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The Effect of Solvent Concentration Against Specific and Non Specific Parameters of Standardization: Ethanolic Extract of Papaya Seed *(Carica papaya* Linn.*)*

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Abstract

Papaya plants (*Carica papaya* L.) can be used as traditional medicine. The parts of the plant commonly used as medicine are the seeds and leaves. Assurance of the quality of the extract obtained needs to be carried out by testing specific and non-specific parameters. The aim of the study was to determine the effect of the percentage of ethanol solvent concentration on specific parameters (organoleptic, phytochemical screening, and total phenolic content) and non-specific parameters (moisture content, ash content, and specific gravity) of papaya seed extract (*Carica papaya* L.). This type of laboratory experimental qualitative research was done to determine the effect of the percentage of ethanol solvent concentration (70% and 95%) on specific and non-specific parameters. Specific and non-specific parameter testing is based on work procedures listed in the Indonesian Herbal Pharmacopoeia. The results of testing the specific parameters on organoleptics showed no difference in the extract obtained, which was viscous and dark brown in color with a characteristic odor. Based on qualitative phytochemical screening, 70% ethanol extract contains alkaloids, flavonoids, tannins, and saponins. However, the 95% ethanol extract was only positive for alkaloids, flavonoids, and tannins. The phenolic content test of 70% ethanol and 95% ethanol extracts was 4.31 ± 0.65 mg GAE/g and 2.69 ± 0.41 mg GAE/g, respectively (Sig 0.022 < 0.05). Extract non-specific parameter test results. The ash content test for 70% and 95% ethanol extracts was 9.5% ± 0.21% w/w and 8.5% ± 0.06% w/w (Sig 0.043 < 0.05). The respective water contents were 8.7 ± 0.11% w/w and 9.7 ± 0.26% w/w (Sig 0.046 < 0.05). The specific gravity of each obtained was 0.88 ± 0.006 % g/ml and 0.75 ± 0.006% g/ml (Sig 0.043 < 0.05). The conclusion of this study is that the concentrations of 70% ethanol and 95% ethanol in papaya seed ethanol extract have an effect on specific parameters, namely the measurement of total phenolic content. While the non-specific parameters affect the water content, total ash content, and specific gravity.

Keywords: Papaya Seed, Specific, Non Specific, Standardization of extract

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1 Introduction

Indonesia is a country that has approximately 17,000 islands, consisting of large and small islands. Of the many islands, Indonesia is rich in various plants and fruits, one of which is the papaya plant (*Carica papaya* L.). The papaya plant (*Carica papaya* L.) is a very popular plant among the people, this plant can also live in various tropical areas in Indonesia, has a relatively fast growing time, and is also easy to find in Indonesian regions [1]. Papaya plants (*Carica papaya* L.) can be used as traditional medicine. The parts of the plant that are often used as treatment are the seeds and the leaves [2].

The method of extraction involves removing the substance from the mixture using a suitable solvent to create an extract [3]. To ensure safety, efficacy, and quality while also having the potential to sustain health, address health issues, and restore health, an extract must adhere to extract quality standards. A standardized process can ensure extract quality in accordance with the general standard extract quality criteria since the fulfillment of extract quality requirements and process control are inextricably linked. These parameters are made up of both specific and general parameters [4]. Specific parameters include organoleptic, phytochemical screening and total phenolic content. While the non-specific parameters include the determination of specific gravity, the determination of water content, and the determination of ash content [5].

In the extraction process, many factors affect the quality of the extraction results, including the type of solvent, solvent concentration, extract method, and temperature used in the extract [6]. The effect of solvent concentration on the extraction results was proven in a study where 96% ethanol was said to be the best at producing total phenolic and total flavonoid content compared to 70% ethanol [7]. Then, in another study, the highest levels of flavonoids were found in 70% ethanol extract compared to 50% and 96% ethanol extract [8]. This is because 70% ethanol is a more polar solvent than 96% ethanol and more non-polar than 50% ethanol, so polar flavonoid compounds tend to dissolve more in 70% ethanol. Differences in the concentration of an ethanol solvent affect the degree of polarity of the solvent [9].

Based on the background above, researchers are interested in testing the effect of the percentage of solvent concentration on specific and non-specific parameters of papaya seed extract (*Carica papaya* L). In this study, the solvent chosen was ethanol with a concentration of 70% and 96%. This research is expected to obtain the results of the concentration of ethanol solvent that is capable of producing quality papaya seed extract (*Carica papaya* L.) according to standards.

2 Experimental section

2.1 Raw and Material

The tools used are an analytical balance, stir bar, dark glass jar, flannel cloth, evaporator cup, rotary evaporator, measuring cup, watch glass, spatula, filter paper, water bath, dropping pipette, electric stove, volumetric flask, pycnometer, incubator, Erlenmeyer, aluminum foil, tissue, and UV-Vis spectrophotometry.

The materials used were papaya seed powder, 96% ethanol technical grade, 70% ethanol technical grade, methanol grade pro analysis (Merck), concentrated hydrochloric acid (HCL) 12M (Merck), 2N HCl, mayer reagent (Supelco), dragendroff reagent (Supelco), magnesium (Mg) (Supelco), iron (III) chloride ($FeCl_3$) (Sigma-Aldrich), distilled water, gallic acid ($H_7H_6O_5$) (Sigma-Aldrich), chloralhydrate ($C_2H_3Cl_3O_2$) (Sigma-Aldrich), chloroform ($CHCl_3$), Folin ciocalteu (Sigma-Aldrich), and sodium carbonate (Na_2CO_3) (Merck).

2.2 Extraction of Papaya Seed

The extraction used in this study was maceration extraction. Maceration is carried out by weighing 150 grams of papaya seed powder and then putting it in a maseration jar and soaking it in 70% ethanol. Then proceed with weighing 150 grams of papaya seed powder and putting it in 96% ethanol with a 1:5 ratio of 750 mL each. Soaking was carried out for 5×24 hours, stirring was carried out 5 times, and remaceration was carried out for 2× 24 hours. Then the maceration results were filtered using a flannel cloth to separate the filtrate from the residue. The filtrate obtained was then evaporated using a rotary evaporator and a water bath at 50°C until a thick extract was obtained. [10]. Ethanol is used as a solvent because it is semipolar, making it possible to attract the desired chemical compound with optimal polarity. After the viscous extract is obtained, the yield calculation is carried out. Each extract was concentrated using a rotary evaporator and continued using a water bath at a temperature of no more than 50°C until a thick extract was obtained [11].

2.3 Specific Parameter Testing

Organoleptic, phytochemical screening, and assessment of the total phenolic content are some of the specific aspects of the extract that are examined.

2.3.1 Organoleptic

Organoleptic observations were carried out using the five senses, including shape, smell, color, and taste [12].

2.3.2 Phytochemical Screening

Phytochemical screening tests include the identification of alkaloids, flavonoids, saponins, and tannins as follows:

a. Alkaloids

A total of 0.5 gram of extract was dripped with 2N HCL and then separated into two test tubes. The first tube was added with Mayer's reagent, and the second tube was added with Dragendorf's reagent. Positive alkaloids are formed when a white precipitate is formed when Mayer's reagent is dropped, and an orange precipitate is formed when Dragendorf's reagent is dropped [12].

b. Flavonoids

As much as 0.5 gram of extract was dissolved in 2 ml of 70% ethanol, followed by magnesium powder and 3 drops of concentrated HCL. Positive for the presence of flavonoids if a color change from orange to red is formed [12].

c. Saponin

A total of 0.5 grams of extract was added to 10 ml of boiling water. Then shake vigorously for 10 seconds. Saponin is positive if foam is formed as high as 1–10 cm for 10 minutes. The foam did not disappear after adding one drop of 2N HCl [12].

d. Tannins

A total of 0.5 grams of extract was shaken with hot water until homogeneous, then FeCl3 was added. Positive for tannins if a change in color to green or blue-green is formed and if blue-black contains pyrogallol tannins [12].

2.3.3 Total Phenolic Content

Measurement of the total phenolic content of the ethanol extract of papaya seeds was carried out based on the method listed in the Indonesian herbal pharmacopoeia. The steps taken included making gallic acid mother liquor as a reference standard solution (400 ppm), then making a series of concentrations for standard curves at concentrations of 30, 40, 60, 80, and 100 ppm [13] [12][14]. Up to 10 mg of each extract were weighed, 10 mL of distilled water were used to dissolve it, and the mixture was then homogenized. After adding 5 ml of Folin-Ciocalteu and 300 L of the solution, the mixture was agitated until homogenous and left to stand for 8 minutes. Then, 1.2 mL of Na_2CO_3 solution was added, and the mixture was once again mixed until homogeneous. The solution is then put on hold until operational time (OT). After three iterations, the solution's absorbance was measured at its highest wavelength [15].

2.4 Non-Specific Parameter Testing

2.4.1 Determination of Total Ash Content

As much as 2 g of the extract was carefully placed in a porcelain cup that had been heated and weighed beforehand. Burn slowly with a moderate temperature rise to 800 ± 25 °C until the charcoal runs out. After that, put it in the desiccator and weigh it until the weight is stable [12].

2.4.2 Specific Gravity

After 5% dilution in ethanol, the specific gravity of the extract was measured using a pycnometer. The employed dry pycnometer was calibrated cleanly first, and then its weight and the weight of the freshly boiled water at 25 °C were both measured. A pycnometer that is empty is filled with the liquid extract, which has been heated to around 20 °C. Extra extract is then discarded. The pycnometer is then weighed after being filled to a temperature of 25 °C [12].

2.4.3 Moisture Content

The gravimetric method was used to determine the water content of 1 g of extract, which was weighed in a container that had been prepared. Weighed after 5 hours of drying at 105°C. Then proceed to dry and weigh every hour until the difference between two successive weighings is less than 0.25% [16].

2.5 Data Analysis

The results of the study show the effect of the percentage concentration of 70% ethanol and 96% ethanol extracts of papaya seeds on the standardization of the extract, which consists of extract-specific parameters (organoleptic and phytochemical screening) and non-specific extracts (total ash content, moisture content, and specific gravity), which were then analyzed by descriptive The analysis of total phenolic content was calculated using linear regression, followed by calculating the total phenolic compounds (Equation 1).

$$C = \frac{X \cdot V \cdot Fp}{g}$$

(equation 1)

C = Total Phenolic Content (mg GAE/g)
X = Concentration of Phenolic Content (mg/mL)
V = Extract Volume (mL)
Fp = Dilution Factor
g = Weight of Sample (gram)

Then the data obtained from the calculation of total phenolic content was processed again using a statistical software program with a one-way ANOVA analysis test (analysis of variants) with a level of confidence and the Kruskal Wallis test [17].

3 Results and Discussion

This study used the maceration method by soaking 150 grams of papaya seed powder and then dissolving it with 70% ethanol and 96% ethanol, respectively. Then soaking was carried out for 5×24 hours with 5 times stirring and remaceration for 2×24 hours, then concentrated using a rotary evaporator and continued with a water bath [10].

Table 1. Recovery of Ethanolic extract From Papaya Seed

Solvent	Weight of Simplisia (gram)	Weight of Extract (gram)	Volume of Solvent (ml)	Recovery (%w/w)
Ethanol 70%	150	15.29	750	10.19
Ethanol 96%	150	15.02	750	10.01

Based on table 1, the yields of 70% ethanol extract and 96% ethanol were 15.29% and 15.02%, respectively. These results indicate that 70% ethanol extract yields more than 96% ethanol. 70% ethanol has a greater degree of polarity than 96% ethanol, so the polar group containing the -OH group will be more dissolved in 70% ethanol[14]. The different types of solvents have a significant effect on the yield of the extraction results. Differences in yield can be influenced by differences in extraction methods, length of extraction time, size of simplicia, and different types of solvents [18].

3.1 Spesific Parameter of Papaya seed

The specific parameters of the extract in this study consisted of organoleptic tests, phytochemical screening, and total phenolic content of papaya seed extract.

3.1.1 Organoleptic

Measurements in the organoleptic test of papaya seed extract use the five senses and describe them. The results of the organoleptic test of papaya seed extract can be seen in Table 2.

Table 2. Organoleptic Test Results of Papaya Seed Ethanol Extract

Solvent	Shape	Colour	Odor
Ethanol 70%	Thick	Brown	Specific
Ethanol 96%	Thick	Brown	Specific

Based on the results of the organoleptic test, both the 70% and 96% ethanol extracts had a thick, dark brown color and a characteristic odor. Organoleptically, there was no significant difference between 70% and 96% ethanol through visual observation.

3.1.2 Phytochemical Screening

Phytochemical screening was carried out as a preliminary test to provide an initial qualitative description of the compounds present in the extract. The results of the phytochemical screening of papaya seed ethanol extract can be seen in Table 3.

Table 3. Phytochemical Screening of Ethanolic Extract From Papaya Seed

Phytochemical Testing	Reagent	Result	
		Ethanol 70%	Ethanol 96%
Alkaloids	Mayer	+	+
	Dragendorf	+	+
Flavonoid	Mg + HCl + Alkohol	+	+
Tannins	$FeCl_3$	+	+
Saponin		+	-

Nb : + : Positive -: Negative

A qualitative analysis of the 70% ethanol extract of papaya seeds found alkaloids, flavonoids, tannins, and saponins. While the 96% ethanol extract of papaya seeds contains alkaloids, flavonoids, and tannins, However, it did not show positive saponin content. Analysis of positive alkaloid compounds is indicated by the presence of a white precipitate when Meyer's reagent is added and an orange precipitate with Dragendrof reagent [19]. When Mg and HCl were added, it showed that the sample contained flavonoids with the formation of a red color [20]. The sample was shaken, and a stable foam was formed, indicating that the sample contained saponins. When $FeCl_3$ was added, a green color was formed, which indicated that the sample contained tannins [21].

The effectiveness of the extraction process is influenced by several factors, including solvent concentration, ratio of solvent to simplicia powder, temperature, and extraction time. The positive results contain saponins in the 70% ethanol extract due to their high polarity, which can be seen in their structure. Saponins, in their structure, consist of two parts, namely the glucidic chain (sugar/glycone group) and the fat-soluble structure (aglycone)[22][23]. The screening results in Table 3 show that the ethanol extract of papaya seeds, both 70% and 96% ethanol, contains phenolic compounds. Phenolic compounds are secondary metabolites obtained from the shimicate or polyketide pathways that play a role in the ability of a plant to scavenge free radicals[24].

3.1.3 Total Phenolic Content

The measurement of the total phenolic content of the ethanol extract of papaya seeds was carried out using the method listed in the Indonesian Herbal Pharmacopoeia. The method used is the Folin-Ciocalteu method. The Folin-Ciocalteu method is a reaction based on electron transfer that measures the reductive capacity of an antioxidant. The -OH group present in phenolic compounds has the ability to reduce phosphomolybdate-phosphotungstate compounds from the Folin ciocalteu reagent, which will form a blue molybdenum-tungstate complex. The number of phenolic ions that will reduce heteropoly acids is determined by the size of the concentration. The greater the concentration, the more phenolic ions will reduce heteropoly acids, so the resulting blue color will be more concentrated [25]. This method has the advantage of being fast and easy and does not require expensive reagents or difficult instrumentation [26].

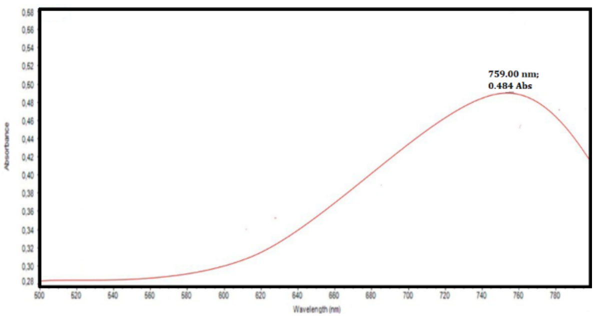

Figure 1. Maximum Wavelength of Gallic Acid

The levels of total phenolic compounds can be seen in Table 5. The average total phenolic compounds of each extract were 4.31 ± 0.65 mg GAE/g for 70% ethanol extract and 2.69 ± 0.41 mg GAE/g for 96% ethanol extract. Based on the results of the analysis with multiple correlations, it shows that there is a relationship between solvent concentration and total phenolic content with a significance value of 0.022 (sig <0.05), which means that there is a significant difference between the total phenolic content in 70% ethanol extract and 96% ethanol extract. The 70% ethanol extract has a higher total phenolic content than the 96% ethanol extract. Solvent concentration is one of the important factors in determining the compounds present in medicinal plants. In this case, the 70% ethanol extract has a greater level of polarity when compared to the 96% ethanol. This difference in polarity has an influence on the solubility of the chemical compounds present in the sample. The choice of solvent polarity is one of the most important factors in determining total phenolics, flavonoids, and the extraction of other bioactive compounds. These results are comparable to a study conducted by Boussoussa et al. (2014) which states that the difference in solvent polarity affects the total phenolic component to be taken [27].

Table 4. Total Phenolic Content of Ethanolic Extract From Papaya Seed

Extract	Replication	Total Phenolic Content (mg GAE/g)	Mean ± SD (mg GAE/g)	*p value* < 0,05
Ethanol 70%	1	3,6	4,31±0,65	0.022*
	2	4,3		
	3	4,9		
Ethanol 96%	1	2,3	2,69±0,41	
	2	2,5		
	3	3,1		

*There is a significant difference with a 95% confidence level (p < 0.05) with Anova Test

3.2 Non Parameter Specific of Ethanol Extract From Papaya seed

The non-specific parameters of the extract in this study consisted of total ash content, moisture content, and specific gravity.

3.2.1 Total Ash Content

Table 5. Total Ash Content of Ethanol Extract From Papaya Seed

Extract	Replication	Total Ash Content (% b/b)	Mean ± SD (%b/b)	*p value* < 0.05
Ethanol 70%	1	9.3%	9.5% ± 0.21	0.043*
	2	9.6%		
	3	9.7%		
Ethanol 95%	1	8.4%	8.5% ± 0.06	
	2	8.5%		
	3	8.5%		

*There is a significant difference with a 95% confidence level (p<0.05) With Kruskal Wallis Test

The results of examining the total ash content of the papaya seed extract are shown in Table 6. The total ash content test was carried out based on what was stated in the Indonesian Herbal Pharmacopoeia [28]. The procedure for determining the ash content is that the material is heated to a certain temperature, which will cause the organic compounds and their derivatives to be destroyed and evaporate so that the mineral and inorganic elements are left behind. Ash content is a parameter in the standardization of herbal medicines that describes the internal and external mineral content originating from the initial process until the extract is formed. The lower the ash content, the higher the level of purity of the extract. Based on Table 6, the total ash content of the 70% ethanol extract has a higher value (9.5% ± 0.21% w/w) compared to the 96% ethanol extract (8.5% ± 0.06 % w/b). Statistical analysis showed that there was a significant difference in the average ash content obtained, with a significance value of 0.043 (Sig < 0.05).

3.2.2 Water Content

The results of the research on the water content of papaya seed extract are shown in Table 7. These results are in accordance with the requirements in the Indonesian herbal pharmacopoeia, which should not be more than 10% [29]. A measurement of water content was carried out to determine the water content of the extract.

Table 6. Moisture Content of Ethanol Extract from Papaya Seed

Extract	Replication	Moisture Content (% w/w)	Mean ± SD (% w/w)	Requirement (% w/w)	*p value* <0.05
Ethanol 70%	1	8.6	8.7 ± 0.11	≤10%	0.046*
	2	8.8			
	3	8.8			
Ethanol 96%	1	9.4	9.7 ± 0.26		
	2	9.8			
	3	9.9			

* There is a significant difference with a 95% confidence level (p<0.05) With Kruskal Wallis Test

The water content obtained in 70% ethanol extract and 96% ethanol extract was 8.7 ± 0.11 % w/w and 9.7 ± 0.26 % w/w, respectively. These results indicate that the water content between the 70% ethanol extract and 96% ethanol has a significant difference (sig. 0.046 <0.05). The water content in 96% ethanol is greater than that in 70% ethanol extract. However, both are within the required range of water content, namely ≤10%. The size of the water content can be influenced by several factors, one of which is the process of extracting or absorbing moisture from the air. In addition, the extraction method can also affect the water content of the ethanol extract of papaya seeds. Previous research stated that the water content makes a significant difference between the maceration method and soxhletation. The maceration method provides a greater water content compared to soxhletation [30].

3.2.3 Specific Gravity

The results of determining the specific gravity of papaya seed extract are shown in Table 8. Specific gravity is the mass per unit volume at a certain room temperature, which is determined with a special tool such as a pycnometer. This test aims to provide a mass limit per unit volume, which is a special parameter for liquid extracts and concentrated extracts that can still be poured [31].

Table 7. Specific Gravity of Ethanol Extract From Papaya Seed

Extract	Replication	Specific Gravity (g/ml)	Mean ± SD (g/ml)	*p value* <0.05
Ethanol 70%	1	0.88	0.88 ± 0.006	0.043*
	2	0.88		
	3	0.87		
Ethanol 96%	1	0.75	0.75 ± 0.006	
	2	0.74		
	3	0.75		

* There is a significant difference with a 95% confidence level (p<0.05) With Kruskal Wallis Test

Based on the results of the tests that have been carried out, The average specific gravity of 70% ethanol extract and 96% ethanol extract was, respectively, 0.88 ± 0.006 g/ml and 0.75 ± 0.006 g/ml with a significance value of 0.043 (sig < 0.05). The results showed that the highest specific gravity value was in the type of solvent, namely 70% ethanol solvent. This is presumably due to the presence of a mixture of water and ethanol in 70% ethanol, so that the specific gravity value is close to the specific gravity value of water, namely 1 g/mL.

4 Conclusions

The conclusion of this study is that the concentrations of 70% and 95% ethanol in papaya seed ethanol extract have an effect on specific parameters, namely the measurement of total phenolic content. While the non-specific parameters affect the water content, total ash content, and specific gravity.

5 Declarations

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5.2 Author Contributions

RW: Collecting and analyzing the data; EM: Preparing the manuscript; MA: Analyzing the data and examining the final manuscript; AF: Analyzing the data.

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5.4 Conflicts of Interest

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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