PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY IN TRADISIONAL BALINESE BABI-GULING SPICES

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ABSTRACT

The traditional Balinese richly spiced swine-grill (Babi-guling) has been increasingly popular even among both the domestic and foreign tourists. The traditional grill is very unique in taste as it contains two main components, i.e. the pork and the unique Balinese spices, which probably could work antagonistically one against the other in affecting people health. The pork because of its rich content of saturated fatty acid is a risk of atherosclerosis, while the spices appear to be rich in antioxidatns. This is a preliminary atherosclerosis experimental study designed to screen qualitatively the phytochemical contents of the spices and to test their antioxidant activity and strength in different concentrations in vitro.

The methods used included Willstater test, NaOH 10% test, Meyer test, Leiberman-Burchard test, test for saponin, and test for phenol. The antioxidant activity was estimated by using DPPH (2,2-diphenyl-1-pricrylhydrazyl) test. The treatment applied to the spice before testing was raw and cooked by heating it inside the pig's body cavity. The concentrations of spices tested were 100, 1000 and 8000 ppm.

The results of our study revealed that the phytochemical content of the spices were flavonoids, terpenoids and phenolic compounds, but saponin and alkaloids were not detected. The antioxidant activity was ineffective in concentration of 100 ppm (percentage of reduction < 50%), effective in concentration of 1000 ppm (percentage of reduction 50-60%), and too thick in concentration of 8000 ppm (percentage of reduction >100%. We inferred that the traditional Balinese richly spiced swine-grill contains flavanoids, terpenoids and phenol which can act as antioxidants in vitro, and the most effective concentrations to decrease the free radicals were between 1000-8000 ppm.

Keywords: Traditional Balinese spices, flavonoids, terpenoids, phenol, antioxidant activity

INTRODUCTION

Balinese Babi-guling has long been known as a Balinese traditional food in Bali. The Balinese usually consume it when they have a big ceremony and after they dedicate it to God in the particular ceremony, therefore they usually eat it quiet rarely. However this particular

food is not just used during ceremony only, but has now been becoming the favorite food, not just for Balinese but even to the foreigner who come to Bali and eating pork.

Looking at the major components composing to this food, it is very unique since it made from pork meat which is known to have a lot of saturated fat (myristic and stearic) and cholesterol. And hence it is very risky in increasing the risk of getting cardiovascular disease (Katsuda *etal.*, 2000, Alpert, 2001). Secondly, it is also composed by plenty of spices. These spices are inserted inside the inner part of the pig's body cavity after they have taken out the entire visceral organ. Then they roast it wholly in the embers of fire. Therefore, it seems that the meat is marinated by those spices while it is cooked.

The spices used are the following: roots (turmeric, alpine galangal, shallots, garlic etc.), flowers (clove, *tabia bun* or *Piper retrofactum*), leafs (*salah ada* leaf and *salam* leaf or *Eugenia Polyantha*), fruits (bird chili, candle nut, tomatoes, tangerine etc). Some of these spices individually have been known to have highly antioxidant activity such as *quercetin*e in shallots, chili, lime, etc (US data base, 2003), *terpenes* in ginger, *alysin* in cashew nut. All of these are belong to antioxidant. This is a preliminary study for a doctoral research on the effect of Babi-guling spice in preventing atherosclerosis induced by the Babi-guling meat, reporting the phytochemical contents and the antioxidant activity of all those mix of spices measured in vitro.

METHODOLOGY

Materials

Material used in this study ultimately was the spices of Balinese babi-Guling and then followed by all chemicals required for antioxidant activity test and materials for screening of the phytochemical content qualitatively.

The spice comprise of three groups of spices or herbs:

1. *Bumbu Genep* (complete spices), include Languas Galanga, Ginger (*Zingiber Officinale*), Kaemferia galangal, Shallot (*Allium Cepa*), Garlic (*Allium Sativum*), Bird Chili (*Capsicum Frutescens*), Big Chili (*Capsicum Annum*), Candlenut (*Aleurites molucana*), Pepper (*Piper Nigru*m), Coriander (*Coriandrum Sativum*).
2. *Bumbu Wangen* (Fragrant spices), include nutmeg (Myristica sp.), *Tabya Bun* (*Piper retrofactum*), *Begarum*, *Cengluh*, Clove, and Cinnamon (cinammomum),

3. *Bumbu penyangluh* (Flavoring spices), include shrimp paste, lime, coconut oil, salt, *Salah ada* leaf and *Salam* leaf (*Eugenia Polyantha*)

The composition of the spice is provided at the table 1

Table 1
The composition of Balinese Babi Guling Spice

MATERIAL	**% weight**
Langua Galanga	24.8
Turmeric (*Alpina Galanga*)	5
Ginger (*Zingiber Officinale*)	3.3
Galingale (*Kaempferia Galanga*)	2.7
Shallot (*Allium Cepa*)	36
Garlic (*Allium Sativum*)	9.1
Bird chili (*Capsicum frutescens*)	9.4
Candle nut (*Aleurites Moluccana*)	3.8
Coriander (*Coriandrum Sativum*))	0.7
Black pepper (*Pepper nigrum*)	0.6
Nutmeg (*Myristica Sp*)	0.6
Tabia Bun	
Begarum	
Cengluh	
Clove	
Cinamon*(Cinammomum)*	
Shrimp paste	1.5
Salt	
Salah Ada leaf	2.6
Salam leaf (Eugenia polyantha)	
Lime juice	
Monosodium	
Coconut oil	

The chemical used were ethanol (ethyl alcohol 90%), reagent DPPH (diphenyl_prikryl hydrazyl), reagent Willstater (for flavonoid test), reagent Meyer (for alkaloid test), Lieberman-Burchard (for terpenoid test), $FeCl_3$ (reagent for testing phenolic compound), NaOH 10% (for confirmation of flavonoid test) and water for saponin compounds.

Equipment

The Equipments used in the study were knife, digital weighing machine, blender, pipette, some glasses stuff such as Erlenmeyer, plate etc., filter paper, new kassa filter, rotary evaporator, UV-vis spectrophotometer.

Working Procedure

Preparing the spice:

The *Bumbu-genep*, after being measured, was peeled arbitrarily, and it then chopped finely. The *Bumbu wangen* (Fragrant spice) were blended until it soft and tender then mixed up with the *Bumbu genep* before it then added with the *Bumbu penyangluh* (flavor) spice. The mixed spices then put into the body cavity of the pig because the entire visceral organ have been taken out. In this study, the spices prepared for the study were cooked (in the body cavity) and raw and the weight was 750 gram each. All the spice preparation was taken place at the babi guling seller in an open place.

Spice extraction

Each mixed spices (raw and cook) was soaked in ethanol 90%, for one hour. Then the spices were blended up, followed by squeezed and filtered by using a kassa filter. The crude extract was collected and put in an Erlenmeyer glass while the remnant was soaked again for the second time in ethanol 90% for a while before it was blend and squeezed again. This treatment was done 3 times until the liquid and the remnant color was getting pale. The liquid then stirred up and filtered again by a fine filter to get a very clear liquid. It then evaporated in a rotary evaporator to get a thick extract of spice. All laboratory work was carried out under room temperature.

Screening for phytochemistry

The phytochemistry screening was carried out by color tests include Willstater test, Meyer test, NaOH 10%, Leiberman-Burchard, $FeCL_3$, and water. 0,01 gram spice thick extract were diluted in ethanol and the volume was made into 10 ml, to have sample material in the concentration of 1000 ppm.

1. Willstater test.

   Some milliliter of diluted sample in ethanol was added with some drop of thick HCl and 2-3 pieces of Magnesium. The changing of color shows the flavonoid content in the solution.

2. NaOH 10% test

   Some milliliter sample was added with 2-4 drops of NaOH 10% solution. Reaction was said to have flavonoid content if there was a color change.

3. Meyer test

   Some cc diluted sample added with some drop of Meyer reagent. If precipitation appears then the sample was saying to have alkaloid.

4. Leiberman-Burchard test

   Some diluted sample added with some drop of Leibermann-Burchard reagent. Test was said to have terpenoid content if there was a color change

5. Test for Saponin content

   2-3 ml of diluted sample added with water and then shaked well. Test is being said as positive to have saponin content when foam is appeared and stable for at least 30 minutes.

6. Test for phenolic content

   2-3 ml of diluted sample dropped with $FeCl_3$ solution. Sample was being said as positive to have phenolic content if the color turned to brownish or brown purplish.

Antioxidant (Radical Scavenging) activity test invitro, using DPPH (2,2-diphenyl-1-pricrylhydrazyl) technique.

Technique to test the scavenging activity of spices was a modification of DPPH test used for algae test that was reported elsewhere (Zahra etal., 2007)

0,08 gram of thick sample was diluted in ethanol 90% and the volume was made into 10 ml. It means that the concentration of the solution was 8000 ppm. Another 0,01 gram of sample also diluted in ethanol 90% to get a 1000 ppm extract solution, then 1 ml of the later diluted sample was added again with ethanol and the volume was made into 10 ml, so then the concentration become 100 ppm.

0,004 gram of DPPH crystal was diluted in methanol and the volume was made into 100 ml in a beaker glass. Therefore the concentration of DPPH was 0,004% (b/v).

Antioxidant activity test was carried out in two steps as follows: firstly DPPH absorbance level was measured, and then secondly it was followed by checking the absorbance level of the sample. The absorbance spectra for DPPH were measured within wave length (λ) of 400-700 nm, while ethanol was use as blank solution. Absorbance levels under the wave length of 797 nm, 517 nm, and 537 nm was then recorded.

The method for measuring the absorbance level of the sample is as follows: 1 mL. of sample solution were dripped in to a cuvette and then added with 2 mL. of 0,004% DPPH solution. This mix then stirred evenly while the time has started to be counted. Within minute 5 and 60 of the reaction, the absorbance level was recorded under the wave length of 497 nm, 517 nm, and 537 nm.

Data analysis

Screening phytochemistry

The screening results can be analyze qualitatively base on the color alteration, the occurrence of sediment or the foam formation of the sample when it was dripped with reagent used in the study.

Counting the scavenging capacity against free radical.

The anti free radical capacity is calculated by the percent reduction of the absorbance level of the sample under the maximum wave-length of 517nm. The formula used to count is the following

$$1 - \frac{\text{Calculated absorbance level on the sample}}{\text{Calculated absorbance level on DPPH}} \text{ X } 100\%$$

And the calculated absorbance level of the sample and the DPPH under the maximum wave length 517 nm can be calculated by using a formula as the following

$$A\,517 - \frac{A\,497 + A\,537}{2}$$

The anti free radical capacity then measured within the concentration of 8000, 1000 and 100 ppm. Each sample from any concentration was measured three times and the mean from the three measurement's result were calculated and recorded.

RESULTS AND DISCUSSION

Screening phytochemistry

The phytochemistry analysis was carried out to find out the active phytochemical content in the Balinese B*abi-Guling* spices either it was raw or cooked inside the body's cavity. The testing was done based on the color changes which then analyze qualitatively.

Table 2.

Screening Phytochemistry of The Raw Material

No	***Reagent***	***Observation results***	***Interpretation***
		Color alteration after adding reagent	
1	Willstater	From brown become orange (++)	(+) **flavonoid** content
2	NaOH 10%	From brown become orange (++)	(+) **flavonoid** content
3	Meyer	No sediment appears within 5 minutes	(-) **alkaloid** content
4	Leiberman-Burchard	From brown become purplish red (++)	(+) **terpenoid** content
5	+ water and shaked	No foam appear within 5 minutes	(-) **Saponin** saponin
6	+ $FeCl_3$	From light brown become purplish brown	(+) **phenol** content

Table 2 and 3 show the phytochemical content of the babi guling spices. Hence one can say that the spices content of flavonoid, terpenoid and phenolic compound, while alkaloid and saponin were absent. And there is qualitatively no difference between the raw and the cooked material, but it seems the cooked material shows higher concentration in comparison with the raw one.

Table 3.
Screening Phytochemistry of The Cooked Material

No	*Reagent*	*Observation results*	*Interpretation*
		Color alteration after adding reagent	
1	Willstater	From brown become orange (+++)	(+) **flavonoid** content
2	NaOH 10%	From brown become orange (+++)	(+) **flavonoid** content
3	Meyer	No sediment appears within 5 minutes	(-) **alkaloid** content
4	Leiberman-Burchard	From brown become purplish red (++)	(+) **terpenoid** content
5	+ water and shaked	No foam appear within 5 minutes	(-) **Saponin** saponin
6	+ $FeCl_3$	From light brown become purplish brown	(+) **phenol** content

There are actually over then 5000 flavonoids naturally accuring in plant have been characterised (Wikipedia, 2009), but according Geissmann, it can be groupped into thirteen groups ie Chalcons, Dihydrocychalcons, Aurone, Flavanone, Flavone, Flavonol, Flavanonol, Leukoanthocyanin, Anthocyanin-Anthocyanidin, Catechin, Isoflavon and Isoflavanon (Geissman, 1962), which then Beecher, classified it into six subclasses only ie Flavanol, Flavanone, Flavones, Isoflavones, Flavonols, Anthocyanindines (Beecher,2003). All of these will provides specific color differences when it is given a test of Willstater or even NaOH 10%. The color changes such as red, magenta, violet, yellowish red, purplish blue, yellowish orange etc. (Geissman, 1962, Padmawinata and Sudiro, 1988).

In this study the color appearance was not specific to one of the flavonoid's color available. It appeared with an orange color, of which it can be combinations from yellow, red and or brown. This is possible because Babi-guling spice is a mixture of various materials and the consequence is that it includes several flavonoid merging into one or domination of one kind of flavonoid plus the others, such as quercetine and kaempferol (flavonoles subclass) from shallots, red bird chili and turmeric and may be the other i.e luteolin

(flavones) which is available in lime juice or eryodictole (flavonones) from turmeric etc. (Narayana etal., 2001, US Data Base, 2003). Therefore, active ingredients need to be evaluated to know what is the most active component compiling the compound.

Plenty of flavonoids give beneficial effect on body's health, since mostly of flavonoids work as antioxidant in human body (Wikipedia, 2009). Should this mix of spices content of flavonoids, though we don't know what composing it, one can say that this mix can be used as antioxidant too. And therefore, it might accidentally by the ancient Balinese before, had been used to fight against the harmfull effect of Babi-guling.

Beside that, this spices also comprise of terpenoid which may be it mostly derived from ginger, cinnamon and cloves (Fuhrman etal., 2002, Linda Lazarides, 2008, Wikipaedia, 2008) or may be someting else. Terpenoid can be sorted agregated into several groups as follows monoterpenoid, seskuiterpenoid, diterpenoid, triterpenoid, isopren and carotenoid (Wikipedia,2008). This kind of terpenoids is still too general, since it can be derived from isoprenoid, vitamin K, vitamin E or even sterol (Leray, 2011).

This mix spices also comprise of phenol. As per definition, phenol means an extremely poisonous compound, C6H5OH, which is caustic and disinfectant; used as a pharmaceutic preservative and in dilution as an antimicrobial and topical anesthetic and antipruritic. Poisoning, due to ingestion or transdermal absorption, causes symptoms including colic, local irritation, corrosion, seizures, cardiac arrhythmias, shock, and respiratory arrest (medical dictionary, 2011). However, in the other way, the naturally plant phenol can comprise of Ferulic, caffeic, chlorogenic, and ellagic acids, which can inhibit the mutagenicity and cytotoxicity of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene (B[α]P 7,8-diol-9,10-epoxide-2), the only known ultimate carcinogenic metabolite of benzo[α]pyrene derived from Salmonella typhimurium (Wood etal., 1982). And in addition, some phenol, like butylated phenolic compound can act as natural antioxidant (Pourmorad etal., 2006)

Active ingredients of all substances above was not yet identified specifically and quantitatively though it works as antioxidant against free radical. However, the anti free radical effect was calculated by DPPH method.

Anti oxidant activity

The anti oxidant activity of the spice was calculated by its damping effect on DPPH (2,2-diphenyl-1-pricrylhydrazyl). Then result of the calculation is drawn in table 4.

Should the substrate or material is able to decrease the absorbance of DPPH by 50 % or above, it determines as an effective antioxidant (Antalovic etal., 2002). However, when it is beyond 100%, it shall be diluted before retesting of its damping capacity (Djatmiko etal., 1998).

Table 4

Calculated Anti Oxidant Activity of Spice Invitro in several Concentration

CONCENTRATION	RAW MATERIALS		COOKED MATERIALS	
	5 minutes (%)	60 minutes (%)	5 minutes (%)	60 minutes (%)
8000 ppm	109,01	116,18	131,09	251,93
1000 ppm	50,50	66,34	62,04	55,63
100 ppm	27,66	34,13	32,38	43,13

(%) : percent reduction

This study shows that the damping capacity of the sample in concentration of 1000 ppm is in between 50-70%. The raw material shows improving capacity following to the time while the cooked one shows the opposite. There is not any explanation of this situation as it is only appeared in the concentration of 1000 ppm only and even it still very effective to work as anti free radical. In concentration of 8000 ppm, the percent reduction shows exceeding from 100% even over then 200%, on the cooked materials after 60 minute. It means that 8000 ppm was too thick and need to be diluted or it might becoming prooxidant invivo. The percent reduction in 100 ppm looks ineffective to work as anti oxidant since the percent reduction was below 50%. Therefore, the concentration of spice mixture between 1000 ppm – 8000 ppm sounds very effective to work as antioxidant.

The conclusion can be drawn as the phytochemistry of babi-guling spice comprise of flavonoids, terpenoid and phenolic compound. And the best concentration to give an anti oxidant activity is between 1000 – 8000 ppm. Since this mix comprise of beneficial and valuable compound from flavonoids and phenol as antioxidant, it can be also give harmfull effect from phenol content, when it is taken too much. And additionally, it is also eaten widely by human being. Therefore, suggestion shall be made to find out the active ingredients of the spice mixture specifically and quantitatively, and how far the antioxidant of the mixture is effective in vivo.

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