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Research Article

The Encapsulation Process of Flavonoid from Kepel Seeds (*Stelechocarpus burahol*)

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Abstract: Flavonoid contained in Kepel seeds was extracted and encapsulated using a crosslinker agent of natrium tripolyphosphate (NaTTP) aiming for protection of the flavonoid content as an antioxidant. Extraction was carried out in variation of ethanol concentration and time extraction to obtain the best extract flavonoid. Fourier Transform Infrared Spectrometry (FT-IR) method was used to determine the presence of flavonoids in the extract. Total flavonoids (TF) analysis was carried out using a UV-Vis spectrophotometer. The highest total flavonoid content was obtained in the extract with 50% ethanol concentration was 2.427% for one hour and 2.888% for two hours.

Keywords: antioxidant, encapsulation, flavonoid extraction, kepel seed

Introduction

Kepel (*Stelechocarpus burahol*) is a fruit that originated in Indonesia and mostly found in Yogyakarta that their leaves have benefits for health such as renal inflammation, abortifacient, and anti-implantation [1-3]. In addition, due to the natural phenolic, the growth of cancer can be inhibited [4]. There are about 30,000 species of flowering plants in various regions spread throughout Indonesia and Kepel is one of them [5]. Kepel has less preferable due to the size of its seeds is 27% and only 47% of fruit can be consumed [6] so that the seeds to be disposed of then generate waste. Based on the results of phytochemical screening earlier, it is known that the seed of kepel fruit contains flavonoids and tannins [1].

Flavonoids can be found in food, especially in vegetables and fruits contained in fruits, stems, leaves, and even roots. There are many benefits of antioxidant for health for example expected to inhibit the oxidation of low-density lipoprotein (LDL), protect cells against lipid oxidation, reduce the tendency of thrombotic in vitro, and can be used as an antiviral, antifungal, and catcher of free radicals [7-10]. Total flavonoid was found 27.82% w/w in Kepel leaves by methanol solvent extraction [11] while 49.81 ± 0.37 % w/w in Kepel fruit pulp by methanol solvent extraction with 1:10 of sample to methanol ratio [12].

To increase the economic value of the Kepel seed, it needs to be processed using the method of extraction and encapsulation with the aid of a certain solvent to extract the flavonoids. The encapsulation method aims to protect the antioxidant flavonoid compounds that are easily damaged if exposed to heat, air, or other substances. To get the maximum flavonoids, testing needs to be done related to the type and grade-appropriate solvent. The polarity properties of the solvent affect the extraction of polyphenols compound [13] and known that polar solvent oftentimes used to extract flavonoid from biomass, such as methanol is the suitable solvent for polyphenol with lower molecular weight while acetone for flavanols with higher molecular weight [4, 14-17], and to extract flavanoid inside the tea, ethanol is the suitable one than methanol and acetone [18]. In medical requirements and functional food, ethanol is safe and an appropriate polar solvent to be used [19]. Ethanol used in the extraction process because it is capable of dissolving almost all organic compounds in the sample and easily evaporated and ethanol considered to be a very good polar solvent [20].

Therefore, the authors took the initiative to study the effect of solvent concentration variation on the amount of flavonoids in Kepel seeds as a source of alternative antioxidants. There is much previous

research studied about Kepel leaves however there is no research on flavonoids in Kepel seeds so this research can utilize a waste, Kepel seeds, as a new source of flavonoids.

Materials and Methods

Materials

The raw material of this study is Kepel seeds, which were obtained from Yogyakarta, Indonesia. Another chemical used in the extraction process is ethanol, and the encapsulation crosslinker agent is NaTTP, ethyl acetate, and chitosan.

Extraction of kepel seeds

Kepel seeds were cleaned by using water to remove the impurities then cut into small pieces about one centimeter. The small Kepel seeds were dried in an oven at 50°C of temperature for approximately 48 hours then mashed with a grinder and sifted become powder with the size 20 mesh. Five grams of Kepel seed powder was added with 100 mL of ethanol in an erlenmeyer. The mixture was then extracted while stirred at 35°C of temperature with the speed of 1200 rpm for one hour. The extracted sample was filtered then carried out to evaporation in a rotary vacuum evaporator at 60°C for one hour to remove the solvent. The ethanol concentration used in this study was 10%, 30%, 50%, 70%, and 90% and the time was one and two hours.

Flavonoid encapsulation

Extracted flavonoid was dissolved in KCl pH 1 (ratio of flavonoid to KCl 1 : 1) ; chitosan (0.3 w/v) was dissolve in acetic acid solution pH 2.6 (20 v/v) ; pH NaTTP solution (0.3 w/v distilled water) was adjusted to 2.6 by using 0.1 N HCl. Two mL of flavonoid extract was added to 20 mL of chitosan solution pH 2.6 (0.3% b/v) and at the same time, that solution was added to NaTTP crosslinker solution pH 2.6. The mixture was then stirred for 3 minutes. The encapsulated suspensions were separated using a centrifuge at a speed of 10,000 rpm for 10 minutes then dried in an oven at 50°C of temperature to become dry and powder.

Flavonoid Characterization

The samples were analyzed by Fourier Transform Infrared Spectrometry (FT-IR) to detect flavonoid compounds in encapsulated flavonoids. Total Flavonoids (TF) were determined by using the spectrophotometric method at 510 nm wavelength based on Wang et al [21] procedure, with the rutin standard. Amount of flavonoid compound in the seeds obtained rutin standard for flavonoid compound as seen in Figure 1 and by using equation (1) and the yield (%) by using equation (2).

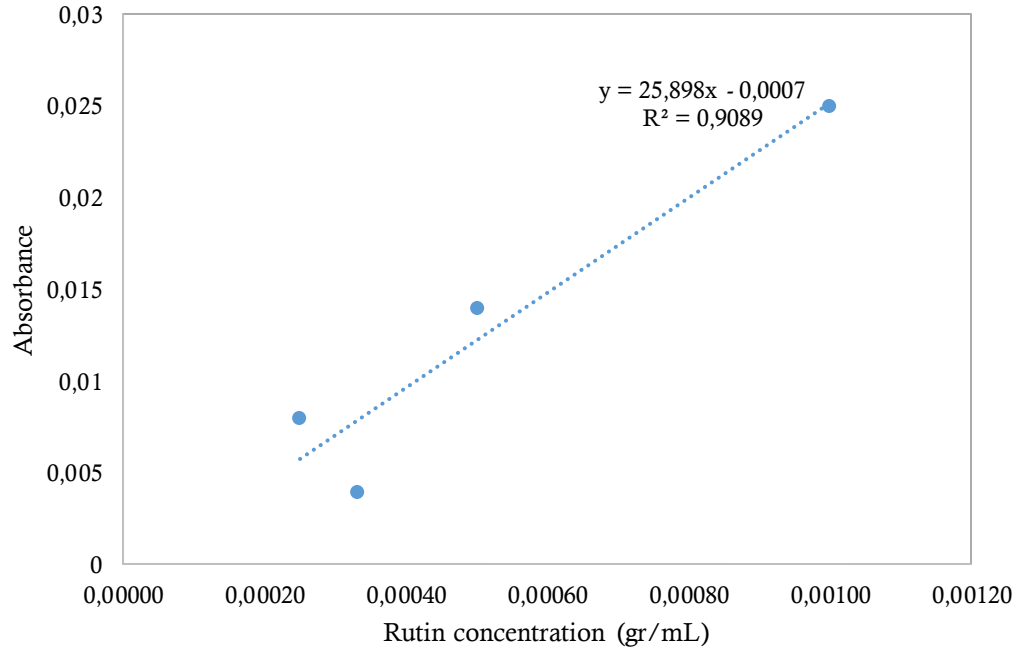

Figure 1. Rutin standard curve for flavonoid

$$TF = \frac{R \; x \; DF}{m} \quad (1)$$

TF : total flavonoid (gr/mL)

DF : 2500

R : rutin concentration (gr/mL)

m : number of Kepel seed

$$\%\, yield\ extract = \frac{flavonoid\ extracted\ (mg)}{kepel\ seed\ powder\ before\ extraction\ (mg)}\ x\ 100 \quad (2)$$

Results and Discussions

Kepel fruit seed has blackish-brown color, large size, and there are 3-4 seeds in one fruit, as well as giving rounded shape, concave-convex, flat-convex, and triangular convex fruit seeds were found to have chemical constituents flavonoid and tannin [1]. Flavonoid compounds can be found in almost all parts of the plant and it is said that there are antioxidants, antibacterial, antiviral, anti-inflammatory, antiallergic, and anticancer [10]. Kepel seed samples were obtained from Sayegan, Sleman, Yogyakarta. The simplistic powder of Kepel seeds has a dark brown color and the peculiar smell of Kepel fruit. Kepel seed powder has been mashed and sifted as seen in Figure 2.

The extracted liquid obtained dark brown. Furthermore, the extraction solvent is evaporated to obtain a thick extract using a rotary vacuum evaporator at 60°C, this is because flavonoids will be damaged at temperatures over 80°C [22]. The results were encapsulated to obtain the extract in powder. The encapsulation process was done using chitosan and natrium tripolyphosphate (NaTPP). Chitosan is a very good coating, but its nature fragile. Therefore, it is necessary for tripolyphosphate compounds as crosslinking agents of the nicest in the manufacture of ionic gelation as not toxic. Results encapsulation in the form of capsules with water levels still high. Furthermore, capsule encapsulation yields dried in an oven at 60°C to obtain a powder extract.

Figure 2. Kepel seeds powder

Analysis of flavonoid compounds was performed using Fourier Transform Infrared Spectrometry (FT-IR). FT-IR works by analyzing the compounds present in the extract of powder-based functional groups. Flavonoid compounds have a chemical structure C6-C3-C6 or benzene rings connected by a three-carbon aliphatic chain. Types are classified by hydroxyl and additional oxygen-heterocyclic rings with different patterns. Based on the use of FT-IR analysis results obtained as shown in Figure 3. As seen in Figure 3a and 3b, infrared spectrum (IR) exist in the area with the wavelength 3750-3000 cm^{-1}, 1900-1650 cm^{-1}, and 1675-1500 cm^{-1} as an indication of the existence of carboxyl group, carbonyl group, and aromatic and aliphatic groups, respectively. Those indications confirm the presence of the phenolic group

which is a flavonoid compound. All variations in the concentration of ethanol showed the same results for the presence of flavonoids in the Kepel seed at one and two hours of the extraction process.

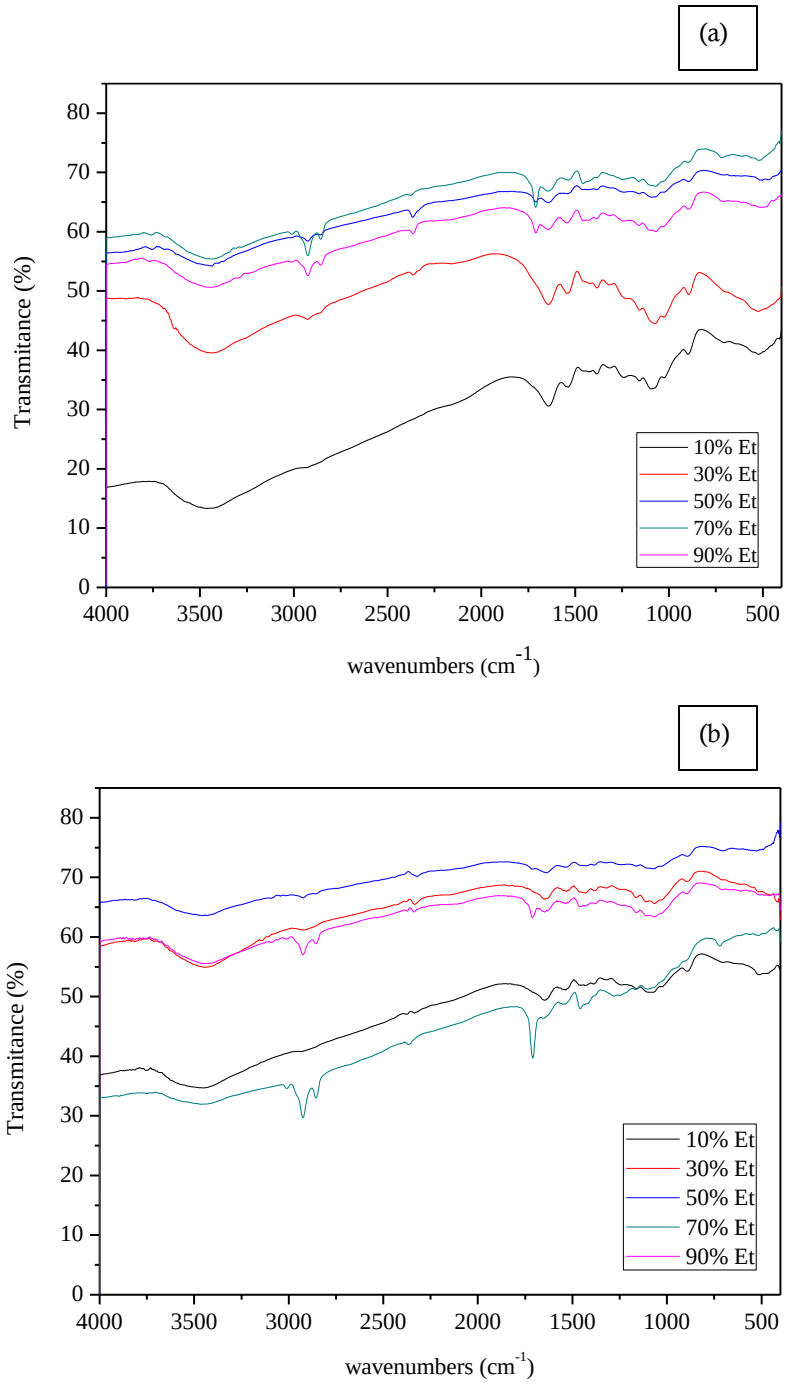

Figure 3. FTIR graph of flavonoid cmpound (a) 1 hr extraction (b) 2 hr extraction

The percent yield results from a comparison of the products produced by the initial raw material used. The following show the influence of solvent concentration on the percentage yield of the extraction process Kepel five grams of powdered seeds with variation stirring time was one and two hours at a temperature of 35°C seen in Table 1.

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Table 1. Flavonoid compound of extracted kepel seeds

Ethanol (%)	Yield (%)	
	1 hr	2 hr
10	18.4	19.2
30	14.2	14.6
50	13.4	7.8
70	4	4
90	3.6	2.2

As seen in Table 1, the higher the concentration of the solvent causes decreasing in yield in the extraction process. This is because the higher the concentration of ethanol used as a solvent, the more solvent is evaporated in the evaporator so that the products produced at the highest solvent concentration will be smaller. In line with Ramadhan et al [23], the higher the concentration of ethanol would cause the level of the polarity of the solvent to be lower. Therefore, solvents with high ethanol concentrations would be difficult to extract flavonoids in Kepel seeds that are polar.

Table 1 shows the percentage of the highest yield is obtained at the lowest concentration, which is 10% with a yield of 18.4% in one hour and 19.2% at 2 hours. While the percentage produced the lowest yield of the highest solvent concentration, ie a concentration of 90% with a yield of 3.6% at the time of 1 hour and 2.2% at 2 hours.

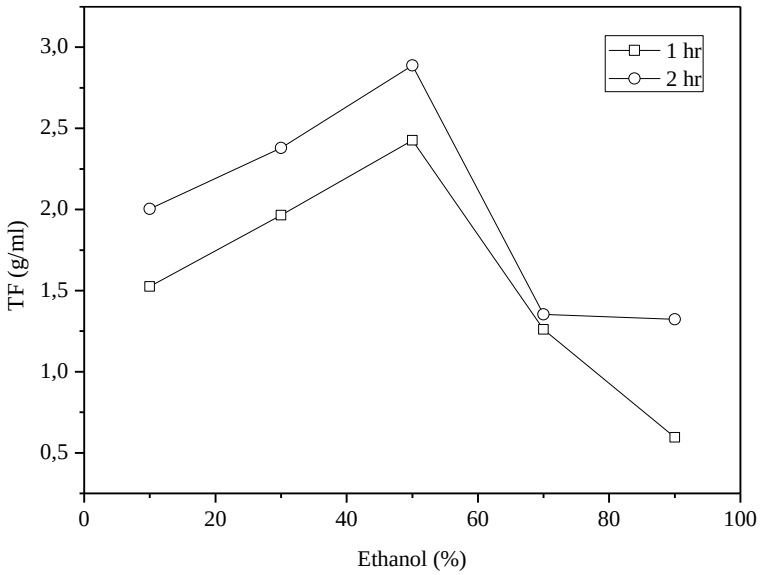

Figure 4. Total flavonoid of kepel seeds in the variation of ethanol concentration

As seen in Figure 4, the highest value of total flavonoids (TF) obtained in ethanol extract 50% with an extraction time of 2 hours is 2.888 gram/ml. This result indicates that the longer the extraction process, the more content of flavonoids extracted. While the influence of the concentration of the solvent is the higher the composition of water in the solvent the polar compounds present in the seeds of the Kepel will diffuse more into the solvent [24]. This is shown in the increase in the concentration of the solvent TF from 90% to 50%. However, this correlation is only reaching 50% solvent concentration conditions and decreased back to the solvent concentration of 30% and 10%. This is because the amount of water that is too large composition while ethanol bit, so that flavonoid compounds diffused too little.

Durability during storage depends on the water content in the extract powder, so to extend the durability it is necessary to remove a certain amount of water content. As seen in Table 2, all the results obtained were not more than 10% so that the powder of Kepel Seed Extract has fulfilled the requirements based on the Ministry of Health of the Republic of Indonesia.

Table 2. The water content of flavonoid encapsulation

Ethanol (%)	Water content (%)	
	1 hr	2 hr
10	8.91	5.26
30	8.97	7.59
50	6.94	7.14
70	4.76	4.76
90	5.26	8.33

Conclusion

The conclusion that can be drawn based on the results of the study is that all powder Kepel seed extract contains flavonoids. Levels of total flavonoids are highest in powder extract with a concentration of solvent of 50% with a time of 2 hours is equal to 2.888 g.mL-1 compared to other concentrations. Extraction time and different concentrations of ethanol as a solvent to affect levels of total flavonoids Kepel seed extract. Even though the extraction process has a varied concentration of ethanol, the water content of flavonoids encapsulated as permitted by the regulation of the Minister of Health of the Republic of Indonesia not more than 10%.

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