Helium: Journal of Science and Applied Chemistry
https://journal.unpak.ac.id/index.php/he_jsac

Volume 4, Number 2, December 2024, Page 72 - 77
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher : Universitas Pakuan

The Effect of Ethanol Polarities on Tannin Content of *Persea americana* Mill Seeds Extract Using Gelatin Precipitation Method

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Article history: *received: 28-11-2024; revised: 23-12-2024; accepted: 30-01-2025; published: 30-01-2025*

ABSTRACT

Persea americana Mill (avocado) seed waste contains tannin compounds with several benefits and high economic value. Tannin is a natural compound widely used in various industries, including the pharmaceutical industry. The difference in concentration of ethanol causes polarity differences. This research aims to determine the effectiveness of tannin extraction from avocado seed using different concentration polarities of ethanol as a solvent. The dry powder of avocado seed was macerated in ethanol at concentrations of 30, 50, 70, and 96% for 72 hours, respectively. The gelatin precipitation method was applied to determine the tannin content in each extract; absorbance was read using a spectrophotometer UV-Vis, and the Folin Ciocalteu reagent was added to the avocado seed extract before spectrophotometer measurement. The results show that the polarity of ethanol has a significant effect ($p<0.05$) on the yield extract and tannin level in each extract. The extract yield and tannin content in avocado seed extracted with 30, 50,70 and 96% ethanol were 21.47%, 26.70%, 30.40%, and 47.67%, while the tannin content was 32.27 mg/g, 50.58 mg/g, 60.94 mg/g, and 85.30 mg/g respectively. The highest extract yield and tannin content are found in avocado seed extracted with 96% ethanol, which has lower polarity. It can be concluded that 96% ethanol is the best solvent to extract tannin from avocado seeds.

Keywords: Ethanol, Gelatin, *Persea americana*, Tannin, Polarity,

1. INTRODUCTION

Avocado plants (*Persea americana* Mill.) proliferate in Indonesia, and the fruit has high economic value and many health benefits. The seeds of an avocado represent 13-18% of the total weight of the fruit, and it can be estimated that there is a lot of avocado seed waste, which will have an impact on the environment [1], [2], [3]. Avocado seeds contain phenols, flavonoids, tannins, saponins, alkaloids, carotene, cardiac glycosides, phytosterols, and stigmasterol [4]. These compounds have biological activities such as antimicrobial, antidiabetic, anti-inflammatory, anti-cancer, antifungal, and anti-neurogenerative [5], [6], [7], [8], [9].

Tannin can protect plants from predators and aid in controlling plant growth. [10], [11]. Potential use of tannin in pharmaceutical or medical applications was as an anti-cancer, antimicrobial, anti-virus, antioxidant, antidiabetic, and antidiarrheic [11].

The tannin content of avocado seeds can be obtained using an extraction method with a suitable solvent, and selecting solvents based on different polarities aims to obtain the best solvent for extracting specific chemical contents maximally. Water alone or combined with additional solvents such as methanol, ethanol, acetone, and sodium hydroxide can extract tannins. Regarding tannin extraction, the solid-to-solvent ratio and the extraction temperature are crucial [10]. Total tannins in acetone, ethanol, and water extract were 0.1989%, 0.2044%, and 0.1804%, respectively. Condensed tannin extracted with ethanol (95% v/v) and ethanol-water mixture (50%-50% v/v) were 65.13 mg/g and 203.62 mg/g. Both research uses maceration as an extraction method [3], [12]. This result indicates that different solvents can influence the amount of tannin in the extract. However, the effect of differences in ethanol polarity on tannin levels has not been studied. Tannis belongs to the polyphenol group, where these compounds are considered relatively polar

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components [13]. Therefore, in this research, ethanol solvents with different polarities will be used by mixing both ethanol and water in different ratios. Compared to distilled water, which has a polarity index of 9, ethanol has a polarity index of 5.2. The mixture of both solvents at different ratios can cause different solvent polarities [14]. The solvent's polarity will affect the solute's ability to dissolve in the solvent [13].

2. METHODS

2.1. Equipment/Tool/Material.

The material used was *Persea Americana* Mill. (Lauraceae) seed from the Cibinong area, District Bogor, Indonesia, was determined in PT Palapa Muda Perkasa, in Cilodong, Depok City, West Java, Indonesia. The chemical compounds used were water distilled, ethanol (Merck), ferric chloride (Merck), Folin Ciocalteu (Merck), gelatin (Brataco), natrum bicarbonate (Na_2CO_3) (Merck), calcium bromide (KBr) (Merck). All reagents were analytical grade. The rotary evaporator (IKA RV10) is used to evaporate the extract, and the UV-visible spectrophotometer (Jasco V-730) is used to analyze tannin.

2.2. Experiments

2.2.1 Preparation of Dry Sample

Fresh avocado seed is collected, peeled, washed, and sliced, then dried in the oven at 40-50 °C for 18-24 hours. Dry seeds were powdered and sieved with a 40-mesh sieve. A certain amount of simplicia avocado seed powder was re-macerated with ethanol solvent (30%, 50%, 70%, and 96%) at a ratio of 1:10 for 72 hours. The filtrate was concentrated using a rotary evaporator to obtain a thick extract. All procedures and tests in this study were carried out in triplicate.

2.2.2 Water and Ash Content Test

A total of 2 grams of sample is heated in an oven at a temperature of 105 °C for 5 hours or more until the weight is constant. The difference in initial and dry weights is calculated as the water content. A total of 2 grams of sample is weighed and slowly incandescent at a temperature of 500-600 °C until charcoal is formed. The difference in charcoal weight and initial weight is calculated as the ash content [15].

2.2.3 Qualitative Test of Tannin in Avocado Seed Powder and Seed Extract

The presence of tannins in the avocado seed powder and extract was carried out according to the method described by Shaikh & Patil (2020) [16].

2.2.4 Determination of Tannin Content in Avocado Extract

A blank solution was made by placing one mL of 10% Folin Ciocalteu in a 10 mL volumetric flask and two mL of 20% saturated Na_2CO_3, then added with distilled water to the limit. The solution was shaken, left for 90 minutes at room temperature, and then measured using a UV-Vis spectrophotometer at a wavelength of 745 nm to detect its absorbance value.

The extract solution was prepared by dissolving 50 mg of avocado seed extract with ethanol in 10 mL of a volumetric flask. Two 10 mL volumetric flasks labeled A and B were prepared. In volumetric flask A (which contains total polyphenols, including tannin), one mL of the extract solution was diluted with ethanol to the mark and shaken until homogeneous. One mL of extract solution was pipetted from flask A and added with one mL of 10% Folin Ciocalteu, two mL of 20% Na_2CO_3 saturated solution, and diluted with distilled water in a 10 mL volumetric flask. The solution was shaken and left at room temperature for 90 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 745 nm.

In volumetric flask B (containing polyphenols and free tannins), one mL of the extract solution was added with five mL of 1% gelatin and diluted with ethanol. The solution was left for a while to form precipitation completely, then filtered to obtain a free tannin solution. One mL of solution was pipetted and put into a 10 mL volumetric flask and treated like the procedure in tube A. A calibration curve was prepared using a series concentration of gallic acid as a standard compound.

Tannin content was calculated using the formula:

$$\text{Tannin content} = (C) = C_A - C_B$$

where:

C_A = Total polyphenol concentration (including tannins).

C_B = Polyphenol concentration without tannins (after gelatin treatment).

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3. RESULTS AND DISCUSSION

3.1 Avocado Seed Dry Powder

Avocado seeds are made into dry powder to extend the shelf life and make the extraction process more efficient. In powder form, the particle surface contact with the solvent will be wider [17]. The organoleptic test was carried out using human senses to evaluate the appearance of avocado seed powder, including smell and color. Avocado seed powder is dark brown (Figure 1) and has a strong but not distinctive aroma. The water content of the dry avocado seed powder was 4.889 ± 0.476, and the ash content was 6.654 ± 0.583.

Figure 1. Dry powder of Avocado Seed

3.2 Avocado Extract

Avocado seed extract was made using the maceration method according to previous research [3],[12]. Although the maceration method has many disadvantages, such as requiring a lot of solvent and a long extraction time, it is still an option used for tannin extraction. The maceration method has simple and low-cost equipment, environmentally friendly characteristics, and is considered safe for extracting thermolabile compounds [18], [19],[20]. The extract yield was calculated in percentage by measuring the weight of the avocado seed extract obtained from each solvent compared to the initial weight of avocado seed powder used in the extraction process. The yield of extract obtained from different solvent concentrations can be seen in Figure 2.

Figure 2 shows that the concentration level of ethanol significantly affects the amount of avocado seed extract yield. Changes in solvent concentration result in changes in polarity. Adding water decreases the concentration of ethanol but increases its polarity. Ethanol 96%, with the lowest polarity, produces the highest extract yield, as much as 47.067 %. This trend occurs probably due to the large number of compounds in the avocado powder that have the same or nearly the same polarity with 96% ethanol.

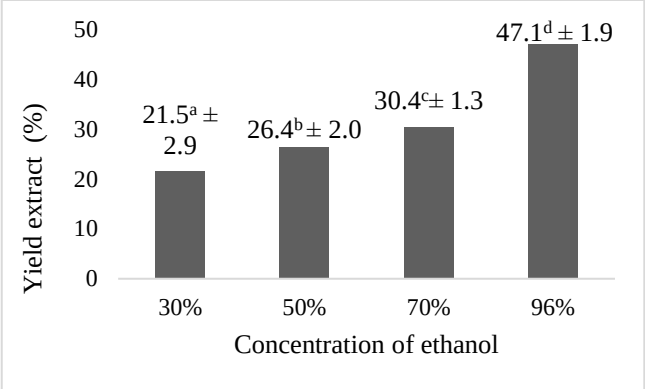

Figure 2. The effect of different concentrations of ethanol on the extract yield of avocado seed

Note: Different superscripts indicate a significant difference between samples ($p<0.05$)

The quality and quantity of compounds extracted by a solvent with the same level of polarity will be more excellent. Avocado extract contains various nutrients such as proteins, crude fibers, carbohydrates, lipids, vitamins, minerals, and numerous phytochemicals [5]. Phytochemical compounds found in avocado extract were flavonoids, polyphenols, tannins, saponins, alkaloids, and steroids [3], [21], and phenolic compounds in the extract were gallic acid, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, benzoic acid, catechin, epigallocatechin, rutinoside-3-O-quercetin, glycoside-3-O-kaempferol, and quercetin [22].

3.3 Tannin Content

The qualitative tannin test in avocado seed powder and extract shows that both samples contain tannins. The qualitative test was carried out by identifying the presence of tannin using ferric chloride ($FeCl_3$). Fe^{3+} ions will form a colored complex when reacting with tannin. The Fe^{3+} ion will act as a central ion that binds three tannin molecules via the O atom at the 4' and 5' dihydroxy positions. The O atom has a single pair of electrons, which can form a coordination bond with the Fe^{3+} ion as the central atom [23].

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The tannin content in avocado seed extract was measured using a UV-Vis spectrophotometer at a wavelength of 745 nm after reacting with a Folin Ciocalteu reagent at room temperature for 90 minutes. Tanin and other Polyphenol compounds will react with Folin Ciocalteu to form a blue-yellow complex compound based on the principles of reduction and oxidation. Molybdenum ions will change from Mo^{6+} to Mo^{5+} because it accept electrons donated by phenolic compounds [24]. This reaction occurs easily in an alkaline environment, so sodium bicarbonate (Na_2CO_3) was added as an alkalizing agent.

Tannin is a polyphenolic compound that can be separated from other forms of polyphenol by applying its ability to precipitate protein [25]. The stabilization of tannin–protein complexes depends on hydrophobic interactions and hydrogen bonding between the hydroxyl groups of tannin and the polar groups of gelatin (amide carbonyl of the peptide backbone) to form a precipitate mass of the tannin-gelatin complex [26]. Therefore, tannin content can be determined by adding gelatin to a separate sample and calculating the difference between total polyphenols in the sample with and without the addition of gelatin. The solvent concentration variation was made by adding water to the ethanol. The addition of water not only changes the concentration of the solvent but also changes its polarity. Water has the highest polarity, so the addition of water will decrease the concentration but increase the polarity [27]. The more water added to the ethanol solvent, the higher the polarity. The extract of avocado seed obtained with different concentrations of ethanol solvent contains different tannin levels, as shown in Figure 3.

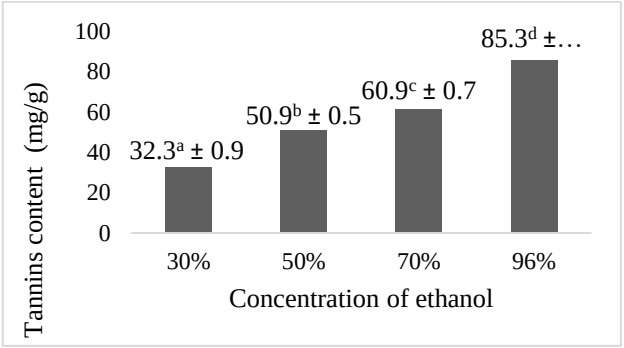

Figure 3. The effect of different concentrations of ethanol on the tannin content of avocado seed extract
Note: Different superscripts indicate a significant difference between samples ($p<0.05$)

The differences in tannin levels in extracts occur due to differences in the polarity of the solvent. The highest tannin content, which is 85.303 mg/g ± 0.322, was obtained from ethanol solvent at a concentration of 96%, and the lowest tannin content, which is 32.273 mg/g ± 0.849, was obtained from ethanol solvent at a concentration of 30%. These results indicate that decreasing solvent polarity could increase its ability to extract high tannin concentrations. This finding aligns with other studies that confirmed that ethanol is a suitable solvent for extracting tannins. The 96% ethanol is most appropriate for extracting tannin from avocado seeds.

4. CONCLUSION

The ethanol polarities significantly affect the extract yield and the tannin content of the avocado seed. The lower the polarity of the solvent, the higher the extract yields. 96% ethanol, which has the lowest polarity, is confirmed as the best solvent compared to 30, 50, and 70% ethanol to extract tannin from avocado seed. The 96% ethanol solvent produces 47.67% extract yield, with tannin content reaching 85.30 mg/g ± 0.32.

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