Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 6 Number 2, 2026
Journal Homepage: http://ejurnal.ung.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v8i2.38090

Antioxidant Activity of *Carica pubescens* Seed Extracts Using Different Extraction Solvents

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ABSTRACT

Mountain papaya (*Carica pubescens*) is one of Indonesia's native plants that grows in the highlands of Dieng, Wonosobo. Its seeds are commonly regarded as agricultural waste and have not been widely utilised, although they may contain bioactive compounds with antioxidant potential. This study aimed to evaluate the antioxidant activity of *Carica pubescens* seed extracts obtained using different extraction solvents. This laboratory experimental study employed the reflux extraction method using four solvents: water, 70% ethanol, ethyl acetate, and n-hexane. The extraction process was carried out for 3 hours. Antioxidant activity was evaluated using the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) radical scavenging method with UV–Vis spectrophotometry at 517 nm, and the activity was expressed as the IC_{50} value. The antioxidant activity was classified based on the IC_{50} criteria applied in this study: very strong (<50 ppm), strong (50–100 ppm), moderate (100–150 ppm), weak (150–200 ppm), and very weak (>200 ppm). The ethanol extract showed the strongest antioxidant activity among the tested extracts, with an IC_{50} value of 122.9486 ppm, indicating moderate antioxidant activity. In contrast, the water, ethyl acetate, and n-hexane extracts showed very weak antioxidant activity, with IC_{50} values greater than 200 ppm. One-way ANOVA showed a significant effect of extraction solvent on the antioxidant activity of *Carica pubescens* seed extracts ($p < 0.001$). These findings indicate that ethanol is the most suitable solvent among those tested for extracting antioxidant-active compounds from *Carica pubescens* seeds.

Keywords:
Carica pubescens; DPPH; IC_{50}; antioxidant activity; reflux extraction; solvent polarity; UV–Vis spectrophotometry

Received:	*Accepted:*	*Online:*
2026-04-07	*2026-05-10*	*2026-05-26*

1. Introduction

Free radicals are unstable and highly reactive molecules or molecular fragments that contain one or more unpaired electrons. These molecules can damage lipids, interfere with DNA synthesis, disrupt blood vessels, and contribute to the development of degenerative diseases [1]. Excessive free radicals may also induce cellular and tissue damage, leading to organ dysfunction and the progression of chronic diseases [2]. Although the human body has endogenous defence mechanisms to counteract oxidative stress, excessive free radical production may overwhelm these protective systems.

Therefore, exogenous antioxidants are required to support the neutralisation of free radicals and reduce oxidative damage [3].

The growing understanding of free radical-mediated damage has increased the use of antioxidant compounds in food, pharmaceutical, and health-related applications. Antioxidants can protect biological systems from the harmful effects of reactive oxygen species and may suppress the progression of degenerative conditions, including diabetes, cancer, inflammation, immune disorders, cardiovascular diseases, and premature ageing [4]. Since endogenous antioxidants are produced in limited amounts, additional antioxidant sources from natural materials are required to maintain cellular protection against oxidative stress. Plant-based materials, including grains, fruits, vegetables, spices, and herbal plants, are widely recognised as natural sources of antioxidant compounds [5].

Mountain papaya (*Carica pubescens*) is one of Indonesia's native plants that grows in the highlands of Dieng, Wonosobo. Various parts of this plant, including the roots, leaves, flowers, fruits, and seeds, have been associated with potential medicinal properties. The seeds of *Carica pubescens* are often considered agricultural by-products and have not been optimally utilised, although they have been reported to contain several secondary metabolites, including alkaloids, steroids, flavonoids, saponins, tannins, and triterpenoids [6]. These secondary metabolites are important because several of them, particularly phenolic and flavonoid compounds, are known to contribute to antioxidant activity. Therefore, *Carica pubescens* seeds represent a potentially valuable natural source of antioxidant compounds rather than merely an unused waste material.

The extraction process is an important stage in obtaining antioxidant compounds from plant materials. The type and amount of secondary metabolites extracted from a sample are strongly influenced by the polarity of the solvent used [7]. Polar compounds, such as flavonoid glycosides, are generally more soluble in polar solvents, including water, methanol, and ethanol, whereas less polar compounds, such as isoflavonoids, flavanones, flavones, and methoxylated flavonoids, tend to be more soluble in semi-polar or non-polar solvents such as ethyl acetate and n-hexane [8]. Thus, solvent selection affects not only the extraction yield but also the phytochemical composition and biological activity of the resulting extract. For this reason, comparing different extraction solvents is scientifically relevant to determine which solvent is most suitable for obtaining antioxidant-active compounds from *Carica pubescens* seeds.

Antioxidant activity in plant extracts can be evaluated using several methods, one of which is the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) radical scavenging assay. The DPPH method is widely used because it is simple, rapid, sensitive, and requires only a small sample volume. This method measures the ability of antioxidant compounds to reduce DPPH radicals, which is indicated by a decrease in absorbance measured using UV–Vis spectrophotometry at 517 nm [9]. The antioxidant activity is commonly expressed as the IC_{50} value, which represents the concentration of an antioxidant compound or extract required to inhibit 50% of DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity [10].

This study addresses the underutilisation of *Carica pubescens* seeds as an agricultural by-product and explores their potential as a natural source of antioxidants. Since solvent polarity strongly influences the extraction of secondary metabolites, comparing different solvents is essential to identify the solvent that produces an extract with the most favourable antioxidant activity. Therefore, this study aimed to evaluate

the DPPH radical scavenging activity of *Carica pubescens* seed extracts obtained using water, ethanol, ethyl acetate, and n-hexane.

2. Methods

This study was conducted as a laboratory experimental study to evaluate the antioxidant activity of *Carica pubescens* seed extracts obtained using different extraction solvents. Four solvents with different polarity characteristics were used, namely water, 70% ethanol, ethyl acetate, and n-hexane. Antioxidant activity was determined using the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) radical scavenging method and measured using UV–Vis spectrophotometry. The DPPH method is based on the reduction of purple-coloured DPPH radicals in methanol solution by antioxidant compounds. When DPPH reacts with an electron- or hydrogen-donating antioxidant compound, the DPPH radical is reduced, resulting in a decrease in purple colour intensity and a colour transition toward yellow derived from the picryl group [11]. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer [9]. Antioxidant activity was expressed as the IC_{50} value, which represents the concentration of extract required to inhibit 50% of DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity [10].

Materials

The materials used in this study included *Carica pubescens* seeds, DPPH solution, 70% ethanol, ethyl acetate p.a., n-hexane p.a., water, methanol p.a., ascorbic acid, and distilled water.

Preparation of *Carica pubescens* Seed Simplicia and Powder

Carica pubescens seeds were cleaned and dried in an oven at 60°C until adequately dried. The dried seeds were then ground using a grinder and sieved through a 50-mesh sieve to obtain uniform seed powder. The resulting powder was used as simplicia material for the extraction process.

Extraction of *Carica pubescens* Seeds

The extraction of *Carica pubescens* seed powder was carried out using the reflux method. The crude powder was extracted with each solvent at a sample-to-solvent ratio of 1:10. The solvents used were water, 70% ethanol, ethyl acetate, and n-hexane. The reflux extraction process was performed for 3 hours using a boiling stone to maintain stable boiling conditions. Each extraction was carried out in triplicate for every solvent. After extraction, the filtrate was collected and concentrated using a water bath at 78°C to obtain the crude extract. The extracts were labelled according to the solvent used: A1 for the water extract, A2 for the ethanol extract, A3 for the ethyl acetate extract, and A4 for the n-hexane extract.

DPPH Antioxidant Activity Assay

The antioxidant activity of *Carica pubescens* seed extracts was evaluated using the DPPH radical scavenging assay. The assay solutions consisted of 50 ppm DPPH solution, negative control solution, positive control solution, and extract test solutions. Ascorbic acid was used as the positive control, while the negative control consisted of DPPH solution without extract or antioxidant standard.

Five concentration levels were prepared for the positive control and each extract sample. The concentration series were 2, 3, 4, 5, and 6 ppm for ascorbic acid; 150, 250, 350, 450, and 550 ppm for the water extract; 90, 100, 110, 120, and 130 ppm for the ethanol extract; 9,000, 12,000, 15,000, 18,000, and 21,000 ppm for the ethyl acetate extract; and 30,000, 60,000, 90,000, 120,000, and 150,000 ppm for the n-hexane extract.

In the UV–Vis spectrophotometric assay, 2 mL of sample solution was mixed with 2 mL of 50 ppm DPPH solution at a ratio of 1:1 and homogenised. The mixture was

incubated for 30 minutes at room temperature to allow the reaction between antioxidant compounds and DPPH radicals. After incubation, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer [9]. For each solvent series, the absorbance of the negative control solution was measured separately to establish the absorbance baseline, because each extract was prepared using a different solvent system.

The percentage of DPPH inhibition was calculated using the following equation:

$$\% \text{ inhibition} = [(A_0 - A_s) / A_0] \times 100$$

where A_0 is the absorbance of the negative control and A_s is the absorbance of the sample or positive control after reaction with DPPH. The IC_{50} value was determined from the linear regression equation obtained by plotting extract concentration on the x-axis and percentage inhibition on the y-axis. The regression equation was expressed as $y = bx + a$, and the IC_{50} value was calculated as the concentration required to produce 50% inhibition of DPPH radicals [12].

Data Analysis

The data obtained from the extraction and antioxidant activity assays were analysed using IBM SPSS Statistics 25. Quantitative data, including extraction yield, absorbance, percentage inhibition, and IC_{50} values, were presented as mean ± standard deviation. The normality of the IC_{50} replication data was assessed using the Shapiro–Wilk test because the number of data points was less than 50. In this study, the statistical analysis was performed using IC_{50} replication data, with three IC_{50} values obtained for each extract group, resulting in a total of 12 data points. Homogeneity of variance was assessed before conducting one-way ANOVA. One-way ANOVA was used to determine whether different extraction solvents significantly affected the antioxidant activity of *Carica pubescens* seed extracts. When significant differences were observed, Tukey's post hoc test was performed to identify differences among solvent groups. Statistical significance was set at $\mathbf{p < 0.05}$.

The antioxidant activity was classified based on the IC_{50} value as follows: very strong activity for IC_{50} < 50 ppm, strong activity for IC_{50} 50–100 ppm, moderate activity for IC_{50} 100–150 ppm, weak activity for IC_{50} 150–200 ppm, and very weak activity for IC_{50} > 200 ppm.

3. Results and Discussion

Extraction Characteristics and Yield of *Carica pubescens* Seed Extracts

The preparation of *Carica pubescens* seed extract was initiated by collecting seed samples from the Dieng Wonosobo mountainous region, Central Java. The dried seed powder was extracted using the reflux method with four different solvents, namely water, 70% ethanol, ethyl acetate, and n-hexane. Reflux extraction was selected because the application of heat can improve solvent penetration into plant cell structures, thereby facilitating the release of intracellular secondary metabolites into the extraction solvent [12]. Previous research also reported that *Carica pubescens* seeds extracted using the reflux method with 70% ethanol produced a relatively favourable yield compared with other extraction methods, such as maceration, sonication, and Soxhlet extraction [6].

The physical characteristics of the extracts differed according to the solvent used. The water extract and 70% ethanol extract appeared as viscous dark-brown extracts, although the ethanol extract showed a darker colour than the water extract. In contrast, the ethyl acetate and n-hexane extracts appeared as slightly oily yellow liquids. These differences in colour and consistency may be associated with the extraction of different groups of polar and non-polar compounds, including flavonoid-related constituents, which are known to contribute to antioxidant activity [13]. Flavonoids and other

secondary metabolites in *Carica pubescens* have also been reported to possess several biological activities, including antioxidant, antimicrobial, antibacterial, and antiviral effects [14]. In this study, the extracts were coded based on the solvent used: A1 for the water extract, A2 for the 70% ethanol extract, A3 for the ethyl acetate extract, and A4 for the n-hexane extract.

Table 1. Extraction yield of *Carica pubescens* seed extracts using different extraction solvents

Sample	Extraction solvent	R1 (%)	R2 (%)	R3 (%)	Yield mean ± SD (%)
A1	Water	11.38	9.43	6.18	9.00 ± 2.63
A2	70% ethanol	4.46	3.94	6.18	4.86 ± 1.17
A3	Ethyl acetate	29.14	28.49	29.17	28.93 ± 0.38
A4	n-Hexane	29.19	28.30	27.31	28.27 ± 0.94

Source: Research data, 2024.
Note: Values are presented as mean ± SD (n = 3). A1 = water extract; A2 = 70% ethanol extract; A3 = ethyl acetate extract; A4 = n-hexane extract.

As shown in **Table 1**, the extraction yield varied among the solvents used. The highest yield was obtained from the ethyl acetate extract (A3), with a mean yield of 28.93 ± 0.38%, followed by the n-hexane extract (A4), with a mean yield of 28.27 ± 0.94%. In contrast, the water extract (A1) and 70% ethanol extract (A2) produced lower yields, namely 9.00 ± 2.63% and 4.86 ± 1.17%, respectively. These findings indicate that solvent characteristics, particularly solvent polarity, influence the ability of each solvent to dissolve and extract compounds from *Carica pubescens* seeds [16]. However, extraction yield only reflects the total mass of crude extract obtained and does not necessarily represent antioxidant potency. Antioxidant activity depends not only on the quantity of extract produced but also on the type, and effectiveness of bioactive compounds in neutralising free radicals [15]. Therefore, the high yields observed in the ethyl acetate and n-hexane extracts should be interpreted together with antioxidant activity parameters, particularly the IC_{50} values.

DPPH Radical Scavenging Response Based on Absorbance and Percentage Inhibition

The antioxidant response of the positive control and *Carica pubescens* seed extracts was evaluated based on absorbance reduction and percentage inhibition using the DPPH radical scavenging assay. The DPPH method is based on the reduction of purple DPPH radicals by antioxidant compounds through electron or hydrogen atom donation, resulting in a decrease in absorbance intensity at 517 nm [11]. Therefore, lower absorbance values indicate a greater ability of the tested sample to reduce DPPH radicals. The absorbance measurement was performed using UV–Vis spectrophotometry at 517 nm, which is commonly used for DPPH-based antioxidant evaluation [9].

Table 2. Absorbance of control solutions

Sample	Concentration (ppm)	R1	R2	R3	Absorbance mean ± SD
K0	-	0.780	0.768	0.771	0.773 ± 0.006
K1	2	0.615	0.602	0.607	0.608 ± 0.007
K1	3	0.528	0.515	0.517	0.520 ± 0.007
K1	4	0.435	0.424	0.428	0.429 ± 0.006
K1	5	0.372	0.360	0.366	0.366 ± 0.006
K1	6	0.273	0.262	0.266	0.267 ± 0.006

Source: Research data, 2024.
Note: Values are presented as mean ± SD (n = 3). K0 = negative control; K1 = positive control using ascorbic acid.

Table 3. Absorbance of *Carica pubescens* seed extract test solutions

Sample	Extract solvent	K0 baseline absorbance	Concentration (ppm)	R1	R2	R3	Absorbance mean ± SD
A1	Water	0.686	150	0.530	0.528	0.514	0.524 ± 0.009
A1	Water	0.686	250	0.436	0.448	0.432	0.439 ± 0.008
A1	Water	0.686	350	0.412	0.415	0.405	0.411 ± 0.005
A1	Water	0.686	450	0.333	0.357	0.336	0.342 ± 0.013
A1	Water	0.686	550	0.248	0.264	0.246	0.253 ± 0.010
A2	70% ethanol	0.675	90	0.398	0.411	0.392	0.400 ± 0.010
A2	70% ethanol	0.675	100	0.385	0.393	0.381	0.386 ± 0.006
A2	70% ethanol	0.675	110	0.361	0.368	0.358	0.362 ± 0.005
A2	70% ethanol	0.675	120	0.340	0.349	0.340	0.343 ± 0.005
A2	70% ethanol	0.675	130	0.319	0.342	0.313	0.325 ± 0.016
A3	Ethyl acetate	0.723	9,000	0.424	0.420	0.453	0.432 ± 0.018
A3	Ethyl acetate	0.723	12,000	0.371	0.369	0.403	0.381 ± 0.019
A3	Ethyl acetate	0.723	15,000	0.292	0.316	0.346	0.318 ± 0.027
A3	Ethyl acetate	0.723	18,000	0.254	0.263	0.289	0.269 ± 0.018
A3	Ethyl acetate	0.723	21,000	0.205	0.235	0.227	0.222 ± 0.015
A4	n-Hexane	0.726	30,000	0.581	0.582	0.583	0.582 ± 0.001
A4	n-Hexane	0.726	60,000	0.550	0.566	0.569	0.562 ± 0.010
A4	n-Hexane	0.726	90,000	0.528	0.529	0.530	0.529 ± 0.001
A4	n-Hexane	0.726	120,000	0.497	0.509	0.510	0.505 ± 0.007
A4	n-Hexane	0.726	150,000	0.454	0.470	0.488	0.471 ± 0.017

Source: Research data, 2024.
Note: Values are presented as mean ± SD (n = 3). K0 baseline absorbance represents the negative control absorbance measured for each solvent series and used for calculating percentage inhibition. A1 = water extract; A2 = 70% ethanol extract; A3 = ethyl acetate extract; A4 = n-hexane extract.

As shown in **Table 2**, the absorbance of the positive control decreased progressively as the concentration of ascorbic acid increased. The absorbance decreased

from 0.608 ± 0.007 at 2 ppm to 0.267 ± 0.006 at 6 ppm. A similar concentration-dependent pattern was also observed in the extract groups, as shown in **Table 3**. In general, increasing extract concentration resulted in lower absorbance values, indicating increased reduction of DPPH radicals. This pattern supports the principle that higher concentrations of antioxidant compounds can produce greater radical scavenging effects [17].

The reduction in absorbance was further reflected in the percentage inhibition values. The inhibition percentage represents the ability of the positive control or extract sample to inhibit DPPH radicals relative to the negative control. Therefore, a higher percentage inhibition indicates stronger radical scavenging activity.

Table 4. Percentage inhibition of control solutions

Sample	Concentration (ppm)	R1 (%)	R2 (%)	R3 (%)	Inhibition mean ± SD (%)
K0	-	0.00	0.00	0.00	0.00 ± 0.00
K1	2	21.15	21.61	21.27	21.34 ± 0.24
K1	3	32.31	32.94	32.94	32.73 ± 0.36
K1	4	44.23	44.79	44.49	44.50 ± 0.28
K1	5	52.31	53.13	52.53	52.66 ± 0.42
K1	6	65.00	65.89	65.50	65.46 ± 0.45

Source: Research data, 2024.

Note: Values are presented as mean ± SD (n = 3). K0 was used as the negative control and was considered to have 0% inhibition.

Table 5. Percentage inhibition of *Carica pubescens* seed extract test solutions

Sample	Extract solvent	Concentration (ppm)	R1 (%)	R2 (%)	R3 (%)	Inhibition mean ± SD (%)
K0	Negative control	-	0.00	0.00	0.00	0.00 ± 0.00
A1	Water	150	22.74	23.03	25.07	23.61 ± 1.27
A1	Water	250	36.44	34.69	37.03	36.05 ± 1.22
A1	Water	350	39.94	39.50	40.96	40.13 ± 0.75
A1	Water	450	51.45	47.96	51.02	50.14 ± 1.89
A1	Water	550	63.84	61.52	64.14	63.17 ± 1.42
K0	Negative control	-	0.00	0.00	0.00	0.00 ± 0.00
A2	70% ethanol	90	41.04	39.11	41.93	40.69 ± 1.44
A2	70% ethanol	100	42.96	41.77	43.55	42.76 ± 0.90
A2	70% ethanol	110	46.52	45.48	46.96	46.32 ± 0.76
A2	70% ethanol	120	49.63	48.30	49.63	49.19 ± 0.77
A2	70% ethanol	130	52.74	50.07	53.63	52.15 ± 1.85
K0	Negative control	-	0.00	0.00	0.00	0.00 ± 0.00
A3	Ethyl acetate	9,000	40.45	41.91	37.34	39.90 ± 2.34
A3	Ethyl acetate	12,000	47.03	48.96	41.77	45.92 ± 3.79
A3	Ethyl acetate	15,000	59.61	56.29	48.82	54.91 ± 5.56
A3	Ethyl acetate	18,000	62.52	63.62	60.03	62.06 ± 1.83
A3	Ethyl acetate	21,000	68.74	67.50	68.60	68.28 ± 0.67
K0	Negative control	-	0.00	0.00	0.00	0.00 ± 0.00
A4	n-Hexane	30,000	19.97	19.84	19.69	19.83 ± 0.14
A4	n-Hexane	60,000	24.24	22.04	21.63	22.64 ± 1.37
A4	n-Hexane	90,000	27.27	27.14	27.00	27.14 ± 0.14
A4	n-Hexane	120,000	31.54	29.89	29.75	30.39 ± 0.98
A4	n-Hexane	150,000	37.47	35.26	32.78	35.17 ± 2.35

Source: Research data, 2024.

Note: Values are presented as mean ± SD (n = 3). A1 = water extract; A2 = 70% ethanol extract; A3 = ethyl acetate extract; A4 = n-hexane extract.

Based on **Table 4**, the percentage inhibition of the positive control increased from 21.34 ± 0.24% at 2 ppm to 65.46 ± 0.45% at 6 ppm. This confirms the strong and concentration-dependent radical scavenging response of ascorbic acid. A similar trend was observed in the extract samples, although the magnitude of inhibition differed substantially among solvents. As shown in **Table 5**, the 70% ethanol extract (A2) reached 52.15 ± 1.85% inhibition at only 130 ppm, whereas the n-hexane extract (A4) reached only 35.17 ± 2.35% inhibition even at a much higher concentration of 150,000 ppm. This comparison indicates that the ethanol extract produced a markedly stronger DPPH radical scavenging response than the n-hexane extract.

Overall, the absorbance and percentage inhibition data demonstrate that the antioxidant response of *Carica pubescens* seed extracts was concentration-dependent. Increasing extract concentration reduced absorbance and increased percentage inhibition, indicating a greater ability to scavenge DPPH radicals. The relationship between concentration and percentage inhibition was subsequently used to establish the linear regression equation for determining IC_{50} values. A correlation coefficient is considered to meet the requirements if $r \geq 0.98$ and is very strong if the obtained r value is above 0.9 but less than 1.0 [18].

IC_{50} Values and Antioxidant Activity Classification

The IC_{50} value is the main parameter used to evaluate antioxidant activity in the DPPH radical scavenging assay. IC_{50} represents the concentration of a sample required to inhibit 50% of DPPH radicals; therefore, a lower IC_{50} value indicates stronger antioxidant activity [10]. The IC_{50} value was obtained from the linear regression equation generated by plotting sample concentration against percentage inhibition. A good relationship between concentration and percentage inhibition supports the reliability of IC_{50} determination. In this study, antioxidant activity was classified based on the IC_{50} value as follows: very strong activity for IC_{50} < 50 ppm, strong activity for IC_{50} 50–100 ppm, moderate activity for IC_{50} 100–150 ppm, weak activity for IC_{50} 150–200 ppm, and very weak activity for IC_{50} > 200 ppm.

Table 6. IC_{50} values and antioxidant activity classification of *Carica pubescens* seed extracts

Sample	Extract/control	IC_{50} R1 (ppm)	IC_{50} R2 (ppm)	IC_{50} R3 (ppm)	IC_{50} mean ± SD (ppm)	Antioxidant activity
K1	Ascorbic acid	4.6166	4.6166	4.6166	4.62 ± 0.00	Very strong
A1	Water extract	432.2665	446.2029	419.1205	432.53 ± 13.54	Very weak
A2	70% ethanol extract	121.3801	127.7644	119.7015	122.95 ± 4.25	Moderate
A3	Ethyl acetate extract	11,978.4615	12,392.7273	14,104.2308	12,825.14 ± 1,126.92	Very weak
A4	n-Hexane extract	345,920.00	347,730.00	341,200.00	344,950.00 ± 3,371.34	Very weak

Source: Research data, 2024.

Note: Values are presented as mean ± SD (n = 3). K1 = positive control using ascorbic acid; A1 = water extract; A2 = 70% ethanol extract; A3 = ethyl acetate extract; A4 = n-hexane extract. Lower IC_{50} values indicate stronger antioxidant activity.

As shown in **Table 6**, the positive control, ascorbic acid (K1), showed very strong antioxidant activity, with an IC_{50} value of 4.62 ± 0.00 ppm. This result confirms the strong radical scavenging capacity of ascorbic acid and supports its use as a reference

antioxidant in the DPPH assay. Among the *Carica pubescens* seed extracts, the 70% ethanol extract (A2) showed the strongest antioxidant activity, with an IC_{50} value of 122.95 ± 4.25 ppm. Based on the IC_{50} classification used in this study, this value indicates moderate antioxidant activity.

In contrast, the water extract (A1), ethyl acetate extract (A3), and n-hexane extract (A4) showed very weak antioxidant activity, with IC_{50} values of 432.53 ± 13.54 ppm, 12,825.14 ± 1,126.92 ppm, and 344,950.00 ± 3,371.34 ppm, respectively. Although the water extract showed lower antioxidant activity than the ethanol extract, its IC_{50} value was still considerably lower than those of the ethyl acetate and n-hexane extracts. The very high IC_{50} values of A3 and A4 indicate that substantially higher concentrations of these extracts were required to inhibit 50% of DPPH radicals. Therefore, based on IC_{50} values, the antioxidant activity of the extracts followed the order: 70% ethanol extract (A2) > water extract (A1) > ethyl acetate extract (A3) > n-hexane extract (A4).

These findings suggest that the antioxidant-active compounds in *Carica pubescens* seeds were more effectively extracted using 70% ethanol than using water, ethyl acetate, or n-hexane. The moderate activity of the ethanol extract also indicates that solvent selection plays an important role in obtaining extracts with better radical scavenging potential. However, although A2 demonstrated the best antioxidant response among the tested extracts, its activity remained lower than that of ascorbic acid, as indicated by the much higher IC_{50} value compared with K1.

Influence of Solvent Polarity on Extraction Yield and Antioxidant Activity

The comparison between extraction yield and IC_{50} values showed that a higher extraction yield did not necessarily correspond to stronger antioxidant activity. In this study, the ethyl acetate extract (A3) and n-hexane extract (A4) produced the highest extraction yields, namely 28.93 ± 0.38% and 28.27 ± 0.94%, respectively. However, both extracts exhibited very weak antioxidant activity, as indicated by their high IC_{50} values. The ethyl acetate extract had an IC_{50} value of 12,825.14 ± 1,126.92 ppm, whereas the n-hexane extract showed the weakest activity, with an IC_{50} value of 344,950.00 ± 3,371.34 ppm. In contrast, the 70% ethanol extract (A2) produced a much lower extraction yield of 4.86 ± 1.17%, but showed the strongest antioxidant activity among the tested extracts, with an IC_{50} value of 122.95 ± 4.25 ppm.

This finding indicates that extraction yield mainly reflects the total mass of crude extract obtained, rather than the concentration or effectiveness of antioxidant-active compounds. A crude extract with a high yield may contain large amounts of non-antioxidant constituents, such as lipids, waxes, pigments, or other compounds that do not contribute substantially to DPPH radical scavenging activity. Therefore, the antioxidant potency of an extract is more closely associated with the type and concentration of bioactive compounds extracted than with the total extract mass. This interpretation is consistent with the present results, where A3 and A4 yielded larger amounts of crude extract but demonstrated markedly weaker antioxidant activity than A2.

Solvent polarity is one of the most important factors influencing the extraction of secondary metabolites from plant materials. Different solvents have different abilities to dissolve phytochemical compounds depending on their polarity and chemical affinity [7], [16]. Flavonoid compounds, particularly flavonoid glycosides and other polar phenolic constituents, tend to be more soluble in polar solvents such as water, methanol, and ethanol. Meanwhile, less polar flavonoid derivatives, including isoflavonoids, flavanones, flavones, and methoxylated flavonoids, may be more soluble in semi-polar

or non-polar solvents such as ethyl acetate and n-hexane [8]. Thus, the polarity compatibility between the solvent and the target compounds strongly determines the phytochemical profile and biological activity of the extract.

The better antioxidant activity of the 70% ethanol extract may be attributed to the ability of ethanol-water mixtures to dissolve a broad range of polar and semi-polar antioxidant-related compounds. Ethanol at 70% concentration contains both organic and aqueous phases, allowing it to extract phenolic and flavonoid compounds more effectively than either highly non-polar or purely aqueous systems. Since flavonoids and related secondary metabolites have been reported in *Carica pubescens* and are associated with antioxidant and other biological activities, their more effective extraction by 70% ethanol may explain the lower IC_{50} value observed in A2 [13],[14]. However, because total phenolic content, total flavonoid content, and specific marker compounds were not measured in this study, this explanation should be interpreted as a plausible mechanism rather than a definitive phytochemical conclusion.

The very weak antioxidant activity of the ethyl acetate and n-hexane extracts may be explained by the possibility that these solvents extracted more non-polar constituents that were less active in the DPPH radical scavenging system. Although ethyl acetate and n-hexane produced high extraction yields, the extracted compounds may not have had sufficient hydrogen- or electron-donating capacity to reduce DPPH radicals effectively. Previous studies have shown that solvent polarity can influence not only extraction yield but also phenolic content, phytochemical composition, and antioxidant capacity of plant extracts [20],[21],[22]. Therefore, the high yield observed in A3 and A4 should not be interpreted as evidence of strong antioxidant potential.

Overall, the results demonstrate that 70% ethanol was the most effective solvent among those tested for obtaining antioxidant-active compounds from *Carica pubescens* seeds. The discrepancy between yield and IC_{50} values confirms that extraction efficiency should be evaluated not only by the amount of crude extract obtained but also by the biological activity of the extracted compounds. These findings also highlight the importance of selecting an appropriate solvent in antioxidant studies, particularly when the target activity is related to polar or semi-polar secondary metabolites such as phenolics and flavonoids.

Statistical Analysis of the Effect of Extraction Solvent on Antioxidant Activity

Statistical analysis was conducted to determine whether different extraction solvents significantly affected the antioxidant activity of *Carica pubescens* seed extracts. In this study, the IC_{50} replication data from each extract group were used as the primary endpoint for statistical analysis. Before performing one-way ANOVA, the normality of the IC_{50} data was assessed using the Shapiro–Wilk test. The results of the normality test are presented in **Table 7**.

Table 7. Shapiro–Wilk normality test of IC_{50} values

Sample	Shapiro–Wilk statistic	p-value
A1	0.862	0.273
A2	0.898	0.380
A3	0.890	0.353
A4	0.938	0.519

Source: *Research data, 2024.*
Note: *A1 = water extract; A2 = 70% ethanol extract; A3 = ethyl acetate extract; A4 = n-hexane extract. Data are considered normally distributed when $p > 0.05$.*

As shown in **Table 7**, all extract groups had p-values greater than 0.05, indicating that the IC_{50} data were normally distributed. Therefore, the data fulfilled the normality assumption required for parametric analysis using one-way ANOVA.

Table 8. One-way ANOVA of IC_{50} values among extraction solvent groups

Source of variation	F-value	p-value
Between groups	370.73	<0.001

Source: Research data, 2024.
Note: Statistical significance was determined at $p < 0.05$.

The one-way ANOVA results in **Table 8** showed a significant difference in IC_{50} values among the extraction solvent groups, as indicated by $p < 0.001$. This result demonstrates that the type of extraction solvent significantly influenced the antioxidant activity of *Carica pubescens* seed extracts. Therefore, solvent selection is an important factor in determining the radical scavenging potential of the resulting extract.

Since the ANOVA result was significant, Tukey's post hoc test was performed to identify the statistical differences among solvent groups. The results are presented in **Table 9**.

Table 9. Tukey post hoc test of IC_{50} values among extraction solvent groups

Sample	Extract solvent	IC_{50} mean ± SD (ppm)
A1	Water	432.53 ± 13.54[a]
A2	70% ethanol	122.95 ± 4.25[a]
A3	Ethyl acetate	12,825.14 ± 1,126.92[b]
A4	n-Hexane	344,950.00 ± 3,371.34[c]

Source: Research data, 2024.
Note: Values are presented as mean ± SD (n = 3). Different superscript letters indicate statistically significant differences among groups based on Tukey's post hoc test at $p < 0.05$. The same superscript letter indicates no statistically significant difference. Lower IC_{50} values indicate stronger antioxidant activity.

Based on **Table 9**, the Tukey post hoc test grouped the extraction solvent treatments into three statistical categories. The water extract (A1) and 70% ethanol extract (A2) shared the same superscript letter, indicating that there was no statistically significant difference between these two groups based on the IC_{50} replication data. However, descriptively, A2 showed a lower IC_{50} value than A1, suggesting stronger antioxidant activity in the 70% ethanol extract. The ethyl acetate extract (A3) and n-hexane extract (A4) had different superscript letters, indicating that they differed significantly from the other groups.

Overall, the statistical analysis confirmed that extraction solvent significantly affected the antioxidant activity of *Carica pubescens* seed extracts. Although A1 and A2 were not statistically different in the Tukey grouping, the descriptive IC_{50} profile showed that the 70% ethanol extract had the most favourable antioxidant activity among the tested extracts. In contrast, the ethyl acetate and n-hexane extracts showed substantially higher IC_{50} values, confirming their weaker DPPH radical scavenging activity. Therefore, these findings support the importance of selecting an appropriate extraction solvent to obtain extracts with better antioxidant potential.

Study Limitations and Future Validation

This study has several limitations that should be considered when interpreting the antioxidant activity of *Carica pubescens* seed extracts. First, the extraction process was performed using the reflux method, which involves heat exposure. Although this method can facilitate the release of secondary metabolites from plant materials, some antioxidant compounds may be sensitive to high temperature, prolonged extraction time, light exposure, or inappropriate storage conditions. Therefore, the antioxidant

activity observed in this study may have been influenced not only by solvent polarity but also by possible degradation of thermolabile compounds during extraction. Second, antioxidant activity was evaluated only using the DPPH radical scavenging assay. Although DPPH is a simple, rapid, and widely used method, it represents only one antioxidant mechanism, mainly related to hydrogen atom or electron donation. Therefore, the antioxidant potential of *Carica pubescens* seed extracts cannot be fully interpreted based only on DPPH results. Third, this study did not determine total phenolic content (TPC), total flavonoid content (TFC), or specific phytochemical marker compounds. Consequently, the relationship between solvent type, phytochemical composition, and antioxidant activity could not be confirmed directly. Future studies should include complementary antioxidant assays such as ABTS and FRAP, compare reflux with low-temperature or ultrasound-assisted extraction methods, and conduct TPC, TFC, and phytochemical profiling to identify the compounds primarily responsible for the antioxidant activity of *Carica pubescens* seed extracts.

4. Conclusion

The antioxidant activity of *Carica pubescens* seed extracts varied according to the extraction solvent used. Among the tested extracts, the 70% ethanol extract showed the strongest antioxidant activity, with an IC_{50} value of 122.95 ± 4.25 ppm, corresponding to moderate antioxidant activity based on the classification applied in this study. In contrast, the water, ethyl acetate, and n-hexane extracts showed very weak antioxidant activity, with IC_{50} values greater than 200 ppm. Although ethyl acetate and n-hexane produced higher extraction yields, their antioxidant activities were considerably weaker than that of the ethanol extract, indicating that extraction yield does not necessarily reflect antioxidant potency. These findings suggest that 70% ethanol is the most suitable solvent among those tested for extracting antioxidant-active compounds from *Carica pubescens* seeds. Statistical analysis using one-way ANOVA confirmed that extraction solvent significantly affected the antioxidant activity of *Carica pubescens* seed extracts ($p < 0.001$). Future studies should include complementary antioxidant assays and phytochemical profiling to further validate the bioactive compounds responsible for the observed antioxidant activity.

Acknowledgements:

The authors would like to thank Health Polytechnic of the Ministry of Health Malang for funding this research and also all parties who have supported this study. Funding for this research was provided by the Poltekkes Kemenkes Malang Budget Allocation (DIPA) under grant number DP 04.03/F.XX1II/1710.2/2024, issued by the Director of Health Polytechnic of the Ministry of Health Malang.

Conflicts of Interest:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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