

Physicochemical and Antimicrobial Properties of Kinang Extract Microparticle on Various Time and Temperature of Ultrasonication Process

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Abstract

Original Research Article

The research was aimed to determine the chemical and antimicrobial characteristics of the microparticle kinang extract using ultrasonication on various temperature and time ultrasonication process. This study uses a randomized factorial completely design with two treatment. The measuring parameters was consisted of chemical properties (antioxidant activity, total phenol, moisture content and pH value) and antimicrobial analysis (antibacterial activity). The results showed that time of ultrasonication treatment had significant effect on antioxidant activity, total phenol, antibacterial activity, moisture content and pH value. And temperature of ultrasonication treatment had significant effect on total phenol, antibacterial activity, moisture content and pH value. The interaction between time of ultrasonication and temperature of ultrasonication had significant effect on antibacterial activity, moisture content and pH value.

Keywords: Microparticles, kinang extract, ultrasonication.

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INTRODUCTION

Indonesia is one of the tropical countries that have biodiversity known as a warehouse for herbal plants. Many Indonesian people have a healthy habit of consuming a mixture of herbal ingredients such as kinang. Indonesian people call the term "Nginang". Nginang is usually carried out by parents and used for traditional events and is considered to strengthen teeth and inhibit caries in the teeth [1]. Nginang consists of several ingredients mixed from herbal plants such as betel leaf, areca nut, whiting and gambier which are then chewed. A material capable of holding has different benefits. In betel leaf contains 4.5% essential oil which will produce a distinctive aroma [2]. Pinang contains 15% tannin which causes betel nut to have antibacterial and antioxidant benefits [3]. Betel lime has a dry or wet form that is useful to change the acidic environment to alkaline [4]. Gambir contains 7-33% of catechin compounds which have benefits as antioxidants and as an inhibitor of the growth of *Streptococcus mutans* bacteria [5].

The composition of mixed ingredients in kinang have many benefits needs to be streamlined so as to increase added value. One way that can be used is

by applying technology with the application of new technologies such as microparticle. The application of microparticle to kinang can make a new innovation. Microparticles are particles which have a particle size of 1-1000 μm . Microparticles can release more than 80% of the active substance within 10 minutes. Microparticles are one of the delivery systems that can be administered orally, transdermal, intramuscular, intraperitoneal and lung [6].

Microparticles can be made by several methods including solvent evaporation, ionic gelation, dry spray, coacervation, and supercritical fluid extraction [7]. One method that is often used is one with the ionic gelation method. This method is carried out by mixing chitosan polymers with sodium tripolyphosphate polyanion.

Ionic gelation allows the interaction between positively charged amino groups in chitosan with negatively charged tripolyphosphate to enable the formation of modified chitosan with micrometer morphology and even nanometers. Chitosan without cross-linking at micron size has a large surface area of particles which allows greater adsorption capacity but is unstable because it is easily dissolved in water [8]. With

the ionic gelation method using sodium tripolyphosphate material can produce modified chitosan products with micrometer size and are more stable [9].

The use of ionic gelation methods can be modified by the addition of ultrasonication to facilitate the formation of microparticle sizes. Ultrasonication is a method that uses samples with suitable mixing solvents and is placed into an ultrasonic bath at a certain temperature and time [10]. Ultrasonic has become an important implement for microparticle until nanoparticle synthesis. Ultrasonic is a good method for microparticle preparation with controlled morphology [11].

The application of microparticle to kinang extract using ultrasonication was expected to produce new products with better quality and can be applied to food and health products.

MATERIALS AND METHODS

Preparation of Kinang Extract

All constituents of kinang were made into powder form, filtered using 40 mesh sieves. The production of kinang extract was carried out by cold maceration. The extraction process with maceration technique was done by shaking several times or stirring at room temperature [12]. All the ingredients making up the kinang were weighed according to the formulation of kinang [13] that will be used, namely betel leaf (8 g), areca nut (3.5 g), gambier (2.5 g) and whiting (2 g). And mixed all the ingredients that had been weighed. The mixed form of kinang was put into a bottle and a water solvent is added in a ratio of 1: 10. Then shaken and soaked for 24 hours. In the first 6 hours the samples were shaken until evenly distributed and allowed to stand again for the next 18 hours. After soaking for 24 hours, the sample was filtered using filter paper. The waste obtained then remodelled again up to 2x. The results of the first extraction and remaeresation results then mixed and stirred until evenly distributed.

Preparation and Synthesis of Kinang Extract Nanoparticle Solutions

Microparticle were made using chitosan and sodium tripolyphosphate as a coating solution for kinang extract based on the research by [14]. Making chitosan solution was by weighing as much as 0.5 g of chitosan using a watch glass then chitosan is dissolved with 5% v / v acetic acid to 100 mL and stirred with a magnetic stirrer for 5 minutes. And making sodium tripolyphosphate solution by weighing sodium tripolyphosphate as much as 0.2 g then dissolved with distilled water to 40 mL and stirred with a magnetic stirrer for 5 minutes.

Synthesis of microparticle kinang extract using chitosan-STPP as a coating. The steps taken were a modification of the research method by [14, 15]. The

100 mL chitosan solution was stirred with a magnetic stirrer for 10 minutes. During stirring, added 50 ml of kinang extract gradually and after mixing it was stirred again for 15 minutes. Added 40 mL sodium tripolyphosphate solution and stir for 20 minutes until homogeneous.

Kinang-chitosan-sodium tripolyphosphate mixture solution was sonicated to help breakdown the particles contained in the solution using two treatments (temperature and time).

Ultrasonication Treatment

The microparticles solution of kinang extract which was ready then carried out the next process by using the aid of ultrasonic water bath type [16]. The preparation of ultrasonication equipment, the sonication device used is the Elmasonic S 60H sonicator type cleaning sonicator (water bath) with a frequency of 35 kHz. Before using this sonicator, first add enough water as a medium for propagation of ultrasonic sound energy to the sample. A sample solution was placed into the sonicator as much as 1 liter for each treatment. The temperature and time of the instrument was set according to the treatment, the treatment time used is 30 minutes, 60 minutes, 90 minutes and 120 minutes, while the temperature used was 50 ° C, 60 ° C and 70 ° C with three replications per treatment. After the sample solution had been carried out the ultrasonication process, then dried in the sample solution using a freeze drying (capacity of 5 liters). The solution of the dried sample was then smoothed using a softener.

Design of Experiment

Experiment was conducted on completely randomized factorial design, with two treatment, namely time ultrasonication treatment and temperature ultrasonication treatment. The time ultrasonication treatment was consisted of four level, namely 30 minutes (coded by N1), 60 minutes (coded by N2), 90 minutes (coded by N3), and 120 minutes (coded by N4). The sonication temperature treatment was consisted of three level treatment, namely 50°C, 60°C and 70°C (respectively coded by T1, T2, and T3).

The Physicochemical and Antimicrobial Analysis

The physicochemical analysis of microparticle kinang extract was consisted of antioxidant activity [17], total phenol content [18], moisture content [17] and pH value [19]. The antimicrobial analysis of microparticle kinang extract was consisted of antibacterial activity [20].

RESULTS

The values of antioxidant activity, total phenol content, moisture content, pH value, antibacterial activity of microparticle kinang extract was shown in Table-1. Results of analysis of variance showed that N factor (time ultrasonication), T factor (temperature ultrasonication) and their interactions had not significant effect on antioxidant activity, total phenol content, moisture content, and pH value.

Table-1: Antioxidant activity, total phenol content, moisture content, pH value, and antibacterial activity of microparticle kinang extract

Treatment	Antioxidant activity	Total phenol content	Moisture content	pH value	Antibacterial activity
N ₁ T ₁	240.94±80.94 ^a	147.92±4.88 ^{bcd}	3.32±0.06 ^a	4.78±0.29 ^c	7.81±1.01 ^a
N ₂ T ₁	142.66±6.08 ^{bcd}	169.10±14.10 ^{abcd}	2.44±0.13 ^{bc}	5.00±0.19 ^{bc}	5.42±0.10 ^{bc}
N ₃ T ₁	113.62±7.45 ^{bcd}	201.18±6.58 ^a	2.16±0.11 ^c	5.06±0.13 ^{bc}	6.05±0.23 ^{bc}
N ₄ T ₁	101.84±18.42 ^{cd}	203.29±7.73 ^a	1.70±0.14 ^d	5.19±0.06 ^{bc}	5.55±0.28 ^{bc}
N ₁ T ₂	194.46±38.31 ^{abc}	120.47±1.15 ^d	3.49±0.12 ^a	4.81±0.31 ^{bc}	6.44±0.08 ^b
N ₂ T ₂	143.57±43.62 ^{bcd}	128.25±18.18 ^{cd}	2.65±0.22 ^b	5.18±0.14 ^{bc}	5.37±0.15 ^c
N ₃ T ₂	96.64±20.34 ^d	138.74±11.42 ^{cd}	1.72±0.23 ^d	5.19±0.12 ^{bc}	5.25±0.10 ^c
N ₄ T ₂	92.10±6.79 ^d	170.22±14.92 ^{abcd}	1.32±0.06 ^e	5.31±0.06 ^b	5.21±0.13 ^c
N ₁ T ₃	205.68±34.42 ^{ab}	156.77±23.11 ^{abcd}	3.40±0.10 ^a	5.14±0.09 ^{bc}	5.80±0.28 ^{bc}
N ₂ T ₃	98.70±17.09 ^d	164.14±16.89 ^{abcd}	2.66±0.08 ^b	5.26±0.19 ^{bc}	5.62±0.33 ^{bc}
N ₃ T ₃	92.65±6.66 ^d	180.51±44.12 ^{abc}	1.50±0.04 ^{de}	5.85±0.20 ^a	5.15±0.13 ^c
N ₄ T ₃	82.15±12.26 ^d	192.69±4.28 ^{ab}	1.27±0.10 ^e	6.25±0.09 ^a	5.08±0.18 ^c

Note: values followed by the different letter was significantly different, according to Tukey's at the probably level of 5%

DISCUSSIONS

Antioxidant activity of microparticle using ultrasonication had IC₅₀ values ranging from 82.15 ppm to 240.94 ppm. The highest antioxidant activity was found in the treatment sonication time of 120 minutes and sonication temperature of 70°C was 82.15 ppm and the lowest antioxidant activity was found in the treatment of sonication time of 30 minutes and sonication temperature of 50°C which was 240.94 ppm. IC₅₀ value was defined as the magnitude of the concentration of the test compound that can reduce free radicals by as much as 50%. The smaller the IC₅₀ value, the higher the free radical scavenging activity [21]. Antioxidant activity produced microparticle kinang extract using time and temperature variations in the ultrasonication process was included in the category of moderate to strong antioxidants activity. This was due to the long duration of ultrasonication that will multiply cells that break, so that the antioxidant compounds in the kinang will come out. The sonication process was an exothermic process which generates heat and can create interface movements between solid and liquid, thereby causing an increase in mass transfer rate and increasing product yield [22].

Total phenol levels had a relationship with antioxidant activity. This phenolic compound was widely distributed in plants and had been reported to have antioxidant activity. In addition, polyphenols have hydroxyl groups in different amounts and positions that can function as antibacterial compounds [23]. The total phenol content produced showed that the longer the sonication process, the higher the total phenol produced. This was because the cavitation waves generated during sonication can make the cells in the kinang extract split so that the phenol compound can be easily extracted out and then absorbed with a solution of chitosan-stpp microparticles. The principle of ultrasonic extraction was to increase mass transfer caused by increased solvent penetration into plant tissue through the capillary effect. Cavitation bubbles formed on the cell walls of plants due to ultrasonic waves. The

effect of bursting cavitation bubbles resulted in an increase in pores in the cell wall. Cavitation bubbles splited due to the thinness of the glandular parts of plant cells which can be easily damaged by sonication [24].

Analysis of antibacterial activity using *Streptococcus mutans* in this study aims to determine the activity of flavonoids namely catechins and tannins contained in the mixture of kinang powder which acts as an antibacterial and anti-inflammatory. Classification responses of bacterial growth inhibition power seen based on clear zone diameter consists of 4 groups: weak response (diameter <5 mm), moderate (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter < 20 mm) [25]. The antibacterial activity of kinang extract microparticle using ultrasonication has an average value of inhibition diameter of *Streptococcus mutans* ranging from 0.33 mm to 3.06 mm by using paper discs with a diameter of 4.75 mm. This was because the duration of sonication can reduce the antibacterial activity found in the microparticle solution.

The lowest moisture content value was found in treatment sonication time 120 minutes and sonication temperature 70 °C while the highest water content value was found in treatment sonication time 30 minutes and sonication temperature 60 °C. Moisture content of microparticles of kinang extract shows that the longer the ultrasonication process and the higher the ultrasonication process temperature, the lower the value of the water content produced. This was due to the long sonication process which was able to break down particles in solution into smaller particles so that after drying using freeze drying it will produce a low water content value.

Based on the degree of acidity, a food can be classified into three groups of degrees of acidity, namely the first low-acidity food with a pH range of 5.3 to 4.5, the second medium-acidity food with a pH range of 4.5 to 3.7 and the last was high-acidity food with a pH below 3.7 [26]. The resulting pH value indicated the

longer the ultrasonication process and the higher the temperature used, then the pH value produced increases. This was because the ultrasonication process with variations in time and temperature increases the pH value produced due to the amount of amine ions that will be protonated [27].

CONCLUSIONS

The chemical and antimicrobial changes had occurred on microparticles of kinang extract with various time and temperature ultrasonication process. The interaction between time of ultrasonication and temperature of ultrasonication had significant effect on antibacterial activity, moisture content and pH value.

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