ORIGINAL RESEARCH

ANTIOXIDANT ACTIVITY ASSAY ETANOL EXTRACT OF PEPAYA LEAVES (*Carica Papaya* L.) FROM ACEH BESAR

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Abstract

The leaves, fruits, and roots of papaya (Carica papaya L) are mostly used as traditional medicine. Papaya leaves are used by the community to treat diarrhoea, acne, appetite, and digestive problems. Papaya leaf extract has potential as an antioxidant derived from secondary metabolites, namely alkaloid compounds that produce a bitter taste in papaya leaves. This study was conducted to look at antioxidant compounds by conducting antioxidant activity tests. The results of papaya leaf research contain active compounds flavonoids, alkaloids, and steroids. Research conducted on the level of colour brightness in papaya leaves is different, namely 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. Papaya leaf extract has a power that is included in the strong category with the IC_{50} value obtained 8.71 marked by the results of calculations less than 100. Antioxidant activity testing using the DPPH method papaya leaf extract positively contains antioxidant compounds and can be utilised as a natural antioxidant.

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I. INTRODUCTION

In the world there are about 40,000 species of plants and 30,000 of them are in Indonesia. Indonesia is a country where various types of plants are found). Indonesia is ranked second in the category of countries that have the largest biodiversity in the world. Various kinds of plants that thrive because Indonesia is on the equator and surrounded by volcanoes [1].

Plants that grow in Indonesia have both secondary and primary contents that have proven efficacy as a treatment [2]. According to PT Sido Muncul (2015), around 7,500 medicinal plants have known properties, but only 1,200 medicinal plants are used as raw materials for herbal medicines. The utilisation of plants as medicine is one of the community efforts carried out from generation to generation and meets the health needs of the community [3].

Figure 1. Aceh Besar papaya leaves (Personal Publication) (2023)

Papaya leaf (**Figure 1**) is one plant that can be utilised as an herbal medicine. Papaya leaves are single leaves, large, fingered, serrated and have parts of the petiole and leaf blade. The tip of the papaya leaf is tapered, the petiole is long and hollow. The surface of papaya leaves is smooth and slightly shiny. The arrangement of papaya leaf bones is fingered [4]. Papaya leaves are usually processed by the community into vegetables, herbs, herbal medicines and so on. In processing papaya leaves can be boiled or mashed and extracted. Among the people are not too fond of consuming papaya leaves because of their bitter taste. Papaya leaves are rich in nutrients that are very beneficial for the human body, including containing high vitamin C, which reaches 140 mg [5].

In this study, researchers tested the antioxidants contained in the ethanol extract of papaya leaves. Antioxidants can act as free radical scavengers in the body. The presence of free radicals in the body will cause oxidative damage that triggers degenerative diseases such as hypertension, diabetes, obesity, and heart disease [6]. The human body does not have a large supply of antioxidants, so additional antioxidants are needed to protect the body to inhibit free radicals [7]

METHODOLOGY

Materials

The tools used in this research are glassware, blender, filter paper, analytical balance, waterbath, oven, desiccator, bunsen, rotary evaporator, infrared spectroscopy (FTIR Spectrophotometer 8300/8700). The materials used were papaya leaves, ethanol, aquadest, dragendroff, ammonia, chloroform, filtrate, sulfuric acid, concentrated HCl, magnesium, FeCl 1%, anhydrous acetic acid, and concentrated sulfuric acid.

Preparation of sample

The samples used in this study were old male papaya leaves picked from papaya plantations on Jln. Teuku Chik Ditiro, Gampoeng Mureu Bung Ue, Kec. Indra Puri, Kab. Aceh Besar.

Determination of plants

Determination of papaya leaf plants from Jln. Teuku Chik Ditiro, Gampoeng Mureu Bung Ue, Kec. Indra Puri, Kab. Aceh Besar. Performed in the laboratory of MIPA Biology Syiah Kuala University.

Determination of water content

Porcelain cup is heated in an oven at 105oC for 1 hour, then the cup is cooled in a desiccator for 1 hour, 3-5 grams of sample is weighed and then put into the cup, then close and weigh. Samples in an open state in the oven are heated at 105 oC for 3 hours. Then the weighing bottle is closed and moved into a desiccator to cool for 1-2 hours then weigh. Then calculate the water content with :

$$water\ content = \frac{(\mathrm{a} + \mathrm{b}) - \mathrm{c}}{\mathrm{b}}\ x\ 100\%$$

Description:
a = weight of porcelain cup after drying (g)
b = sample weight (g)
c = weight of cup + contents (g)

Determination of ask content

A sample of 3 grams was weighed carefully in a cup (W). The cup containing the sample was heated over a small bunsen flame until it became charcoal, then ignited in a furnace at 600 oC until it was white or gray for 3 hours. After the charring stage, the cup was cooled in a dexicator for 30 minutes and then weighed. Then calculate the ash content with:

$$Ash\ Content = \frac{\mathrm{W2} - \mathrm{W1}}{\mathrm{W} - \mathrm{W1}}\ x\ 100\%$$

Description:
W = weight of cup and sample weight (g)
W1 = empty cup weight (g)
W2 = weight of cup and ash (g)

Ekstraction of papaya leaf

Extraction was carried out by maceration method using 95% ethanol solvent as much as 1 liter and 200 g of papaya leaves. The marinade was allowed to stand for 24-48 hours with stirring every 2 hours, then filtered. Repeated three times until the extraction liquid looks clear, the next way is the separation process using a Rotary evaporator.

Phytochemical Test

Phytochemistry is a stage in determining the class of compounds contained in papaya leaves, this method is carried out by looking at color testing reactions,

some of the phytochemical tests carried out are alkaloids, flavonoids, tannins, saponins, and steroids / triterpenoids.

FT-IR

FTIR (*Fourier Transform Infrared*) FTIR is a method that uses infrared spectroscopy, some of the infrared radiation is absorbed by the sample and some is transmitted. FTIR aims to determine the process of mixing whether physically or chemically, the sample is placed into the set holder, then the corresponding spectrum is sought. The result will be a diffractogram of the relationship between wave number and intensity.

Antioxidant assay

The antioxidant activity test stage of papaya leaf ethanol extract by DPPH radical scavenging method refers to the procedure of Brand [8].

RESULTS AND DISCUSSION

Determination of sample

Determination of papaya leaves from papaya plantations on Jln. Teuku Chik Ditiro, Gampoeng Mureu Bung Ue, Kec. Indra Puri, Kab. Aceh Besar conducted in the laboratory of MIPA Biology Syiah Kuala University are Kingdom : Plantae; Subkingdom : Tracheobionta; Super Divisi : Spermatophyta; Divisi : Magnoliophyta; Kelas : Magnoliopsida; Sub Kelas : Dilleniidae; Ordo : Brassicales; Famili : Caricaceae; Genus : *Carica L.;* Spesies : *Carica papaya L.*. In general, journal papers will contain three-seven figures and tables. The same data cannot be presented as tables and figures.

Water Content

Table 1. Water content analysis data of papaya leaves

Sample Repeatability	Weight of empty	Sample	Weight of cup+content	% Water content
P1	40,84	2,01	42,84	0,5%
P2	45,88	2,03	47,90	0,5%
P3	58,58	2,02	60,58	0,5%
Avarage				0,5 %

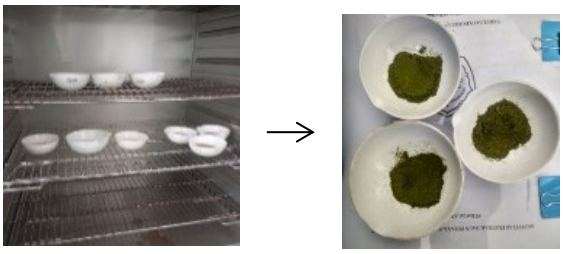

Figure 2. Determination of water content: (a) The process of heating the sample using an oven, (b) Results of moisture content analysis after heating

Table 1 shows that the moisture content of papaya leaves used as samples is 0.5%. Determination of moisture content was carried out three times using the thermogravimetric method (Figure 2). Determination of moisture content aims to determine the moisture content and storage resistance of the sample. A moisture

content of less than 10% indicates that the sample used can be stored for a relatively long time[9]. The data table 4 shows that the papaya samples used in this study can be stored for a relatively long time. the lower the water content, the slower the growth of microorganisms proliferates, so that the decay process will be slower [10].

Ash content

Table 2. Ash content analysis data of papaya leaves

Sample Repeatability	Weight of empty	Sample	Weight of cup+content	% Ash content
P1	41,57	2,01	41,81	11,9%
P2	39,17	2,05	39,40	11,2%
P3	40,80	2,05	41,06	12,7%
Avarage				11,9%

The results of the analysis of total papaya leaf ash content showed that the ash content test did not meet the standard requirements that have been set. The standard requirement for total ash content found in MMI ash content should not exceed 8%. The total ash content in papaya leaves is very high. The high ash content in papaya leaves indicates the high content of internal minerals in the papaya leaves. The higher the ash content, the higher the mineral content in the leaves [11].

Extrac of papaya leaf

The papaya leaf extraction process is carried out by maceration method. Soaking the sample will break the cell wall and membrane due to the difference in pressure inside and outside the cell, so that the secondary metabolites in the cytoplasm will be dissolved with the solvent and the extraction of compounds will be perfect, the period of soaking can be adjusted [12]. The maceration process in this study used ethanol solvent and was allowed to stand for 2 days.

Figure 3. Extraction process of papaya leaves using maceration method

Phytochemical assay

Phytochemistry is one of the steps that aims to reveal the potential of resources contained in medicinal plants as antibiotics, antioxidants and anti-cancer. Chemical compounds contained in plants include secondary metabolite compounds. Secondary metabolites accumulate in small amounts in plant cells. Based on their biosynthesis, secondary metabolite compounds are divided into three main groups, namely, flavonoids, alkaloids, and steroids [13].

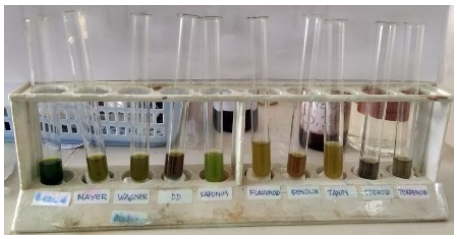

Figure 4. Phytochemical assay results of papaya leaf extracts papaya

Table 2. Phytochemical Content of Papaya Leaf Methanol Extract

Phytochemical	Reagen	Result	Summary
Alkaloids	Mayer	No white precipitate formed	-
	Wagner	No brown precipitate formed	-
	Dragendorf	Formed orange precipitate	+
Flavonoids	Mg+HCl	Formed red color	+
Tanins	NaCl+glatin	No green/bluish color formed	-
Saponins	Aquadest	No froth/foam formed	-
Steroid	Liebermann-Burchad	Forms a green/bluish color	+
Triterpenoids	Liebermann-Burchad	No red color formed	-

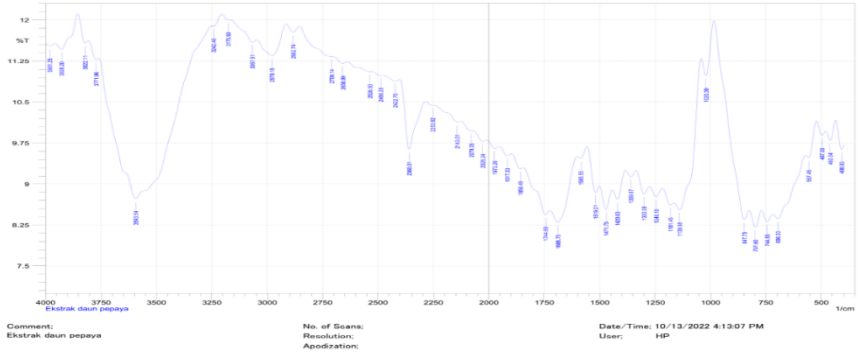

Figure 5. FT-IR spectrum of ethanol extract of papaya

The IR spectrum showed an absorption at wave number 3593.54 cm-1 of OH. The wave number absorption of 2979.18 cm^{-1} and 2882.74 cm^{-1} is CH-aliphatic [14]. C=N was also detected at the absorption of wave number 2360.01 cm-1 (Rakhmawati and Ona, 2019). The absorption at wave number 1744.69 cm-1 shows C=O. The existence of C=C bonds is shown in the absorption of the wave number region 1688.75 cm-1. The absorption at wave number 1359.78 cm-1 indicates CH3- while -CO- is shown at wave number 1245.10 cm-1. The absorption at wave number 1020.39 cm-1 indicates C-N. The infrared spectrum data shows that papaya leaf ethanol extract has OH-, CH- aliphatic, C=N, C-O, C=C, CH3-, CO-, and C-N functional groups [14].

Antioxidant Assay

Spectrophotometer measurement of antioxidant activity is done at a wavelength of 517 nm. The method that is often used to capture free radicals is 1,1-diphenyl-2-picrihydazyl (DPPH) (Prayoga, 2013). In this study, the antioxidant activity of papaya leaf extract was measured using the DPPH method. Figure 6 shows the DPPH test results of papaya leaf extract. The control used is ascorbic

acid. Vitamin C or ascorbic acid (C6H8O6) is known as the largest source of antioxidants found in food and beverages. Vitamin C is a water-soluble antioxidant [7].

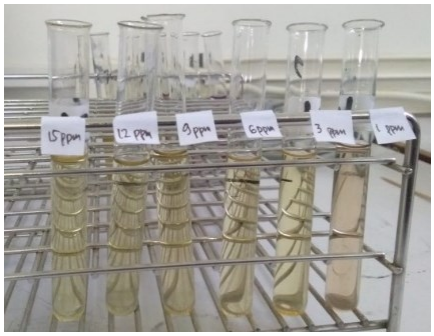

Figure 6. Antioxidant Assay results of papaya extracts

Table 3 and **Figure 6** show that the concentration of 100 ppm decreased absorbance, namely at P1 0.652, P2 0.652 and P3 0.654 absorbance compared to 10 ppm has a high absorbance value. The antioxidant activity of free radical capture can be known from the decrease in absorbance. Comparison between papaya leaf antioxidants with vitamin C can be seen in the percentage results of absorbance.

Figures 6 and **7** show that the comparison between papaya leaves and vitamin C is seen from the amount of ppm concentration. Papaya leaves were tested with a higher concentration than vitamin C, so the ability of vitamin C activity is more accurate than the activity of papaya leaves. This proves that papaya leaves have antioxidant abilities and are very well utilized as a natural antioxidant material.

Table 3. DPPH absorbance of vitamin C

No	Concentrations (ppm)	Control (Abs)			% Inhibisi	IC_{50}
		P1	P2	P3		
		0,943	0,938	0,936		
1	1	0,198	0,198	0,197	79,948	
2	3	0,086	0,087	0,086	90,805	
3	6	0,083	0,082	0,082	91,231	
4	9	0,078	0,080	0,079	91,586	2,35
5	12	0,068	0,069	0,070	92,650	
6	15	0,060	0,060	0,060	93,609	

Vitamin C	
Slope	Intersep
0,753	84,032

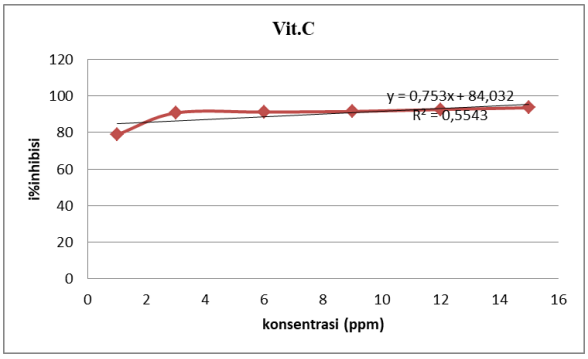

Figure 7. Antioxidant Activity Graph of Vitamin C Extract

The antioxidant activity test in this study uses the IC_{50} (inhibition concentration) parameter to interpret the test results with the DPPH test method. IC_{50} is the number of extract concentrations (ppm) that inhibit the oxidation process by 50% of free radical activity [15]. IC_{50} can be determined from the linear regression curve between papaya leaves and comparator vitamin C at various test concentrations versus % inhibition[16]. The smaller the IC_{50} value, the higher the antioxidant activity. Antioxidant compounds can be said to be very strong if the IC_{50} value is less than 50 ppm, strong for IC_{50} values of 50-100 ppm, moderate values of 100-150 ppm, and weak if the IC50 value is 151-200 ppm [17].

The IC_{50} value is obtained from several stages, namely calculating the log concentration value and the probit value for each percentage of DPPH free radical inhibiting activity of papaya leaf extract and vitamin C. Furthermore, connecting the two data from the calculations obtained in 1 whole graph, where the log concentration value is used as the X axis and the probit value is used as the Y axis. As in this case can be seen in **Figures 6** and 7**.**

Based on Figure 7. a linear regression equation Y = 0.2535x + 6.2571 for papaya leaf extract, Y = 0.753x + 84.032 for vitamin C can be obtained in **Figure 7**. The IC_{50} value of papaya leaf and vitamin C is determined based on the regression equation obtained. The final calculation shows the IC_{50} value of papaya leaf extract has an IC_{50} of 8.71043 ppm. Meanwhile, the IC_{50} value of vitamin C had an IC_{50} of 2.35486 ppm. This shows that the ability to capture free radicals of papaya leaf extract is included in the strong category because the IC_{50} value of the calculation results is less than 100 ppm.

Conclusion

The results of the research that has been done can be concluded that papaya leaves contain 0.5% moisture content so that they can be utilized in a longer period of time and contain high minerals because they have an ash content exceeding 8%. Papaya leaves contain active compounds flavonoids, alkaloids, and steroids. The antioxidant activity test of papaya leaves has a power that is included in the strong category with an IC_{50} value obtained of 8.71 ppm characterized by the calculation results of less than 100 ppm.

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