fruits from Cilacap has no flavonoid. Different with the flavonoid content, most of the samples have no steroid content, only leaves from Nusa Kambangan consist of steroids. Samples which we collect are from

different type of enviromental condition. Kepel from Nusa Kambangan grow in more bases condition, due to a lot of lime in Nusa Kambangan.

All samples were extracted with some solvent. The fractionation step used to get all type of compound varying from polar to non polar compounds. The leaves were extracted first with methanol to get all type of compounds and continue with fractionation with ethyl acetic to get more non polar compounds and with water to get the polar compounds from Kepel leaves. The fruits were extracted with n-hexane first to get the non polar compound and or the volatile compound and then the residue extracted with ethyl acetic to get the semi polar compound and finnally the residue extracted with water to get the polar compounds. The yield of each extract were shown in Table 3. The anti-acne activities of all samples were examined by its property as antimicrobial (againts *P.acnes*), lipase inhibitory activities and antioxidant activities. Based on antimicrobial activities against *P. acnes* and lipase inhibitory activities, Kepel (leaves and fruits) have no activities. Kepel leaves and fruits extracts cannot inhibit *P. acnes* growth until concentration 4 mg/ml. Kepel leaves and fruits extracts also cannot inhibit *P. acnes* lipase activities from concentration 25 μg/ml until 500μg/ml.

Tabel 2. Phytochemical screening data of Samples

No	Samples name	Flavonoid	Alkaloid	Tanin	Saponin	Steroids	Triterpenoid
1	Leaves (Nusa Kambangan)	+	-	+	-	+	-
2	Leaves (Cilacap)	+	-	+	-	-	-
3	Leaves (Karang Anyar)	+	-	+	-	+	-
4	Leaves (Yogyakarta)	+	-	+	-	-	-
5	Fruits (Cilacap)	-	-	+	-	-	-
6	Fruits (Karang Anyar)	+	-	+	-	-	-
7	Fruits (Yogyakarta)	+	-	+	-	-	-

All samples were extracted with some solvent. The fractionation step used to get all type of compound varying from polar to non polar compounds. The leaves were extracted first with methanol to get all type of compounds and continue with fractionation with ethyl acetic to get more non polar compounds and with water to get the polar compounds from Kepel leaves. The fruits were extracted with n-hexane first to get the non polar compound and or the volatile compound and then the residue extracted with ethyl acetic to get the semi polar compound and finnally the residue extracted with water to get the polar compounds. The yield of each extract were shown in Table 3. The anti-acne activities of all samples were examined by its property as antimicrobial (againts *P.acnes*), lipase inhibitory activities and antioxidant activities. Based on antimicrobial activities against *P. acnes* and lipase inhibitory activities, Kepel (leaves and fruits) have no activities. Kepel leaves and fruits extracts cannot inhibit *P. acnes* growth until concentration 4 mg/ml. Kepel leaves and fruits extracts also cannot inhibit *P. acnes* lipase activities from concentration 25 μg/ml until 500μg/ml.

Part of plants	solvent	Yield of extracts (%) based on dry weight of samples			
		Nusa Kambangan	Cilacap	Karang Anyar	Yogyakarta
Leaves	Methanol	14.98	6.79	15.18	14.78
	EtOAc fr.	2.52	2.57	3.89	3.37
	Water fr.	6.69	6.21	3.68	3.43
Fruits	Hexane	-	-	6.16	6.08
	EtOAc	-	-	2.31	2.81
	Water	-	-	19.85	19.86

As antioxidant, Kepel extracts have mild activities. The extracts have activity but in higher concentration compare to vitamin C as positive control. The IC_{50} of samples based on DPPH scavenging activities like shown in Table 4. The most active extracts was Kepel leaves water fraction from Nusa Kambangan with IC_{50} about 14.94 µg/ml. The same fraction from other places shown different activities. It might because of steroid constituent on leaves from Nusa Kambangan. Fruits extracts have more activity as antioxidant especially the non polar extracts. More polar extracts gave higher IC_{50} value of antioxidant.

Table 4. Antioxidant activity data of all Kepel extracts, control positive Vitamin C (IC_{50}: 4.56 µg/ml)

Part of plants	solvent	Antioxidant activity (IC_{50} in µg/ml)			
		Nusa Kambangan	Cilacap	Karang Anyar	Yogyakarta
Leaves	Methanol	105.67	143.45	143.89	133.67
	EtOAc fr.	95.41	113.33	111.12	104.59
	Water fr.	14.94	50.67	51.45	52.11
Fruits	Hexane	-	-	17.85	17.99
	EtOAc	-	-	29.32	31.23
	Water	-	-	52.31	56.44

CONCLUSION

Extracts from leaves and fruits of Kepel had mild anti-oxidant activity, no antimicrobial activity against *P.acnes* and no lipase inhibitory activity. The most active leaves extracts as antioxidant is leaves from Nusa Kambangan while the most active fruits compounds is the nonpolar compounds.

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