

ISOLATION, IDENTIFICATION, AND ANTIOXIDANT ACTIVITY OF CHEMICAL COMPOUND IN ETHANOL EXTRACT OF PAPAYA LEAVES (*CARICA PAPAYA* L.)

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## ABSTRACT

**Objective:** The purpose of this research was to isolate and identify the active antioxidant compound in ethanol extract of papaya leaves (*Carica papaya* L.).

**Methods:** The methods used were fractionation using vacuum liquid chromatography (VLC), and the active fraction was purified using column chromatography (CC). The pure isolate was obtained with preparative thin-layer chromatographic (TLC) identified by spectroscopy. Its antioxidant activity was evaluated using 2,2-diphenyl-picryl-hydrazyl. The presence of phenols was analyzed using ultraviolet (UV)-visible, Fourier-transform infrared (FTIR), and gas chromatography (GC)-mass spectrometry (MS).

**Results:** The results showed that ethanol extract of papaya leaf has a strong antioxidant activity with  $IC_{50}$  value of  $100.0 \pm 0.07$  ppm. The result of VLC fractionation using mobile phase of chloroform-methanol indicated that the active fraction was EtOH.3 that has antioxidant activity with  $IC_{50}$  of  $121.6 \pm 0.66$  ppm. The purification using CC produced active fraction EtOH.3.3 with  $IC_{50}$  value of  $176.4 \pm 0.76$  ppm. After identified, the active isolate compound EtOH.3.3.1 from preparative TLC, the results of UV-visible spectrophotometry, FTIR spectrophotometry, and GC-MS show the presence of the phenol compounds.

**Conclusion:** The ethanol extract has antioxidant activity and the extract was suspected to contain 2-methoxy-4-vinylphenol compound.

**Keywords:** Papaya leaves, Antioxidant activity, Active compound, Fractionation.

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## INTRODUCTION

Free radicals are molecules that contain one or more unpaired electrons in their outer orbital, very reactive and unstable. In an attempt to achieve their stable state, the free radicals will react with atoms or molecules around it to obtain electron pairs [1]. Free radical formation cannot be avoided; therefore, our bodies need an antioxidant. Antioxidant is a neutralizer of free radicals in the body and can inhibit oxidation. This substance is needed in the body to combat the trigger of chronic disease caused by free radicals. Natural antioxidants can be obtained from fruits and vegetables one of them is papaya (*Carica papaya* L.). In addition to the fruit used by the community as food, almost all parts of papaya plants can be used as a natural remedy. Many reports have also shown that the leaves of *C. papaya* have activity as an anticancer [2], antimicrobial [3-6], anti-inflammatory [7], analgetic activity [8], antiplasmodial or antimalarial activity [9-11], and antioxidant [12-14]. Papaya leaves water extract is proved as dengue fever drug [15,16]. The purpose of this research was to isolate and identify the active antioxidant compound in ethanolic extract of papaya leaves (*C. papaya* L.).

## METHODS

## Materials

Papaya leaves (*C. papaya* L.) were collected in Balitro, Bogor, Indonesia, ethanol, methanol, n-hexane, ethyl acetate, chloroform, Vitamin C, 1,1-diphenyl-2-picrylhydrazyl (DPPH), silica gel 60, Sephadex LH20, 30% ammonia, hydrochloric acid, 1% iron (III) chloride, Dragendorff's reagent, Mayer reagent, and Stiasny reagent.

Rotary vacuum evaporator, chromatographic columns, ultraviolet (UV) lamps, chamber, GF254 silica gel plates, UV-visible spectrophotometry, Fourier-transform infrared (FT-IR) spectrometer, and gas chromatography-mass spectrometry (GC-MS) were used.

## Extraction

About 1 kg of dried powder of *C. papaya* leaves was extracted with n-hexane, ethyl acetate, and ethanol by maceration method at room temperature for 24 h (3 times), then collected solution was filtered. The crude ethanolic solution was subsequently concentrated using rotary vacuum evaporator. The ethanol extract was ready for further analysis.

## Phytochemical screening

Phytochemical screening is meant to identify compounds such as flavonoids by the reduction test (Mg-HCl/amyl alcohol), saponins by the foam formation test, tannins by the iron (III) chloride reagent, quinones by the NaOH reagents, steroids/triterpenoids by the Liebermann-Burchard's reagent, alkaloids by the Dragendorff's reagent/Mayer reagent, coumarins by the fluorescence test with ammonia, and essential oils by the odor test, based on the method of Farnsworth [18,24].

## Thin-layer chromatographic (TLC) analysis

The ethanol extract was analyzed by TLC method using silica gel GF<sub>254</sub> with three solvents mixture: n-hexane-ethyl acetate (9:1, 4:1), ethyl acetate-methanol (3:7, 4:6), and chloroform-methanol (7:3, 6:4). The spots were visually identified under 254 nm and 366 nm UV lamp. The solvent that produced good separation on TLC chromatograms was chosen for vacuum liquid chromatography (VLC) and then used in TLC monitoring of fractions taken from column chromatography (CC).

## Antioxidant activity test

The antioxidant activity of *C. papaya* extract was determined by the method of William *et al.* [19] and Blois [20] with slight modification using the stable DPPH scavenging. Briefly, samples extract with various concentrations was prepared 30, 60, 90, 120, and 150 µg/mL. Each sample was mixed with 1.0 mL of 0.4 mM DPPH solution. All the solutions were prepared with methanol to 5.0 mL. Experiment was done in triplicate. The test sample was incubated for 30 min at room temperature and the absorbance was measured at 517 nm. Ascorbic acid

was used as a standard at concentration of 2, 4, 6, 8, and 10 µg/mL and DPPH in methanol was used as a control. The different in absorbance between the test and the control was calculated and the expressed as % scavenging of DPPH radical (% inhibitions). Then, % inhibitions were plotted against respective concentration used and from the graph  $IC_{50}$  was calculated.

#### Fractionation

The ethanol extract was separated on silica gel 60 using VLC with a step gradient elution of following composition chloroform-methanol with a ratio as shown in Table 1. All of the fractions were tested antioxidant using DPPH scavenging method. The active fractions were further purified by CC. The stationary phase was made up of glass column packed using Sephadex LH20 with methanol as mobile phase. The chemical composition of fraction was evaluated using TLC and visualized with UV (254 nm and 366 nm).

#### Purification and identification

Purification was conducted using TLC preparative ( $SiO_2$ , chloroform-methanol=6:4). Furthermore, the identification by UV-visible spectrophotometer, FTIR spectrometer, and GC-MS.

#### RESULTS AND DISCUSSION

Phytochemical screening of the ethanol extract of *C. papaya* leaves contains flavonoids, tannins, alkaloids, and steroids/triterpenoids.

The antioxidant activity of ethanol extract of papaya leaves has a strong antioxidant activity with  $IC_{50}$  value of  $100.0 \pm 0.07$  ppm. Crude extract with  $IC_{50}$  values <200 ppm showed mild antioxidant activity. The  $IC_{50}$  is the concentration of antioxidant activity to scavenge DPPH free radical 50%. Vitamin C as positive control has highest antioxidant activity that Vitamin C has the power of attenuation against free radicals with  $IC_{50}$  value of 2.4 ppm. VLC results obtained nine fractions, using a mobile phase chloroform-methanol (Table 1), whereas the mobile phase used for the TLC analysis of fraction using chloroform-methanol (6:4) which gave the best separation. Antioxidant activity test results to see percentage of inhibition for the all fractions using concentration of 120 ppm are given in Fig. 1.

Based on the data (Fig. 1) showed that the EtOH.3 fraction had the largest % inhibition (67.91%), indicates that the EtOH.3 fraction is the most powerful fraction of its activity. Next, the fraction was tested for antioxidant activity, results showed  $IC_{50}$  value of  $121.6 \pm 0.66$  ppm. According to Blois [20], in Hanani *et al.* [21], a substance can be expressed to be a strong antioxidant if it has an  $IC_{50}$  value <200 ppm, thus EtOH.3 is a fraction that has antioxidant capabilities such as those associated with phytochemical screening results that include flavonoids. Flavonoids have been studied to have various activities such as antioxidants and antibacterials [17].

The EtOH.3 fraction was further purified by CC using Sephadex LH20 as stationary phase and methanol as a mobile phase. The purification using CC produced six fractions. Monitoring of fractions obtained from CC was performed by TLC method (silica gel  $GF_{254}$ , chloroform-ethanol=6:4). Furthermore, the fractions tested the antioxidant activity using DPPH free radical to determine the most active fraction to be identified further. The results showed in Table 2.

The fraction of EtOH.3.3 is the most active fraction was further purified by preparative TLC (silica gel  $GF_{254}$ , chloroform-ethanol=6:4) to get pure isolate. The chromatogram that obtained of preparative TLC can be seen in Fig. 2.

The possibility of antioxidant activity decreases due to the content of nutritious compounds as an increasingly weakened antioxidant and reduced synergistic compounds that increase antioxidant activity because isolates are thought to have been a single compound. This is in accordance with the theory of secondary efficacy enhancing substance.

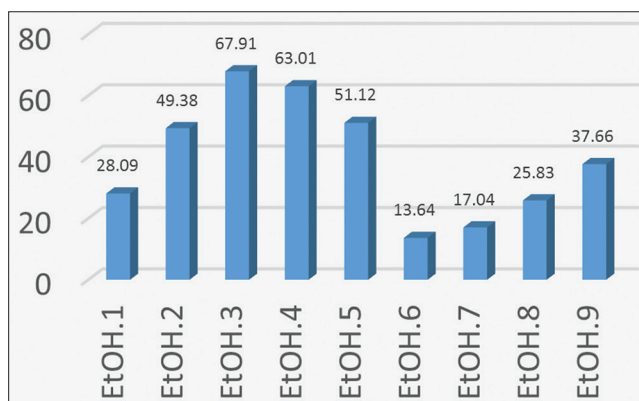
**Table 1: VLC fractionation of ethanol extract papaya leaves**

No.	Fraction	Solvent for VLC (chloroform-methanol)	Weight (g)
1.	EtOH.1	90:10	0.1019
2.	EtOH.2	80:20	1.5487
3.	EtOH.3	70:30	1.1380
4.	EtOH.4	60:40	1.1034
5.	EtOH.5	50:50	2.2428
6.	EtOH.6	40:60	2.4207
7.	EtOH.7	30:70	1.3738
8.	EtOH.8	20:80	2.8902
9.	EtOH.9	10:90	1.1157

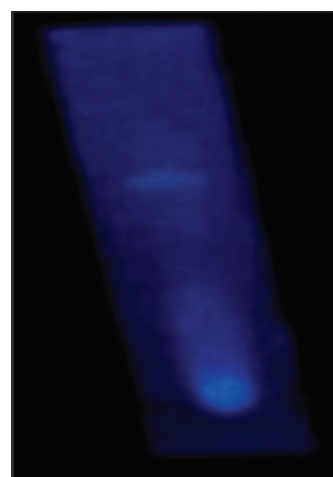
VLC: Vacuum liquid chromatography

**Table 2: Percentage of inhibition and  $IC_{50}$  of the EtOH.3 fraction**

No.	Fraction	Inhibition (%)	$IC_{50}$ (ppm)
1.	EtOH.3.1	31.2	$176.4 \pm 0.76$
2.	EtOH.3.2	44.97	
3.	EtOH.3.3	60.35	
4.	EtOH.3.4	41.92	
5.	EtOH.3.5	56.32	
6.	EtOH.3.6	52.32	



**Fig. 1: The % inhibition 2,2-diphenyl-picryl-hydrazyl of the nine fractions ethanol extract from vacuum liquid chromatography**



**Fig. 2: Chromatogram of preparative thin-layer chromatographic EtOH.3.3 fraction**

Based on Table 1, ethanol extract of papaya leaves has a moderate strength of antioxidant activity with  $IC_{50}$  value of  $100.0 \pm 0.07$  ppm, the EtOH.3 fraction from VLC showed decreased antioxidant activity

compared with  $IC_{50}$  produced by ethanol extract with  $IC_{50}$  value of  $121.6 \pm 0.66$  ppm. Based on Table 2, the EtOH.3.3 fraction produced by separation of CC yield  $IC_{50}$  of  $176.4 \pm 0.76$  ppm which increasingly indicates weak strength of antioxidant activity contained in isolate EtOH.3.3.1 having % inhibition of 52.83%.

The UV-visible spectrum of isolate (Fig. 3) showed maximum wavelength at 206.0 nm with absorbance 0.7140. The UV-visible spectrum results can be estimated by the presence of the conjugated double bond present in the compound because the maximum measured wavelength is above 200 nm. The results of the spectrum of the isolate can be seen in Fig. 3.

The FTIR spectrum of isolate (Fig. 4) showed the presence of functional groups C=C (aromatic ring) at  $1571.88/cm$ , -OH (alcohol) at  $3282.62/cm$ , and C=O (carbonyl) at  $1666.38/cm$  [22,23]. These results are supported by the prediction of identification data that EtOH.3.3.1 isolates contain phenol compounds. To identify more

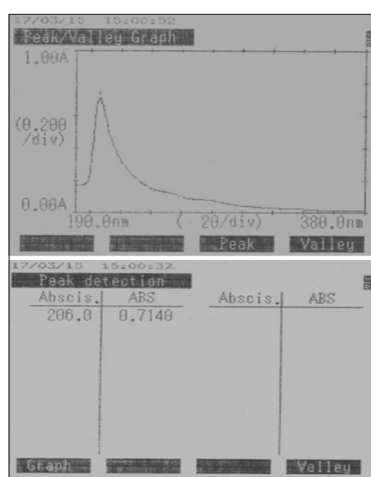


Fig. 3: Ultraviolet-visible spectrum of isolate from the EtOH.3.3.1 fraction

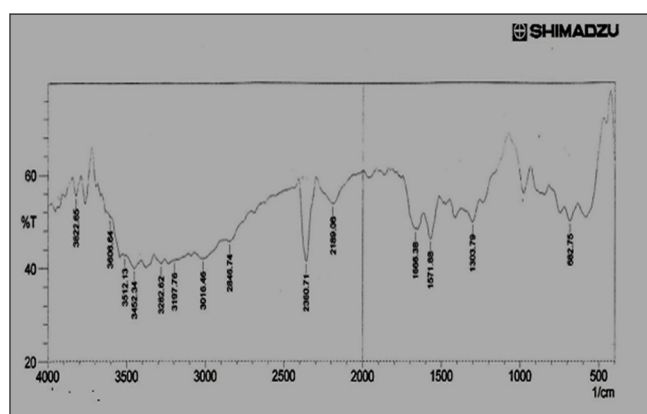


Fig. 4: Fourier-transform infrared spectrum of isolate

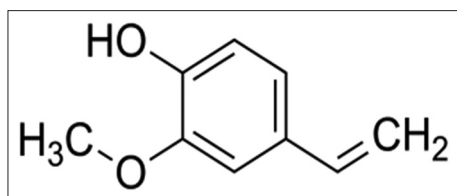


Fig. 5: Molecular structure of 2-methoxy-4-vinylphenol

clearly the isolate compounds contained in EtOH.3.3.1 isolates, it is necessary to analyze using GC-MS. The isolate shows the presence of the phenol compounds with % inhibition of 52.83%. Based on this similarity, the structure of isolate was therefore predicted as 2-methoxy-4-vinylphenol (Fig. 5).

## CONCLUSION

Based on the results of isolation, identification, and antioxidant activity test of the chemical compound of ethanol extract of *C. papaya* leaves, it could be concluded that

1. The ethanol extract was suspected to contain 2-methoxy-4-vinylphenol compound ( $C_9H_{10}O_2$ ).
2. The ethanol extract has antioxidant activity using DPPH free radical scavenging.

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