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(RESEARCH ARTICLE)

Assessment of antioxidant activity and bioactive compound identification of methanol and petroleum ether extracts of male Papaya Leaves (*Carica papaya* L.) From Indonesia

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Abstract

Papaya (*Carica papaya* L.) leaves are a potential source of bioactive compounds that possess various pharmacological properties, including antioxidant activity. The utilization of these compounds is crucial for the development of natural products that promote wellness. This study aimed to evaluate the antioxidant activity and bioactive compounds in methanol and petroleum ether extracts of papaya leaves. Extraction was performed using the maceration method with both solvents. Antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, while bioactive compound identification was conducted through phytochemical screening and GC-MS (Gas Chromatography-Mass Spectrometry) analysis. Phytochemical screening indicated that the methanol extract contained flavonoids, alkaloids, steroids, and saponins, whereas the petroleum ether extract contained alkaloids and steroids. GC-MS analysis identified 26 bioactive compounds in the methanol extract, with Neophytadiene (25.56%), 1,4-Eicosadiene (8.84%), and Benzyl nitrile (7.70%) as the major components. The petroleum ether extract contained 18 bioactive compounds, predominantly Hexadecanoic acid-methyl ester (45.92%), Benzene, (isothiocyanatomethyl)- (12.57%), and Neophytadiene (10.02%). IC50 values indicated that the antioxidant potential of the methanol extract was very weak (280.319 ppm), while that of the petroleum ether extract was moderate (142.565 ppm). These results suggest that the petroleum ether extract of papaya leaves is more effective as a source of antioxidants than the methanol extract and thus has potential for development as a bioactive ingredient in pharmaceutical or nutraceutical applications.

Keywords: *Carica papaya*; Antioxidant activity; Bioactive compound; Secondary metabolite

1. Introduction

As virus-related diseases increase, prevention measures that focus on enhancing the body's immune system are needed. Various studies have been carried out to explore effective and sustainable methods, one of which is through the use of natural materials. Plants have been used as a source of traditional medicine for a long time because of the content of bioactive compounds and have the potential to provide protective effects on health, including in countering oxidative stress and supporting immune system function [1].

Oxidative stress occurs when the quantity of free radicals in the body exceeds the ability of the antioxidant system to neutralize them. This condition can disrupt the physiological balance of cells and result in decreased immune system function. Free radicals can be derived from the environment or from normal metabolic processes in the body [2].

One of the plants that has potential as a source of natural antioxidants is papaya (*Carica papaya* L.), a tropical plant that grows in Indonesia. Papaya plants have various types of flowering, namely male, female, and hermaphrodite. Unlike the female and hermaphrodite papayas that produce fruit, male papayas do not bear fruit so their utilization is still limited.

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Papaya leaves, especially from male plants, are known to contain various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and steroids that have potential biological activities, including as antioxidants [3-5].

The biological activity of a plant extract can be influenced by the method and type of solvent used in the extraction process. The difference in solubility of the solvent will determine the type of compounds contained, thus directly affecting the content of bioactive compounds and the resulting antioxidant activity. Polar solvents are generally more effective at dissolving polar compounds, while nonpolar solvents tend to extract lipophilic compounds. Therefore, this study was conducted to assess the antioxidant activity and identify the content of bioactive compounds of male papaya leaves extracted using solvents with different levels of polarity, namely methanol and petroleum ether.

2. Material and methods

2.1. Plant material and extraction

Fresh male papaya (Carica papaya L.) leaves were collected from Trawas, Mojokerto Regency, East Java, Indonesia. The leaves were washed thoroughly under running water to removal of surface contaminants, air dried at room temperature without direct exposure to sunlight, and then milled into powder to extraction.

Extraction was conducted using the maceration method with methanol and petroleum ether as solvents. The powdered material was soaked in each solvent for 9 days at room temperature. Filtration was carried out every 3 days, and the remaining plant material was macerated using the same type and volume of the solvent. The combined filtrates were collected as methanol and petroleum ether extracts.

2.2. Phytochemical screening

Qualitative phytochemical assays were performed to identify the presence of major secondary metabolites, including flavonoids, alkaloids, terpenoids, steroids, and saponins, in both extracts. Flavonoids were identified using Wilstätter (cyanidin) reaction by adding a concentrated hydrochloric acid and magnesium band. Alkaloids were identified using Wagner's reagent. Terpenoids and steroids were determined by addition of chloroform followed by Liebermann-Burchard reagent. Saponins were identified by adding 5 mL of hot distilled water and concentrated hydrochloric acid, followed by vigorous shaking for 30 seconds and observation of stable foam formation. All procedures followed standard phytochemical screening protocols [6,7].

2.3. GC-MS analysis

GC–MS analysis using an Agilent Technologies 7890B gas chromatograph coupled with an Agilent Technologies 5977A mass selective detector equipped with an electron impact (EI) ionization source. Separation was achieved on an HP-5MS capillary column (5% phenyl, 95% methylpolysiloxane; 30 m × 250 µm × 0.25 µm). The oven temperature program was initially set at 40 °C and held for 5 min, followed by a temperature increase at a rate of 10 °C/min to 300 °C, which was maintained for 20 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, with a purge flow of 3 mL/min. Sample injection was performed in pulsed splitless mode at an injector temperature of 290 °C, using an injection volume of 1 µL. The mass spectrometer operated with an ion source temperature of 230 °C, a scan speed of 1562 (N_2), and a mass scan range of m/z 44–750. Compound identification was carried out by comparing the obtained mass spectra with those available in the NIST 2014 and Wiley 9 mass spectral libraries [8].

2.4. Antioxidant activity assays

Antioxidant activity assays were carried out utilizing the DPPH method. Methanol and petroleum ether extracts of papaya leaves, as well as silymarin as a comparison, were prepared in various concentrations, such as 200, 150, 125, 100, 75, 50, 25, 15, 10, and 6.25 ppm. Each sample solution was inserted into a 96-well microplate, then 100 µL of DPPH stock solution was added. The mixture was incubated for 45 minutes under dark conditions. After the incubation process, the absorbance of each solution was measured using a UV-Vis microplate spectrophotometer at a wavelength of 517 nm [9].

The percentage of antioxidant activity was calculated based on the difference between control absorbance and sample absorbance using the following equation:

$$Radical\ scavenging\ activity\ (\%) = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} x\ 100\%$$

The percentage value of antioxidant activity obtained was then used to construct a relationship curve between sample concentration (x-axis) and percentage of antioxidant activity (y-axis) by linear regression analysis. The IC_{50} value of each test sample was then compared with the IC_{50} value of the test sample.

3. Results and discussion

3.1. Phytochemical profile of papaya leaves extracts

Qualitative phytochemical screening was conducted to identify the major classes of secondary metabolites present in the methanolic and petroleum ether extracts of male *Carica papaya* leaves. The results of the phytochemical screening are presented in Table 1.

Table 1 Phytochemical screening results of male *C. papaya* leaf extracts

Secondary Metabolite	Methanolic Extract	Petroleum Ether Extract
Alkaloids	+	+
Flavonoids	+	-
Steroids	+	+
Terpenoids	-	-
Saponins	+	-

(+): detected; (-): not detected

Flavonoid screening was conducted using magnesium metal and concentrated hydrochloric acid. A positive reaction was observed in the methanolic extract of papaya leaves, indicated by the formation of an orange coloration, whereas the petroleum ether extract showed no color change, suggesting a negative result. The appearance of orange or red coloration in this test is attributed to the reduction of the benzopyrone nucleus of flavonoids by magnesium under acidic conditions, leading to the formation of flavilium salts [6]. Flavonoids are well known for their biological activities, including antioxidant, antimutagenic, and cytotoxic effects, which are mainly associated with their ability to donate hydrogen atoms to free radicals [10,11].

Alkaloid identification was carried out using Wagner's reagent. Both methanolic and petroleum ether extracts produced a brownish coloration, indicating the presence of alkaloids in both samples. This reaction occurs due to the formation of triiodide ions from iodine and potassium iodide, which subsequently interact with the nitrogen atoms of alkaloids to form coordination complexes and precipitates [7]. Alkaloids have been reported to exhibit various bioactivities such as antihypertensive, antimalarial, anticancer, analgesic, and antioxidant properties. Their antioxidant activity is associated with their ability to donate hydrogen atoms, allowing them to act as primary antioxidants [12,13].

Terpenoids and steroids were identified using the Liebermann–Burchard reagent. The methanolic extract showed a color change from green to dark bluish-green, while the petroleum ether extract changed from yellow to green, indicating positive results for steroid compounds in both extracts. These color changes are characteristic of oxidative reactions involving conjugated double bonds in terpenoid or steroid structures [14,15]. Terpenoids and steroids are classified as lipophilic antioxidants and are known to contribute to the antioxidant capacity of plant-derived extracts [16].

Saponin screening was performed by adding concentrated hydrochloric acid and hot distilled water to the extracts, followed by vigorous shaking. The methanolic extract produced stable foam lasting for approximately 10 minutes, confirming the presence of saponins, whereas the petroleum ether extract showed a negative result. Foam formation in this test is caused by glycosidic compounds that are capable of forming stable froth in aqueous solutions [17]. Saponins have been reported to possess antibacterial, antiviral, and antioxidant activities, with their antioxidant mechanism involving the scavenging of superoxide radicals and the prevention of oxidative damage [11,18].

3.2. GC-MS analysis of bioactive compounds

GC–MS analysis revealed distinct differences in the chemical composition of the methanolic and petroleum ether extracts. A total of 26 compounds were identified in the methanolic extract, whereas 18 compounds were detected in

the petroleum ether extract. The differences in chemical profiles between the two extracts were evident from the chromatographic patterns. Figure 1 shows the GC–MS chromatogram of the methanolic extract of male *Carica papaya* leaves, while Figure 2 presents the chromatogram of the petroleum ether extract.

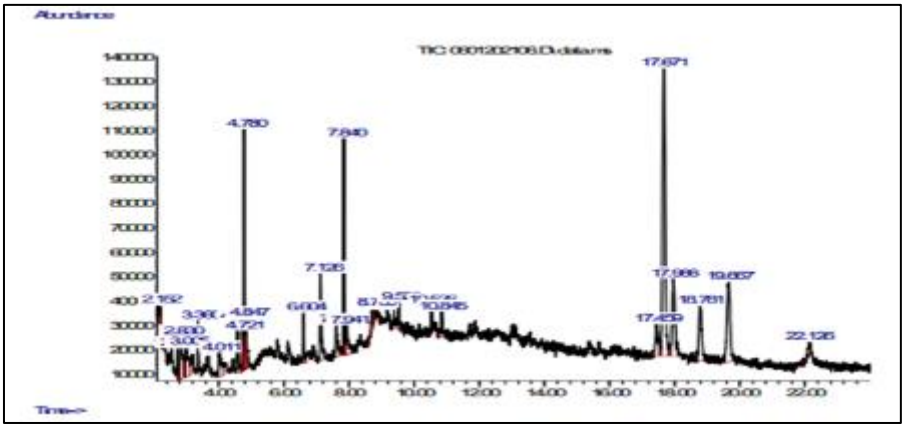

Figure 1 Chromatogram profile of male papaya leaf methanol extract

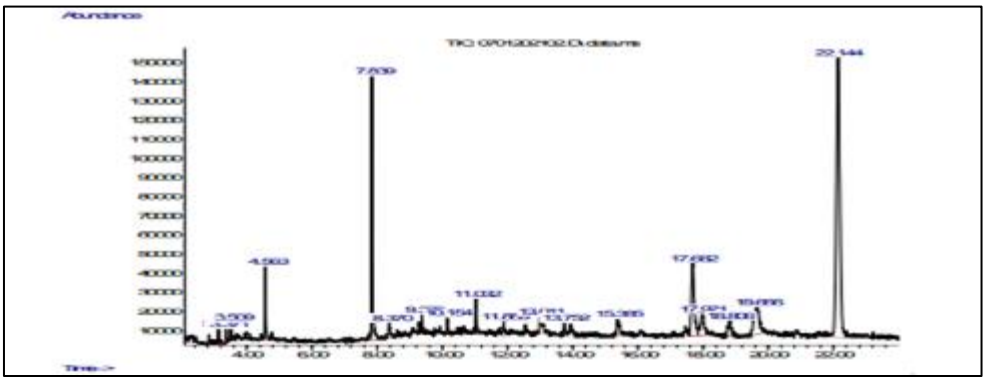

Figure 2 Chromatogram profile of male papaya leaf petroleum ether extract

The bioactive compounds identified in the methanolic extract are summarized in Table 2. Neophytadiene was the predominant compound in the methanolic extract. This diterpenoid compound has been reported to possess antioxidant, anti-inflammatory, and antimicrobial activities [19]. The presence of fatty acid derivatives such as 1,4-eicosadiene also contributes to the antioxidant potential of the extract [20].

Table 2 Bioactive compound of male *Carica papaya* leaf methanol extract

No	Retention Time (Minute)	Area (%)	Compound
1.	2.152	1.55	2-methyl-2-Azabicyclo[2.2.0]hex-5-ene
2.	2.726	0.65	2-(2-aminoethyl)piperidine
3.	2.830	3.49	Butyric acid-2-D1
4.	3.005	3.10	2-Pentanethiol
5.	3.360	1.63	Benzylamine
6.	4.011	1.98	Guanidine
7.	4.572	2.42	Benzyl isocyanate
8.	4.721	1.35	Methylene cyclohexane-2,2,6,6-D4
9	4.780	7.70	Benzyl nitrile

10.	4.847	2.39	3,5-Dihydroxy-2-methyl-5,6-dihydropyran-4-one
11.	6.604	1.73	n-Ethyl-4-piperidinecarboxamide
12.	7.126	4.36	2-Methoxy-4-vinylphenol
13.	7.616	1.98	2,6-dimethoxy-Phenol
14.	7.840	6.51	Benzene-(isothiocyanatomethyl)
15.	7.941	2.48	Methyl 2,3-diacetamido-2,3-dideoxy-. *α*.-D-glucopyranoside
16.	8.729	1.20	D-Glucose, 4-O-*β*.-D-galactopyranosyl
17.	8.760	0.42	9,10-Secochola-5,7,10(19)-triene-,24-diol, (3. *β*.,5Z,7E)
18.	9.528	1.28	3-methyl-thiophene-2-carboxamide
19.	10.536	1.33	Benzene,1,1'-(1,2-ethenediyl)bis
20.	10.845	1.75	Quinoline, 2-ethyl-
21.	17.459	3.09	2-Hexadecene, 3,7,11,15-tetramethyl
22.	17.671	25.56	Neophytadiene
23.	17.966	7.04	2-Hexadecene, 3,7,11,15-tetramethyl
24.	18.781	5.01	2,6,10-trimethyl, 14-ethylene-14-pentadecne
25.	19.667	8.84	1,4-Eicosadiene
26.	22.126	1.14	methyl 10-ethyltetradecanoate

The bioactive compounds identified in the petroleum ether extract are presented in Table 3. The petroleum ether extract was dominated by hexadecanoic acid methyl ester (methyl palmitate), which has been reported to exhibit antioxidant and anti-inflammatory activities [14]. Benzyl isothiocyanate, a glucosinolate-derived compound, is widely recognized for its antioxidant and chemopreventive properties [21]. The predominance of lipophilic compounds in the petroleum ether extract confirms the effectiveness of nonpolar solvents in extracting nonpolar bioactive constituents [7,22].

Table 3 Bioactive compound of male *Carica papaya* leaf petroleum ether extract

No	**Retention Time (Minute)**	**Area (%)**	**Compound**
1.	3.137	0.47	1,3,5-Trimethylbenzene
2.	3.371	0.64	1-Hexanol, 2-ethyl-
3.	3.509	1.08	Benzyl Alcohol
4.	4.563	3.47	Benzyl isocyanate
5.	7.839	12.57	Benzene, (isothiocyanatomethyl)-
6.	8.370	1.80	Methyl ester of benzylcarbamic acid
7.	9.379	1.12	3-Buten-2-one
8.	10.154	1.08	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
9	11.032	2.21	Propanoic acid
10.	11.889	1.25	Sorbitol hexaacetate
11.	13.011	1,95	Pyrethrin I
12.	13.752	0.94	Tridecanoic acid
13.	15.385	2.13	Loliolide
14.	17.682	10.02	Neophytadiene

15.	17.971	3.83	2-Hexadecene, 3,7,11,15-tetramethyl
16.	18.806	2,31	lavandulyl acetate
17.	19.666	7.20	2,6,10-trimethyl, 14-ethylene-14-pentadecne
18.	22.14	45.92	Hexadecanoic acid, methyl ester

3.3. Antioxidant activity assays

The antioxidant activity of the methanolic and petroleum ether extracts was evaluated using the DPPH radical scavenging assay. The IC_{50} values and antioxidant activity classifications are presented in Table 4.

Table 4 IC_{50} values and antioxidant activity classification

Sample	**IC_{50} (ppm)**	**Antioxidant activity**
Silymarin (control positive)	22,185	Very strong
Methanolic extract	280,319	Very weak
Petroleum ether extract	142,565	Moderate

Silymarin was used as a reference compound in the antioxidant activity assay. Silymarin is a natural compound derived from *Silybum marianum* and is well known for its strong antioxidant properties, particularly its ability to stabilize reactive oxygen species (ROS) [23]. Based on the antioxidant activity test, silymarin exhibited very strong antioxidant activity with an IC_{50} value of 22.185 ppm.

The methanolic extract of papaya leaves showed very weak antioxidant activity, as indicated by an IC_{50} value of 280.319 ppm. This finding is consistent with previous studies reporting that methanolic extracts of papaya leaves generally possess weak antioxidant activity, characterized by IC_{50} values greater than 200 ppm. Maisarah et al. reported an IC_{50} value of 7.8 ± 0.06 mg/mL for the methanolic extract of papaya leaves [24], while Sepriyani et al. [25] reported an IC_{50} value of 884.8272 ppm, which also falls within the very weak antioxidant category. Although the antioxidant activity observed in the present study was higher than that reported in some previous studies, it remains within the same classification. Differences in antioxidant activity may be influenced by several factors, including sample type, geographical origin, and extraction method. This is supported by Edison et al. [26] who stated that antioxidant activity is affected by solvent type, extraction technique, seasonal variation, sampling location, and plant species.

In contrast, the petroleum ether extract of papaya leaves exhibited moderate antioxidant activity, with an IC_{50} value of 142.565 ppm. The difference in antioxidant activity between the two extracts can be attributed to the variation in bioactive compounds extracted by solvents with different polarities. Phytochemical analysis indicated the presence of steroid compounds in the petroleum ether extract. Steroids are reported to function as lipophilic antioxidants by scavenging reactive species such as superoxide radicals and by chelating metal ions, including Fe^{2+} and Cu^{2+} [14]. Furthermore, GC–MS analysis of the petroleum ether extract identified three major bioactive compounds—hexadecanoic acid methyl ester, benzene (isothiocyanatomethyl), and neophytadiene—as shown in Table 3, all of which have been reported to exhibit antioxidant activity. Supporting these findings, Mardiyah et al. [27] reported that petroleum ether extracts demonstrated higher antioxidant activity compared to extracts obtained using other solvents, which may be attributed to the ability of petroleum ether to dissolve low-polarity antioxidant compounds.

4. Conclusion

This study demonstrates that papaya leaf extracts exhibit measurable antioxidant activity, with petroleum ether extract showing superior potency compared to methanolic extract as indicated by lower IC_{50} values. The observed differences in antioxidant capacity are closely associated with the polarity of the extraction solvent, which significantly influences the phytochemical profile and dominant bioactive constituents. Methanolic extraction yielded a broader range of secondary metabolites, including flavonoids and saponins, and a more diverse composition of bioactive compounds. In contrast, petroleum ether extraction preferentially isolated lipophilic constituents, particularly fatty acid derivatives and isothiocyanate-related compounds, which are likely responsible for the enhanced antioxidant activity observed. These findings provide new insight into the role of solvent selection in optimizing the recovery of antioxidant-related compounds from papaya leaves. Overall, papaya leaves represent a promising natural source of antioxidant compounds,

warranting further investigation to isolate key active constituents and evaluate their biological efficacy for potential applications in food and pharmaceutical systems.

Compliance with ethical standards

Disclosure of Conflict of interest

The authors declare no conflict of interest

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