

Karakteristik Bubuk Instan Cemcem (*spondiaz pinnata* Lf kurz) *Characteristics of Cemcem (spondiaz pinnata l.f. kurz) Instant Powder*

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ABSTRACT

One of tropical plant used as a traditional beverage in Bali is Kecemcem or Cemcem (*Spondias pinnata* (Lf) Kurz) or in Indonesian called Kedondong Hutan. Empirically, the beverage is mentioned for treating coughs, increasing appetite, and body refreshment. Besides used as a beverage, Cemcem leafs are also used as a flavor enhancer of fish products. The study was carried out to determine the characteristics of Cemcem instant powder produced by encapsulation using maltodextrin and dried by the thin layer drying method. Cemcem instant powder was then analyzed its content of vitamin C, total phenols, tannins, antioxidant capacity, water content, ash content, and solubility. The flavor compounds in Cemcem instant powder obtained in Bukit Jimbaran area are identified by GC-MS devices. The result showed that cemcem instant powder which was produced by 24 hours of maceration time has the best characteristics and potential to be developed as a natural antioxidant products. It has the highest of total phenol, tannin, vitamin C, and antioxidant capacity. The content of vitamin C, total phenol, and tannin were 135.06 mg/100g, 38.95 mg GAE/g, and 11.01% respectively. The antioxidant activity of cemcem instant powder which analyzed by DPPH method was about 43.80%. There are 17 compounds detected by GC-MS, these compounds belonged alkenesalcoholsphenols compounds and esters of fatty acids. The whole compounds were the building blocks of flavor cemcem leaves.

Keywords : *Characteristic of cemcem instant powder, antioxidant capacity, and flavor compounds of cemcem leaves*

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INTRODUCTION

Indonesia is a tropical country with many kinds of herbs and spices. Herbs are used in many domains, including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics. Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, antiinflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential (Cai et al., 2004; Djeridane et al., 2006). Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Wojdylo et al. 2007).

Group of compounds that are considered to have certain physiological functions in the functional food ingredients namely: (1) dietary fiber, (2) oligosaccharides, (3) sugar alcohols (polyols), (4) multiple unsaturated fatty acids, (5) certain peptides and proteins, (6) and isoprenoid glycosides, (7) polyphenols and flavonoids, (8) choline and lecithin, (9) lactic acid bacteria, (10) phytosterol, (11) vitamins, and (12) pigments (chlorophyll, anthocyanin) and certain minerals (Muchtadi, 2004).

One of the plants that are often used as a traditional beverage in Bali is Kecemcem or Cemcem (*Spondias pinnata* (Lf) Kurz) or in Indonesian called Kedondong Hutan. Empirically, this drink is mentioned in treat coughs, increase

appetite and gives the effect of refreshing the body. Besides used as a beverage, Cemcem leaves are also used as a flavor enhancer in fish products. Phytochemical compounds contained in the genus *Spondias* have been widely studied. Crude extract of the bark is reported to have antibacterial activity and is able to treat dysentery. Methanol and ethyl acetate extracts of *Spondias* rod has a hepatoprotective activity (Rao and Raju, 2010). Furthermore, Das et al. (2011) stated that methanol and chloroform extracts of the stems consist of phytochemical compounds such as glycosides, alkaloids, carbohydrates, saponins, steroids, and resin. Research conducted by Keawsa-ard and Liawruangrath (2009) and Hazra et al. (2008) stated that the fruit pulp of Cemcem has potential as antimicrobial and potentially bark as a natural antioxidant. Research conducted by Gupta et al. (2010) showed that resin extract of *Spondias* able to inhibit the growth of gram-positive (+) bacteria. Research on the content of bioactive compounds from the ethanol extract of Cemcem leaf has been done by Ariantari dan Yowani (2012). They stated that the extract contains steroids, flavonoids and triterpenoids and extract at concentrations of 10 to 100 mg / mL as an antituberculosis ability. Ariati (2012) stated that the extract of Cemcem leaf has an antibacterial ability to *Erwinia chrysanthemi*, causes soft rot in the aloe vera plant.

Cemcem leaves have become widespread and readily available in traditional markets in Bali. Cemcem beverages processed with a very simple

way is to use the water extract of fresh leaves with a ratio of 1 : 10 to 1 : 20 , then added katuk leaf extract or Suji leaf extract as a green dye, salt and sugar , then filtered, put into a plastic bottle and stored in the refrigerator. This traditional drink has unstable in a flavor, color and aroma due to the raw materials, production processes, and formulations used inaccurate every production time. In addition, drinks are processed in this way has a shelf life of 24 to 48 hours . If the drink is left at room temperature, after 3-4 hours it changes color, there is a sediment in the bottom of the bottle, bubbly, and its flavor begins to change to be more acidic. The process development of presenting a Cemcem leaf extract as commercial instant beverage has never been done. That is why in-depth research is needed on the characteristics of bioactive compounds and antioxidant capacity of instant Cemcem powdered obtained from time varying maceration.

The aims of this study are to identify and define the characteristics of Cemcem instant powdered which was produced by the encapsulation using maltodextrin and dried by the thin layer drying method. The results from this research are expected to provide information on the potential of Cemcem leaf extract to be developed as natural antioxidant which can be mass produced and consumed.

METHODS

Materials and Chemicals

Materials used in this research were Cemcem leaves were collected from Bukit Jimbaran Regency, methanol, hexane,

etil acetate, aquadestilata, maltodextrin (MD), tween 80, whatmann filter paper, kits of total phenol, vitamin C, total tannin, pH, and antioxidant capacity (DPPH radical scavenging).

Equipments

The equipments used in this study are spectrophotometer, analytical balance, separation, flask, rotary vacuum evaporator, incubator, oven drier, vacuum filter, magnetic stirrer, GC-MS, and glass ware.

Preparation of Samples

The dried Cemcem leaf powder was extracted using water with a ratio of 1: 10 and maserated until 6, 12, 18, 24, and 36 hours. Extraction in this research was done using maceration at 28oC in an incubator and protected from the sun. After that, it was filtered with a whatmann filter paper. Filtrate was added 1% Tween 80 and encapsulated by MD (20%), and dried using thin layer drying method (500C). The dried extract of Cemcem called Cemcem instant powder. Cemcem instant powder was analyzed levels of vitamin C, total phenols, tannins, antioxidant capacity (DPPH radical scavenging), water content, ash content and solubility. All determinations were performed in triplicate (n = 3).

Data analysis was tested by the effectiveness index method to get Cemcem instant with the best characteristics. Sample is also identified by GC-MS instrument to find their active compounds of the Cemcem extract.

Total phenolic content

Total phenolics content was determined by the Folin–Ciocalteu method, which was adapted from Swain and Hillis (1959) and modified by Thaipong et al. (2006). The 150 mL of extract, 2400 mL of pure water, and 150 mL of 0.25 N Folin–Ciocalteu reagent were combined in a plastic vial and then mixed well using a Vortex. The mixture was allowed to react for 3 min then 300 mL of 1N Na₂CO₃ solution was added and mixed well. The solution was incubated at room temperature (23 °C) in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass) using a gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Antioxidant Capacity (Free radical scavenging activity)

The antioxidant capacity assay was carried out according to Okawa et al. (2001). The DPPH method was used to determine for radical scavenging activity of Cemcem leaf extracts. Various concentrations of samples (0.05 ml) were added to 1.95 ml of methanolic DPPH (0.1 mM) and mixed thoroughly. The mixture was left to stand for 45 min in the dark at room temperature and the absorbance was measured at 517 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging effect was expressed as scavenging activity ($1 - A_c/A_d$), where A_c is the absorbance of solution when the extract is added at a particular concentration, and A_d is the absorbance of

the DPPH solution. The results were reported in percent (%).

Vitamin C or ascorbic acid (mg/100 g)

The vitamin C assay was carried out according to Sudarmadji et al., 1984. Ascorbic acid or vitamin C content was measured using spectrophotometer method described previously by Apriyantono et al. (1989). Irreducible Ascorbic acid changed entirely into ascorbic acid by dichlorofenol dehidro indofenol. In a sour ambience, the whole dehidro ascorbic acid reacts with dinitrofenilhidrazin, the reaction form colored complex that can be measured by spectrophotometer at 530 nm wavelength.

Tannin (%)

Tannin levels were analyzed using Follin - Dennis reagent and measured using a spectrophotometer. Samples in the form of dry extract, powder or crude drugs of plant and weighed as much as 1 g dissolved in 100 ml of distilled water. Samples were boiled for 10 minutes and then cooled and filtered with a filter paper. The filtrate screening results incorporated into the measuring flask and add 250 ml of distilled water until the calibration mark. The filtrate was diluted 5 ml taken, put in a pint flask of 100 ml, then diluted again with distilled water until the calibration mark. Solution of 1 ml of dilution taken, put in a pint flask 10 ml, was added 0.5 ml Follin - Denis reagent and 1 ml of saturated sodium carbonate, and distilled water was added to the calibration mark, shaken for 3 min and left to stand for 40 minutes. The mixture absorbance was measured at a wavelength of 725 nm. Levels of tannins (%) is calculated as tannic acid.

Water content (AOAC, 1995)

5 grams of sample (a) is introduced into a cup that has been weighed and dried in an oven drier at $103^{\circ} \pm 2^{\circ} \text{C}$ for 6 hours. Cup containing the sample was then transferred to a desiccator, cooled and weighed (b). Repeat drying up the difference between the two weighing results do not exceed 0.005 grams. Water content was calculated by using the formula:

$$(\% \text{ bb}) = a - b \times 100 \%$$

Ash content (AOAC, 1995)

2-3 g of sample (w1) was put into a porcelain dish and evaporated in a water bath until dry. After that, the sample was put into an electric furnace at a maximum temperature of 550°C until ashing is complete. Chill in a desiccator and weigh up to fixed weights (w2).

$$\text{Ash content} = \frac{w1 - w2}{w1} \times 100 \%$$

Solubility (SNI 01-2891-1992)

A total of 5 grams of material (w) put into 100 ml of water and stir until dissolved. The solution is then filtered with a filter paper of known weight (w2). Filter paper containing the residue dried in an oven drier at a temperature of 105°C for 2 hours and then weighed (w1). The part that does not dissolve in water are:

$$W1 - W2 / W \times 100\%$$

solubility (%) = 100 - (%) part that doesn't dissolve in water

Identification of the flavor compound by GS-MS

This study aims to determine the flavor compounds in the Cemcem leaf extract. The research was conducted through the insulating phase fractionation followed by GC-MS analysis phase. Cemcem leaf

extract prepared by mixing 5 g of powder and put into a glass beaker, then 96% ethanol was added to a volume of 200 ml. This mixture was put in an incubator with a temperature of 28°C and left to 24 hours. After that, the mixture was filtered with Whatmann paper and evaporated with a vacuum rotary evaporator at a temperature of 45°C until all of the solvent evaporates. Solvent-free extract was mixed with 100 ml ethanol and put into a flask, add 100 ml separating hexane and shaken for 10 minutes until homogeneous. Subsequently the mixture is allowed to stand for two hours resulting in the partition between hexane and ethanol fractions. Hexane and ethanol fractions were separated, and the fraction of ethanol re-inserted into the flask split and partitioned again with 100 ml of ethyl acetate, and separated in the same manner with the fractions of hexane above. Fraction of hexane, ethyl acetate, and ethanol was then evaporated with a rotary vacuum evaporator to obtain extracts with a volume of about 10 ml. Already vaporized, the fraction is then analyzed by GC-MS devices.

GC-MS analysis carried out by the method of Erdem and Olmez (2004). The carrier gas used was helium with a flow rate of 30 ml per minute. GC temperature is set as follows. Injector temperature 300°C , oven initial temperature was 100°C , the rate of temperature rise $10^{\circ}\text{C}/\text{menit}$, and the final temperature of the oven to 300°C . Identification of compounds is done with using software Wiley 229, NIST 12 and NIST 62 Library.

RESULT AND DISCUSSION

The results of Cemcem instant powder characteristics are presented in Table 1. Cemcem powdered instant results are presented in Figure 1. After drying with thin layer drying method, extracts of Cemcem have brownish color. This is caused by the breakdown of chlorophyll due to the drying process.

Characteristics of Cemcem Instant Powder

The compounds were measured on Cemcem products are total phenols, tannin levels, vitamin C, antioxidant capacity, water content, ash content and solubility. There were presented in Table 1. The content of total phenols, tannins, vitamin C and antioxidant capacity has increased to the maceration time of 24 hours, then decreased at 30 hours maceration time. The content of total phenols, tannins, and vitamin C was highest at 24 hours of maceration. According Sudjadi (1986), separation of the active compounds from plants using maceration method is generally done for a few hours up to a maximum of three days (72 hours). Prolonged maceration time is resulting in oxidation or hydrolysis reactions on the active substances, so that the content of active substances decreases.

In the opinion of Padayatty *et al.* (2002) Vitamin C is a vitamin that is soluble in water and is very often found in plants as L-ascorbic acid and natural sources of vitamin C are fruits and vegetables. These vitamins are very labile to temperature and oxygen so that the presence of heat exposure and the

conditions in the open air very quickly broken.

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables (Hodzic *et al.*, 2009). The compounds usually have antioxidant activity are phenolic compounds that have a hydroxy group substituted in the ortho position and the group-OH and-OR (*et al*2001). According to Cowan (1999) the most effective solvent to extract polyphenolic compounds are ethanol and methanol. While the water is an effective solvent to extract polyphenolic compounds such as tannins and anthocyanins class. Paceration damage polyphenolic compounds. Okawa .), . rolonged mtime is resulting in to the

Identification of Flavor Compounds

Identification of the flavor compounds in Cemcem instant powder obtained in Bukit Jimbaran area are presented in Table 2. There are 17 compounds were detected using GC-MS instrument. 10 compounds detected hexane fraction, detected fractions of ethanol (Figure 2,3, and 4). In general, the compounds were detected are volatile and have less than 1000 of molecular weight. These compounds belonged alkenes, alcohols, phenols compounds and esters of fatty acids. The whole compounds were the building blocks of flavor leaves cemcem leaves.

Antioxidant Capacity of Cemcem Instant Powder

The radical-scavenging power could

Table 1. The Characteristics of Cemcem Instant Powder

characteristics	Time of Maceration (h)				
	6	12	18	24	30
Total phenol (mg GAE/100g)	32.95±0.12	36.23±0.09	37.07±0.11	38.95±0.09	38.92±0.09
Tannin (%)	10.01±0.03	10.63±0.06	10.86±0.03	11.01±0.04	10.72±0.04
Ascorbic Acid (mg/100g)	130.06±1.05	130.82±1.08	132.28±1.05	135.06±1.06	117.31±1.05
Antioxidant Capacity (%)	40.32±0.06	40.72±0.04	41.51±0.04	43.80±0.05	40.63±0.06
Water content (%)	2.87±0.02	2.85±0.03	2.87±0.03	2.83±0.03	2.88±0.02
Ash content (%)	0.42±0.02	0.45±0.02	0.42±0.02	0.42±0.02	0.44±0.02
Solubility (%)	97.11±1.05	97.04±1.12	97.22±1.04	97.32±0.84	97.35±0.92
effectiveness index	0.33	0.41	0.96	1.48	1.00



Figure 1. Cemcem Instant Powder

Table 2. Flavor Compounds in Cemcem Instant Powder

No	Hexane fraction	Etyl Acetate fraction	Ethanol fraction
1	Antisal (C ₇ H ₈)	2,5-Dimethyl 2- hexane(C ₈ H ₁₆)	Boric acid ethyl ester (C ₆ H ₁₅ BO ₃)
2	Beta-citral (C ₁₀ H ₁₆ O)	Acetic Acid Isobuthyl Ester (C ₆ H ₁₂ O ₂)	
3	Pentena (C ₆ H ₁₂)	Ethyl 2-butenoate (C ₆ H ₁₀ O ₂)	
4	1,3-Benzodioxole (C ₁₀ H ₁₀ O ₂)	Myristyl chloride (C ₁₄ H ₂₉ Cl)	
5	1,3,4-Eugenol (C ₁₀ H ₁₂ O ₂)	Palmitic acid methyl ester (C ₁₇ H ₃₄ O ₂)	
6	2-Propenyl benzene (C ₁₁ H ₁₄ O ₂)	Methyl-9-octadecenoate (C ₁₉ H ₃₆ O ₂)	
7	Methyl octadecanoate (C ₁₉ H ₃₈ O ₂)		
8	11,14-Eicosadienoate (C ₂₁ H ₃₈ O ₂)		
9	9-Octadecenoate (C ₁₉ H ₃₆ O ₂)		
10	Methyl docosanoate (C ₂₃ H ₄₆ O ₂)		

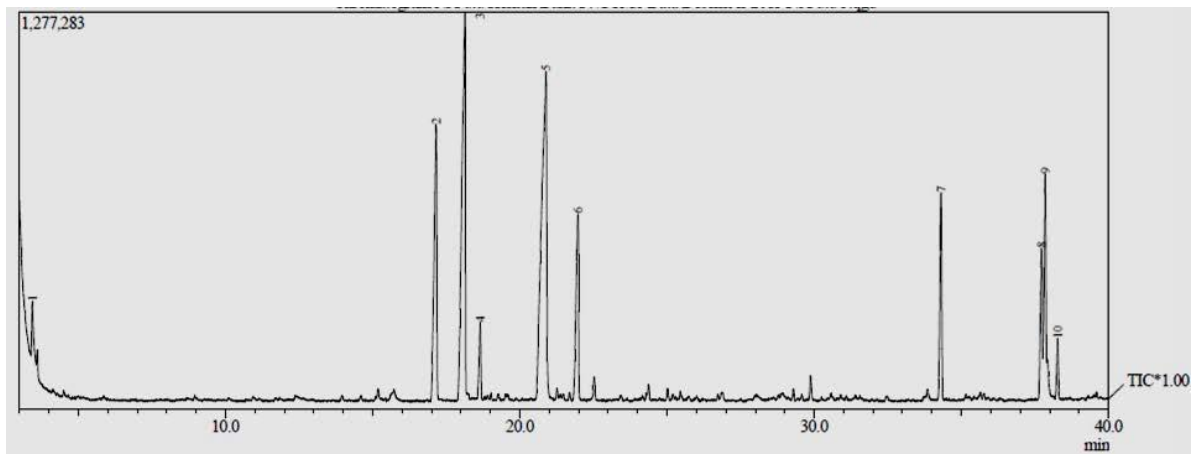


Figure 2. Chromatogram of Hexane Fraction

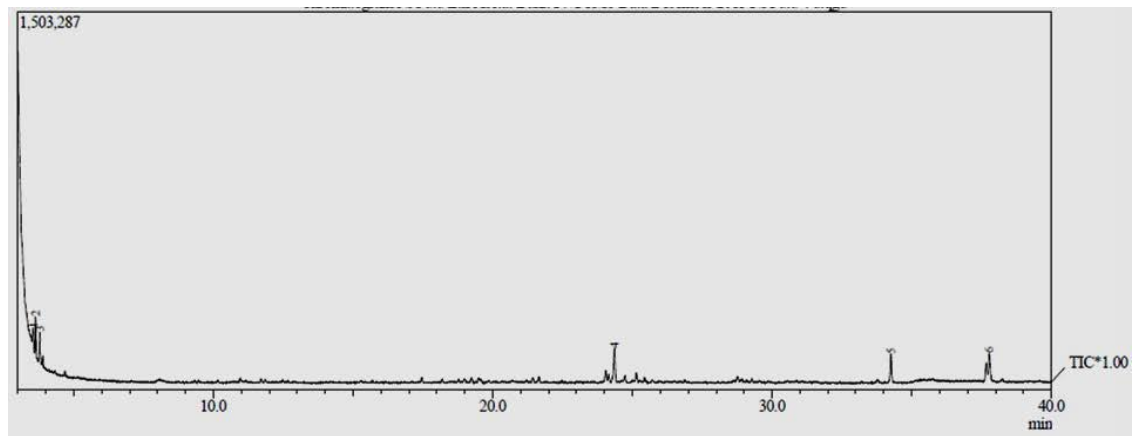


Figure 3. Chromatogram of Ethyl Acetate Fraction

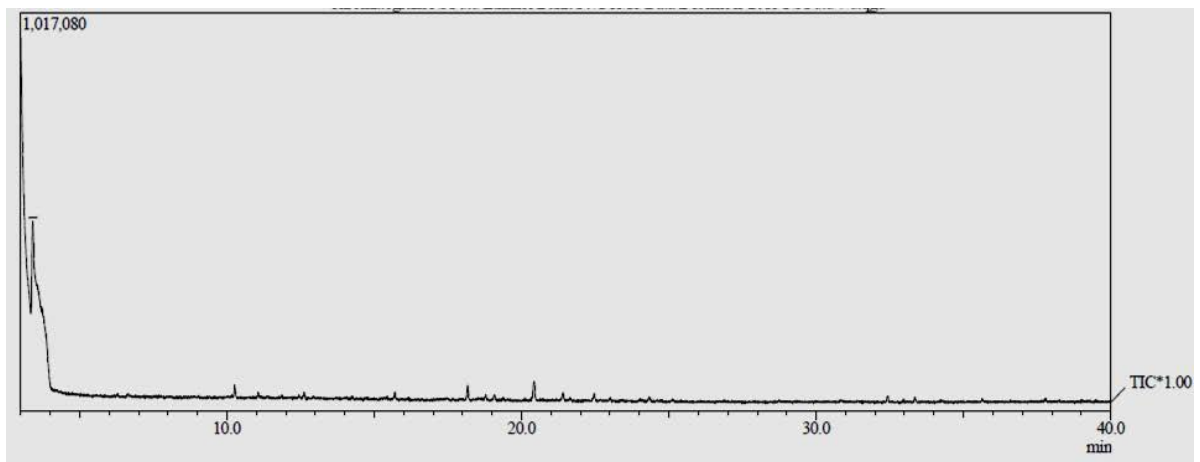


Figure 4. Chromatogram of Ethanol Fraction

be easily determined by DPPH. Highly antioxidative substances will make the conversion from the purple chromogen radical (DPPH●) to the pale yellow hydrazine (Singh and Rajini, 2004). The ability to scavenge free radicals from cemcem instant is caused by the content of active compounds such as vitamin C, tannins, and total phenols. Prakash (2001) and Kumalaningsih (2006) states that a natural antioxidant compounds in general form of vitamin C, vitamin E, carotenoids, phenolic compounds, and can be a polyphenolic flavonoid, cinnamic acid derivatives, cuomarin, tocopherol, and polyfunctional organic acids. Class of flavonoids that have antioxidant activity include flavones, flavonols, isoflavones, catechins, and calkon. While cinnamic acid derivatives include cafeat acid, ferulic acid, chlorogenic acid, and others. Cemcem antioxidant capacity are presented in Table 1. At the time of maceration 6 to 24 hours there was an increase in antioxidant capacity, while it was at the time 30 hours maceration decreased antioxidant capacity. Antioxidant capacity was highest at 24 hours maceration time. This is consistent with the results of measurements of total phenols, tannins, and vitamin C on cemcem instant, that up to 24 hours of maceration time an increase in the content of total phenols, tannins and vitamin C. The content of these compounds began to decline at the time of maceration 30 hours. The decline largely attributable to a decrease in the antioxidant capacity of the total content of phenols, tannins, and vitamin C. Some authors (Cai et al., 2004; Djeridane et al., 2006; Katalinic et al.,

2006; Katsube et al., 2004) have demonstrated a linear correlation between the content of totalphenolic compounds and their antioxidant capacity.

Based on the analysis of the effectiveness index (deGarmo et al., 1984), it was found that the 24 hours of maceration time had the highest index value is 1.48. This means that cemcem instant powder which has 24 hours of maceration time resulted in the most potential instant powder to be developed as a natural antioxidant products.

CONCLUTION

Cemcem instant powdered which was produced by 24 hours of maceration time has the best characteristics and potential to be developed as a natural antioxidant products. It has the highest of total phenol, tannin, vitamin C, and antioxidant capacity. There are 17 compounds detected by GC-MS, these compounds belonged alkenes, alcohols, phenols compounds and esters of fatty acids. The whole compounds were the building blocks of flavor cemcem leaves.

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