Jurnal Eduhealth
Volume 16, Number 04 2025 DOI 10.54209/eduhealth.v16i04
ESSN 2808-4608 (Online)
https://ejournal.seaninstitute.or.id/index.php/healt

Antioxidant Testing of Solid Soap Preparations Containing Ethanol Extract from California Papaya Leaves (Carica Papaya L.)

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Article Info	ABSTRACT
Keywords: Papaya Leaves, Solid Soap Preparation, Antioxidant.	Air pollution is a global environmental problem that is getting worse due to the mixing of natural atmospheric components with pollutants from human and natural activities. One of the main causes of air pollution in Southwest Papua is the increase in the number of motor vehicles, especially in cities such as Sorong, which also contributes to increased pollutant emissions. One plant that has antioxidant activity is papaya, which contains flavonoids, tannins, and alkaloids. Papaya (Carica papaya L) is a fruit from the Caricaceae family. This study aims to test and determine the antioxidant activity in solid soap preparations and preparation concentrations. This study uses two concentrations, namely 8% and 24%. Positive control used quercetin as a comparison. The results of the study conducted on 8% concentration solid soap preparations showed an IC50 value of 37.77, while 24% concentration preparations showed an IC50 value of 36.81. From the results obtained, it can be concluded that California papaya leaf ethanol extract solid soap preparations have high antioxidant activity When compared to the positive control quercetin, the IC_{50} value of quercetin is 0.93 ppm, meaning that the positive control quercetin has stronger antioxidant activity.
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INTRODUCTION

Air pollution is a serious environmental problem that is increasing in various parts of the world. Air pollution occurs when natural components of the atmosphere, such as oxygen and nitrogen, mix with pollutants produced by human and natural activities (Asyhar & Minarni, 2023). The WHO notes that around 7 million people die each year from the direct and indirect effects of air pollution (Rahmawati & Pratama, 2023).

One of the main causes of air pollution in Southwest Papua is the increase in the number of motor vehicles, especially in cities such as Sorong, which also contributes to increased pollutant emissions. Emissions from vehicles include carbon monoxide (CO), hydrocarbons, and particulates.

Fine particulate matter, adding to the air pollution burden in this region (Statistics, 2022). Research conducted by Suhartini et al. (2021) revealed that air quality in the city of

Sorong has deteriorated significantly due to human activities, particularly from the transportation and industrial sectors (Suhartini et al., 2021).

Free radicals are highly unstable and reactive molecules, which can cause serious damage to the body.nOxidative stress occurs when free radicals in the body are produced in quantities that exceed the ability of antioxidants to neutralize them. This oxidative stress can damage important cellular components, including DNA, proteins, and lipids, which can potentially lead to various diseases (Dominica et al., 2019).

Antioxidants are substances that function to inhibit the oxidation process in other molecules. The oxidation process that occurs in the body can trigger the formation of free radicals, which have the potential to damage cells and play a role in the development of various chronic diseases such as heart disease, cancer, and premature aging (Haerani et al., 2018). The human body can produce antioxidants naturally, but the amount is often insufficient. Antioxidants obtained from external sources are called exogenous antioxidants, which are divided into two types based on their origin, namely natural antioxidants and artificial (synthetic) antioxidants (Hani & Milanda, 2016). Natural antioxidants are compounds that are naturally contained in various foods, especially fruits, grains, vegetables, nuts, and various spices (Silvia et al., 2016).

Previous research by Irma et al. in 2021 revealed that papaya plants (Carica papaya L.) have antioxidant properties. Among all parts of the plant, papaya peel showed the highest antioxidant activity, with an IC50 value of 13.769 µg/mL in its ethanol extract (Santi et al., 2021). Based on this explanation, the researcher intends to make a solid soap preparation from California papaya leaf ethanol extract as an antioxidant, given that there has been no research related to solid soap made from California papaya leaf ethanol extract.

METHODS

Tools and Materials

The tools used are stirring rods, funnels, measuring cups, beaker glasses, blenders, hand mixers, filter paper, knives, soap molds, containers, digital scales, masks, lab coats, gloves, glass jars, trays, glass funnels, aluminum foil, rotary evaporators, stoves, baking pans, hotplate, dropper pipette, micropipette, aluminum foil, water bath, vial, ruler, petri dish, pH meter, UV-Vis spectrophotometer. The materials used in this study were ethanol extract of papaya leaves (Carica papaya L.), sodium hydroxide (NaOH), coconut oil, 1-diphenyl-2-picrylhydrazil (DPPH), distilled water, glycerin, 96% ethanol, P.A ethanol, and quercetin.

Method

Sample Extraction Process

The sample extraction process uses the maceration method. Fresh California papaya leaves are collected, cleaned with running water, and dried in an oven at 40°C. After drying, the leaves are reduced in size using a blender until they become powder. A total of 513 grams of dry powder is placed in a maceration vessel and macerated for 3 days with 2L of 96% ethanol solvent, after which it is filtered using filter paper to obtain filtrate 1 and residue 1. The residue is re-soaked using 96% ethanol solvent with the same amount of solvent for 1 day (stirring occasionally), then filtered to obtain filtrate 2 and residue. Next, filtrate 1 and 2 are combined, then evaporated with a water bath until a thick extract of 30 grams is obtained.

Phytochemical Screening Test for Flavonoid Compounds

One milliliter of California papaya leaf sample was placed in a test tube, and 0.2 ml of lead II acetate was added. Flavonoids are indicated by the formation of a white precipitate.

Tannin Compounds

One milliliter of California papaya leaf sample was placed in a test tube, and 0.2 ml of FeCl3 reagent was added. Tannin is indicated by the formation of a blue/greenish-black color.

Alkaloid Compounds

A 1 ml sample of California papaya leaves was placed in a test tube and 0.2 ml of the first reagent, Mayer's reagent, was added. In Mayer's reagent, alkaloids are indicated by the formation of a yellow precipitate. In 0.2 ml of Bouchardat's reagent added to the test tube, alkaloids are indicated by the formation of a black-brown precipitate.

Saponin Compounds

One milliliter of California papaya leaf sample was placed in a test tube and HCl2N reagent was added. Saponin is indicated by the formation of foam that lasts for several minutes (Indonesian Ministry of Health, 1995).

Formula

Table 1. Formulation of California Papaya Leaf Extract (*Carica papaya* L.)

Material	Concentration	
	F1	FII
California papaya leaf extract	8 g	24 g
Coconut Oil	50 ml	50 ml
NaOH	30 g	30 gr
Glycerin	20 g	20 gr
Aquadest	95 ADD	95 ADD

Solid Soap Making

The method for making solid soap is to weigh all the ingredients according to the formula, then dissolve NaOH in distilled water and mix it into heated coconut oil. Add glycerin and 8% and 24% concentrated extracts and stir until it reaches the trace stage, which is when the mixture begins to thicken and harden. Pour the soap mixture into molds and let it set for one week. After that, remove it from the molds and package the soap (Dalimunthe et al., 2024).

Free Radical Activity Testing DPPH

A 0.4 mM DPPH solution was prepared by taking 1 ml of DPPH solution and placing it in a 5 ml volumetric flask covered with aluminum foil. The volume of the solution was then adjusted with p.a. ethanol to the mark. The solution was then left to stand for 30 minutes (Erawati et al., 2024).

Testing the Antioxidant Activity of Quercetin

A 100 ppm quercetin stock solution was taken in amounts of 0.005 ml, 0.01 ml, 0.015 ml, 0.02 ml, and 0.025 ml, then placed in a 5 ml measuring flask wrapped in aluminum foil. Each was mixed with 1 ml of 0.4 mM DPPH solution, then ethanol p.a. was added until the marked volume was reached, resulting in solutions with final concentrations of 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, and 0.5 ppm. The solutions were tightly sealed and left for 30 minutes. Next, the absorbance values were measured using ultraviolet-visible spectrophotometry at a wavelength of 500 nm (Erawati et al., 2024).

Antioxidant Activity Testing of Preparations

To measure the antioxidant activity of solid soap preparations containing papaya leaf extract, a 1000 ppm stock solution was used by taking volumes of extract of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml, respectively. The solution was placed in a 5 ml measuring flask covered with aluminum foil, then 1.0 ml of 0.4 mM DPPH solution was added and diluted with p.a ethanol to a volume of 5 ml, resulting in final concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The mixture was left for 30 minutes, then the absorbance was measured using ultraviolet-visible spectrophotometry at a wavelength of 500 nm (Muslihin et al., 2022).

RESULTS AND DISCUSSION

Extraction Results

Sample extraction using the maceration method. First, fresh green California papaya leaves were collected, washed with running water, and dried in an oven at 50°C. After drying, the leaves were reduced in size using a blender until they became powder. A total of 513 grams of dry powder was placed in a maceration vessel and macerated for 3 days with 2L of 96% ethanol solvent, after which it was filtered using filter paper to obtain filtrate 1 and residue 1. The residue is re-soaked using 96% ethanol solvent with the same amount of solvent for 1 day (stirring occasionally), then filtered to obtain filtrate 2 and residue. Next, filtrate 1 and 2 are combined in a solvent ratio of 1:4 (513 g: 2000 ml). Evaporation was then carried out using a water bath at a temperature of 40°C to obtain a thick extract. Based on the maceration results, 30 grams of thick extract was obtained from 513 grams of dry simplisia, resulting in an extract yield of 5.8%.

Phytochemical Screening Results

In this study, phytochemical screening tests were conducted to identify the compounds contained in California papaya leaf extract (Carica papaya L.), targeting the active compounds flavonoids, tannins, alkaloids, and saponins. This testing aimed to confirm that these compounds are expected to influence the antioxidant activity of solid soap preparations. The test results showed that California papaya leaf ethanol extract contained flavonoids, tannins, and alkaloids. The test results can be seen in Table 2.

Flavonoids are a group of plant polyphenols that play an important role as antioxidants through several main mechanisms, namely, directly capturing free radicals through hydrogen atom or electron transfer (H- atom transfer/single electron transfer), binding transition metal ions (metal chelation) thereby preventing the formation of radicals through

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the Fenton reaction, and regulating endogenous antioxidant cellular pathways (Panche et al., 2016). The antioxidant activity of tannins mainly stems from their ability to capture free radicals through the donation of hydrogen atoms or electrons from phenolic hydroxyl groups, thereby neutralizing free radicals such as hydroxyl radicals (OH) and superoxide (O_2^-) that have the potential to damage cells (Serrano et al., 2019). Alkaloids are known to have antioxidant properties. Their mechanism of action involves the capture of free radicals, where functional groups in the alkaloid structure can donate electrons or hydrogen atoms to neutralize reactive radicals such as hydroxyl radicals, superoxide radicals, and peroxyl radicals (Santos et al., 2019).

Table 2. Results of phytochemical screening tests of California papaya leaf extract (*Carica Papaya* L.)

Testing	Reagent	Observation	Description
Flavonoids	Timbal II asetat	White sediment	+
Tanins	FeCL3	Dark green	+
Alkaloids	Mayer	Yellow deposits	+
	Bouchardat	Dark chocolate deposits	+
Saponins	HCL 2N	Absence of foam	-

Table 3. Results of Quercetin Antioxidant Activity Test

Concentration (ppm)	Absorbance	%Inhibisi	IC50 (ppm)
DPPH	0,560		
0,1	0,498	11,07	
0,2	0,483	13,75	0,93
0,3	0,450	19,64	
0,4	0,413	26,25	
0,5	0,400	28,57	

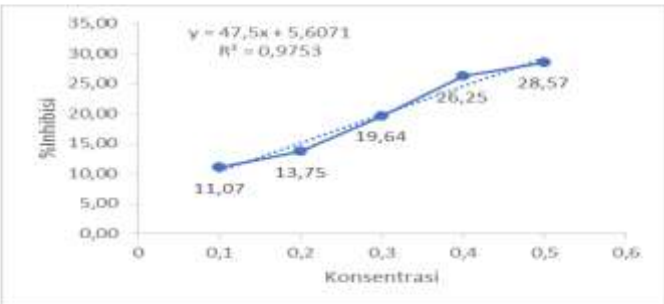

Figure 1. Graph showing the relationship between % inhibition of quercetin and concentration.

Quercetin is one of the most biologically active flavonoids found in papaya leaves. Quercetin has very high antioxidant properties due to its structure, which is rich in hydroxyl groups (–OH), which can donate protons to neutralize free radicals. In an antioxidant test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, an IC_{50} value of 0.93 ppm indicates that quercetin is a very strong antioxidant compound. This means that only 0.93 ppm of quercetin is needed to reduce the activity of DPPH free radicals by 50% (Kumar, et al., 2013).

Table 4. Antioxidant Test Table for Solid Soap Preparation of 8% Concentration Ethanol Extract of Papaya Leaves

concentration (ppm)	Absorbance	%Inhibisi	IC50 (ppm)
DPPH	0,560		
10	0,499	10,89	
20	0,398	28,93	37,77
30	0,346	38,39	
40	0,268	52,14	
50	0,196	65,00	

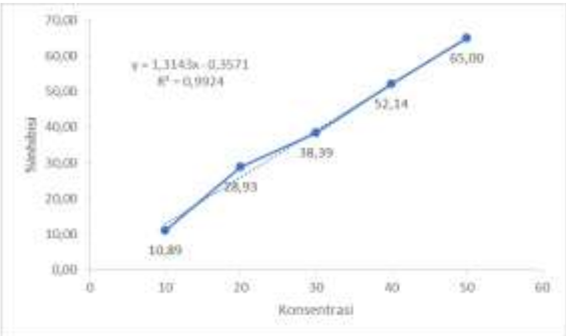

Figure 1. Graph of the Average Inhibition Relationship of Samples at a Concentration of 8%

Antioxidant test results for solid soap formulated using papaya leaf extract produced an IC_{50} value of 37.77 ppm. Based on the antioxidant activity criteria according to Molyneux (2004), this value is categorized as very strong antioxidant activity, as it is below 50 ppm.

Table 5. Antioxidant Test Table for Solid Soap Preparation of 24% Concentration Ethanol Extract of Papaya Leaves

Concentration (ppm)	Absorbance	%Inhibisi	IC50 (ppm
DPPH	0,560		
10	0,489	12,68	
20	0,410	26,79	36,81
30	0,387	30,89	
40	0,253	54,82	
50	0,199	64,46	

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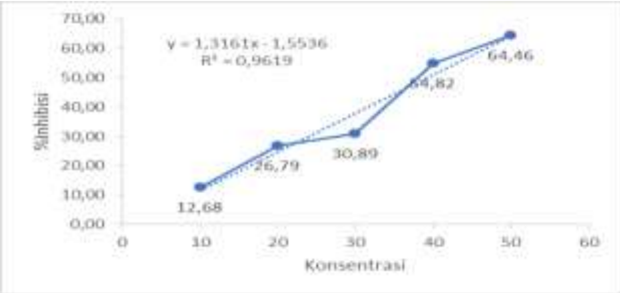

Figure 2. Graph of the Average Inhibition Relationship of Samples with a Concentration of 24%

In this study, solid soap preparations containing papaya leaf extract showed an IC_{50} value of 36.81 ppm. This value falls into the category of very strong antioxidant activity based on Molyneux's (2004) classification, which states that antioxidant activity is very strong if the value is below 50 ppm, strong between 500 and 100 ppm, moderate between 101 and 150 ppm, and weak above 150 ppm.

CONCLUSIONS

Based on the results of the study, solid soap formulations containing California papaya leaf extract (Carica papaya L.) were found to have antioxidant activity. Phytochemical screening tests showed that California papaya leaves contain flavonoids, tannins, and alkaloids that contribute to this activity. The concentration of extract used in the preparation affects the IC_{50} value, where a concentration of 24% produced an IC_{50} value of 36.81 ppm, lower than the 8% concentration preparation which had an IC_{50} value of 37.77 ppm. This indicates that the lower the IC_{50} value, the stronger the antioxidant activity. In the solid soap preparation of California papaya leaf ethanol extract, when compared to the positive control quercetin, the IC_{50} value of quercetin was 0.93 ppm, indicating that the positive control quercetin had stronger antioxidant activity than the solid soap preparation of California papaya leaf ethanol extract. In addition, the results of the quality evaluation of the preparation, including organoleptic testing, pH, foam height, and irritation testing, showed that the solid soap preparation of California papaya leaf ethanol extract met the requirements for a good solid soap preparation.

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