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Research article

Characterization of nano particles ethanol extract of guava (Psidium guajava) fruit with variation of chitosan-alginate

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ABSTRACT

The ethanol extract of Guava (Psidium Guajava) fruit is known to have strong antioxidant activity so that it has the potential to be developed into a nanoparticle delivery system. The purpose of this study was to determine the effect of variations in the concentration of chitosan - alginate polymer on the physical characteristics of guava fruit extract nanoparticles (Psidium Guajava). Guava Fruit Extract was formulated in the form of nanoparticles by ionic gelation method with variations in the concentration of chitosan:alginate polymer, namely 0.5% : 0.5% (F1), 0.75% : 0.5% (F2), and 1% : 0.5% (F3). The test parameters include the percentage of transmittance. The optimal formulation is based on the level of clarity of the solution and the percentage of transmittance obtained in the formulation ratio of 0.75%: 0.5% (F2), with an average transmittance of 91.9%. Based on the results of the research that has been done, it can be concluded that guava fruit extract can be formulated in nanoparticle size with varying physical characteristics depending on the concentration of chitosan and alginate used.

Keywords: Nanoparticles, Chitosan, Alginate, Guava Extract.

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INTRODUCTION

Indonesia is a tropical country that has abundant natural resources. One of the wealth of natural resources is the diversity of tropical fruits. These tropical fruits are generally grown alone or in special plantations. Guava is a type of local tropical fruit that is widely consumed by the community and is easy to find, either sold in the market or grown by the community themselves. Based on previous research, guava fruit has a higher antioxidant activity than papaya fruit, based on ascorbic acid content, total phenol, and DPPH radical scavenging activity[1].Guava is a source of vitamin C, where the role of vitamin C in the process of iron absorption helps reduce ferric iron to ferrous.

There is the effectiveness of guava extract on hemoglobin levels in pregnant rats given the extract orally as much as 1%, 2%, and 3%[2]. Besides having a high source of vitamin C (59.25-76.85mg/100g) compared to papaya (46.20mg/100g), guava fruit also has a higher source of vitamin C than tomatoes (27.13mg/100g)[3]. Some chemical constituents, such as phenolic compounds, carotenoids, and vitamins, especially ascorbic acid (vitamin C) and tocopherols (vitamin E), are effective free radical scavengers[4]. Based on this, guava fruit has enormous potential to be used as medicinal ingredients, and it is necessary to standardize medicinal plant extracts to protect the public from the use of herbal medicines that do not meet quality requirements.

Among the various types of delivery systems, many researchers use nanoparticle delivery systems because of various advantages, including particle size and surface characteristics of nanoparticles that can be easily modified as needed, nanoparticles can control and maintain the release of active compounds during transportation thereby reducing side effects, the release of compounds. Controlled actives and the content of active compounds can be entered into the system without chemical reactions which are important factors for maintaining compound activity [5].

Based on the literature review, it is necessary to develop guava fruit extract into nanoparticle preparations, which aims for the efficiency of drug use, where there are often obstacles to the ability of the drug itself to reach its site of action. In most cases (normal size), only a small amount of the drug can reach the target site of action, while most of the drug is distributed throughout the body according to its physicochemical and biochemical content. In this study, characterization was carried out as the first step in standardizing the ethanol extract of guava fruit. The purpose of this study was to determine several specific and non-specific parameters so as to

ensure that the extract has measurable values and parameters and to determine the effect of variations in the concentration of chitosan - alginate polymer on the physical characteristics of guava fruit ethanol extract nanoparticles.

MATERIALS AND METHODS

Plant determination

The determination test was carried out to determine the correctness of the identity of the plants used in the study. Plant determination was carried out at the Biology Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University (SK.No. 215/Lab Bio/B/VI/2021, with results showing that the simplicia used, was Psidium Guajava L based on Flora of Java (1958): 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14b-16a-239b-243b-244b-248b-249b-250a-251b-253b-254b-255b-256b-261a-262b-263b-264b Myrtaceae 1b-2a Psidium,1 Psidium guajava L

Guava Extraction

The beginning of the manufacture of guava fruit extract (Psidium guajaval) was carried out, namely 5 kg of guava, washed, then sliced thinly and dried in an oven at 60ºC for 24 hours. The dried fruit slices were then blended so that they became powder, then the fine powder was weighed as much as 100 grams. A total of 100 g of dried guava powder was put into a macerator, plus 1 liter of 95% ethanol, soaked for 6 hours while stirring, then allowed to stand for 24 hours. The macerate was separated, and the process was repeated 2 times with the same type and amount of solvent. All the macerates were collected and evaporated with a vacuum vaporizer to obtain a thick extract. The yield obtained is weighed and recorded [6]:

Manufacture of guava extract nanoparticles

Each 0.5%, 0.75% and 1% chitosan solution were put into a 100 ml beaker. Then at each concentration of the solution, 1 ml of polysorbate 80 was added and stirred using a homogenizer at 1000 rpm for 10 minutes. After that, 0.5%, 0.75%, and 1% chitosan solution was added to each 0.1 g guava extract and stirred using a homogenizer at 3000 rpm for 30 minutes. After that, 20 ml, 30 ml, and 40 ml of 0.5% alginate solution were added, respectively, and then homogenized at 4000 rpm for 90 minutes.

Table 1: Nanoparticle formula

Materials	F1	F2	F3
Guava fruit extract	1000 mg	1000 mg	1000 mg
0.5% chitosan solution	100 ml		
0.75% chitosan solution	-	100 ml	-
1% chitosan solution	-	-	100 ml
Alginate Solution 0.5%	20 ml	30 ml	40 ml
Polysorbate 80	1 ml	1 ml	1 ml

Evaluate the transmittance of nanoparticle solution

A total of 100 µLof fruit extract nanoparticles was added with aquabides to a final volume of 10 ml. Homogenization was carried out with the help of a magnetic stirrer for 1 minute. The guava fruit extract nanoparticles were then measured for transmittance using a spectrophotometer at a wavelength of 650 nm [7].

RESULTS AND DISCUSSION

Extraction

Extract concentration was carried out with a water bath until the extract was thick and dark brown in color. The results of the randemen calculation are listed in Table 2:

Table 2: Yield calculation

Fresh guava fruit weight	Weight of simplicial powder	Extract weight	% Yield
5 kg	350 g	45.47 g	12.99 %

Source**:** Laboratory data (2021)

The yield of guava fruit extract is 12.99% so that it meets the requirements of the Monograph of Indonesian Medicinal Plant Extracts Volume I. The yield produced in this study is lower than in previous studies, where the thick extract of red guava flesh was 14.1885 g yielded 15.765% and the thick ethanolic extract of white guava flesh 16.354 g yielded 18.2% yield[8].One of the factors that affect the yield is the extraction time, the accuracy of the length of time used affects the efficiency of the process [9].

Qualitative test of flavonoids

Qualitative test of flavonoids using thin-layer chromatography. The mobile phase used was a mixture of n-butanol: acetic acid: water in a ratio of (4:1:5), with the stationary phase using silica gel GF 254 nm. A qualitative test aims to determine the content of secondary metabolites. The TLC results are shown in Figure 1 and Table 3.

Table 3: Flavonoid examination results

Secondary metabolites	Sample	UV Light366	Rf value
Flavonoid	Quercetin	Blue	0.8
	Guava fruit extract	Blue	0.9

The Rf value of the standard and the sample obtained are almost the same, namely 0.8 in the standard and 0.9 in the sample, so it is known that guava fruit extract contains flavonoid compounds. The selection of mobile phase, which is used in Thin Layer Chromatography is a mixture of eluent n-butanol: acetic acid: water (BAA) with a ratio (4:1:5) capable of providing the best separation.

Evaluation of nanoparticle solution transmission

The percentage (%) transmittance was used to measure the clarity of the nanoparticle preparations. The % transmittance value close to 100 indicates a smaller particle size with a larger surface area, making it easier to read the absorption. The small particle size causes the brown movement that occurs faster to prevent the sedimentation process and result in a clearer solution [10].

Table 4: Test Results % transmittance

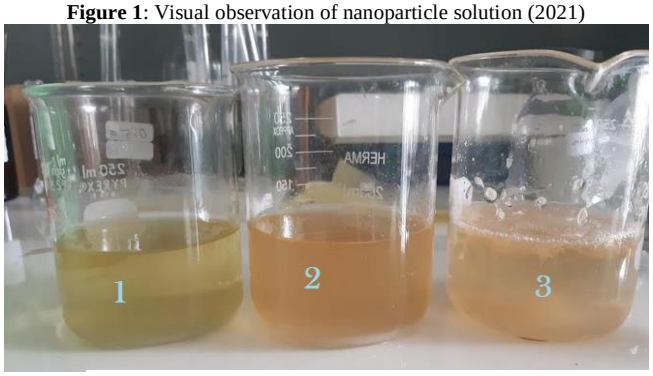
Sample	% Transmittance	Nanoparticle solution visual
1	96.5	Clear +++, Sediment
2	90.7	Clear++
3	88.5	Clear+

Description: (+): level of clarity

Based on the results of the percentage of transmittance test, the second formulation used a variation of 0.75% chitosan and 0.5%

alginate as much as 30 ml, with an average transmittance percentage approaching 100, namely 91.9%.

Figure 1: Visual observation of nanoparticle solution (2021)



In drug carrier systems, polymers such as chitosan and alginate are more often used because they are non-toxic, biocompatible, and good biodegradable. Chitosan and alginate can react together because they have opposite charges, the alginate-chitosan complex solves some of the limitations of individual polyelectrolytes [11].The easy solubility of chitosan at low pH can be prevented by alginate tissue because alginate does not dissolve at low pH conditions. The possibility of disintegration of alginate at higher pH is prevented by chitosan, which is stable over a higher pH range[12].Previous research has succeeded in making a ketoprofen drug carrier system that is easier to make using chitosan modified with alginate and TPP as a crosslinking agent 26.81% and 23.90% respectively[13].

Guava Fruit Extract Gas Chromatography Mass Spectrometry (GC-MS)

The GC-MS chromatogram of the guava fruit extract showed that there were31 compounds contained therein as shown in Figure 2, some compounds having peak areas greater than 1% are listed in Table. 5. GC-MS analysis showed the presence of several important compounds. From the chromatogram, different peaks were obtained at different retention times. Based on standard internal data from MS, compounds are displayed using molecular weights. Guava Fruit Extract contains compounds d-Glycero-d-tallo-heptose, melezitose, lactose, octadecadiynoic acid, heptadecanoic acid, 16-methyl-, methyl ester, and hexadecanoic acid, methyl ester. In a previous study, d Glycero-d-tallo-heptose became one of the main components in date palms, where the use of a combination of grape juice, methanol extract of dates (1:2) could cause a hepatoprotective effect which was confirmed by histopathological examination[14]. Another major component of guava fruit extract is melezitose, which is a trisaccharide and many low molecular weight oligosaccharides[15]. The octadecadiynoic acid component was also found in the methanol extract of Mentha Viridis using the GC-MS method, where Mentha Viridis contains chemicals that are useful for various herbal formulations as a cardiac tonic, analgesic, antiasthmatic, anti-inflammatory, and antipyretic [16].Previous studies using GC-MS, AdathodaiChooranam plant was found to have heptadecanoic acid, 16-methyl, methyl ester, where these components have antioxidant, anti-microbial, and anti-inflammatory activities and contribute to a therapeutic effect on bronchial asthma

Figure 2: Guava fruit extract GC-MS

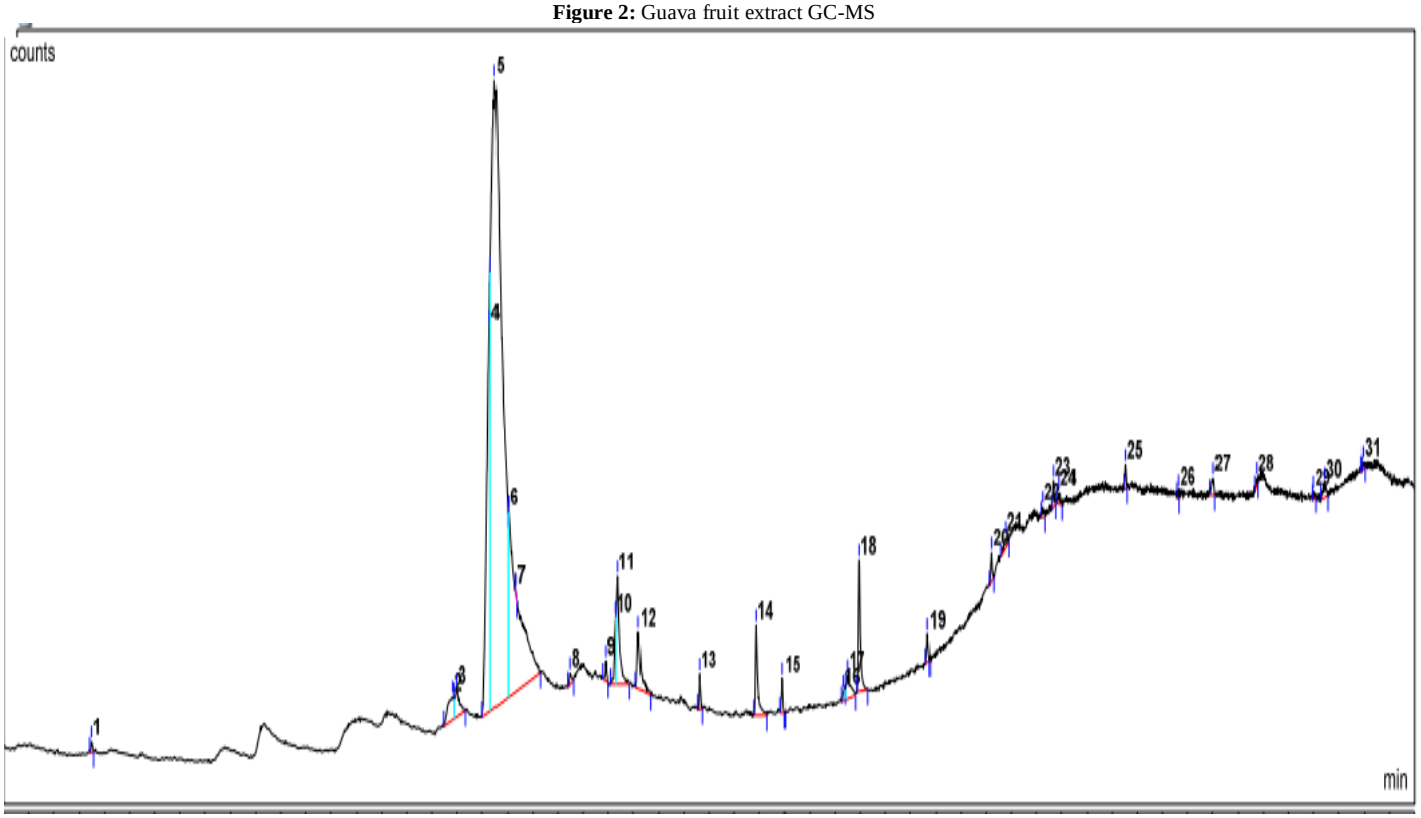


Table 5: Compounds that are mostly found in guava fruit extract

Retention Time	Compound Name	% Peak Area
13.74	d-Glycero-d-tallo-heptose	55.59
14.02	Melezitose	15.83
13.64	Lactose	11.29
16.19	10,13-Octadecadiynoic acid, methyl ester	2.99
20.98	Heptadecanoic acid, 16-methyl-, methyl ester	2.25
18.95	Hexadecanoic acid, methyl ester	1.83
16.60	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	1.54
12.91	Melezitose	1.28
12.99	Melezitose	1.07

In this study, three types of nanoparticle formulation were made with varying concentrations of the combination of chitosan and alginate polymers. The mixing of chitosan and alginate polymers will result in an interaction between the positive charge on the chitosan amino group and the negative charge of the tripolyphosphate. Where the concentration of chitosan and alginate polymer used can affect the physical characteristics of the nanoparticles[7].

CONCLUSIONS

The pharmacological activity of the compounds contained in guava fruit (Psidium guajava) can be optimized through the formation of nanoparticles. The optimal formulation is based on the level of clarity of the solution and the percentage of transmittance obtained in the formulation ratio of 0.75%:0.5%), with an average transmittance of 91.9%. Based on the results of the research that has been done, it can be concluded that guava fruit extract can be formulated in nanoparticle size with varying physical characteristics depending on the concentration of chitosan and alginate used. Guava Fruit Extract can be formulated in nanoparticle size with varying physical characteristics depending on the concentration of chitosan and alginate used.

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Conflicts of interest

The authors declare no conflict of interest

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