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Antioxidant and trombolitic activity of etanol extract and fractions of carica culver (Carica pubescens) in vitro

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ABSTRACT

The prevalence of degenerative diseases arising from cell damage and free radicals continues to increase. The reactivity of oxidant compounds that exceed the limit can form a chain reaction capable of damaging parts of blood vessel cells that cause thrombolysis. Carica skin waste (*Carica pubescens*) has not been widely utilized, carica skin has the potential to have the ability as an antioxidant and thrombolytic. This study aims to determine the potential antioxidant and thrombolytic activity of carica peel. Carica peel samples were extracted by remaceration method using 70% ethanol and then fractionated with n-hexane, ethyl acetate, and water solvents. Antioxidant activity testing was carried out using the DPPH (2.2 Diphenyl-1-picrylhydrazyl) method and thrombolytic activity with the clot lysis method. The results showed that the ethyl acetate fraction had the highest antioxidant activity compared to ethanol extract and other fractions with an IC50 value of 37.04 ppm with an AAI value of 0.54. The thrombolytic activity test of ethyl acetate fraction is also the highest thrombolytic agent with clot lysis value reaching 46.06% close to the clot lysis value of nattokinase positive control of 52.39%.

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1. Introduction

The prevalence of degenerative diseases in Indonesia continues to increase and becomes a health problem every year. The results of the 2018 Riskesdas show that several degenerative diseases caused by blood vessels and cell damage are among the three largest diseases suffered [1]. In 2016, the World Health Organization (WHO) stated that degenerative diseases accounted for 43% of the causes of death in 2021, an increase of 71%.

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Degenerative diseases can be caused by an imbalance of free radicals with antioxidants in the body. This happens because degenerative diseases are mostly initiated by the presence of excessive free radicals in the body that can form highly active free radicals and can damage cell structure and function [2].
Cellular damage caused by free radicals is very harmful to the body. Free radicals are unstable, short-lived and highly reactive which can damage some cell components such as DNA, proteins and lipids. The reactivity of oxidant compounds that exceeds the limit can form a chain reaction that can damage parts of blood vessel cells, resulting in disruption of the organ work system and the emergence of degenerative diseases [3]. Damage to blood vessel cells can cause thrombosis or fibrin clots in blood vessels, as one of the factors causing cardiovascular disease [4], so substances are needed that help protect the body from free radical attacks and reduce their negative impact [5]. Antioxidants are compounds that can prevent the oxidation process of other compounds caused by free radicals, so that plaque that appears due to cell damage can be prevented. Other substances are also needed to reduce the risk of disease due to thrombus blockage, namely to break down plaques that have appeared due to cell damage can be given thrombolytic therapy so as to reduce the risk of other diseases [6], [7].

The use of synthetic drugs is decreasing due to negative side effects so its use is diverted with natural antioxidants obtained from natural ingredients. Secondary metabolites that have potential as natural antioxidants in plants are flavonoids, alkaloids, terpenoids, and saponins [8]. Meanwhile, compounds as thrombolytic agents from plants that have strong potential are phenol group compounds, namely flavonoids [9].

Carica (*Carica pubescens*) is one of the plants that act as a source of antioxidants and natural thrombolytics because this plant contains many flavonoid compounds, polyphenols, triterpenoids, and tannins [10]. The fruit of the carica plant has known antioxidant activity due to the presence of flavonoid compounds [11]. The fruit of the carica plant also has potential as a thrombolytic agent due to the proteolytic enzymes contained in the fruit [12]. Clinical studies have reported that carica fruit peel extracts contain flavonoids, alkaloids, tannins, and phenols [13]. Papaya fruit peel extract is classified as strong in providing antioxidant activity at concentrations of about 50-70 μg/mL [14]. Flavonoid and alkaloid compounds contained in papaya fruit also have thrombolytic effects [15]. Carica peels may also have great potential for antioxidant activity, as the closer the relationship the more similar the phytochemical compounds.

The utilization of carica fruit peels that contain antioxidants and thrombolytics has never been done. Therefore, this study will conduct extraction, fractionation, and testing of antioxidant and thrombolytic activities of carica fruit peels.

2. Method

2.1. Materials

The samples used were carica fruit peels taken from the Dieng area, Wonosobo Regency, Central Java. The samples were extracted by maceration, and then fractionated by liquid-liquid extraction. The chemicals used in this study are rabbit blood, ethanol analysis grade 70% and 96%, n-hexane p.a, ethyl acetate p.a, aquadest, n-butanol, ether, acetic acid, Vitamin C, Nattokinase, and DPPH.

2.2. Tools

Tools for maceration consist of maceration vessels, beaker glass, flannel cloth, mesh 40 sieve, aluminum foil, and vessels for maceration results. Tools for making extracts and fractions of carica fruit peel are separatory funnels. Other tools used are analytical balance, oven, separating funnel, grinder, rotary evaporator, drop pipette, stative, other glassware, and micropipette.

2.3. Extraction of carica fruit peel

Peel powder preparation, weighed 900 grams plus 70% ethanol in a ratio of 1: 10 b / v, then macerated in a vessel for 1 day, while stirring gradually for 6 hours. The results of the macerate were remacerated by repeating the process at least once with the same type of solvent and the amount of solvent volume was half the amount of solvent volume in the first 400 ml. After filtering the results of the maserat then evaporated at low pressure with a temperature of no more than 500C using a rotary evaporator.

2.4. Fractionation of carica fruit peel extract

To make fractions of carica fruit peel extract, carica fruit peel extract was taken and then partitioned successively using n-hexane, ethyl acetate and water solvents [16]. The soluble part of the solvent is then evaporated at low pressure with a temperature of no more than 500C using a rotary evaporator and used as a fraction to test antioxidant activity with the standard DPPH method of vitamin C and used to test thrombolytic activity with the Clot Lysis method.

2.5. Antioxidant activity

Stock solutions of ethanol extract of carica fruit peel, n-hexane fraction, ethyl acetate fraction, water fraction of carica fruit peel and nattokinase were made with 5 concentration series of extract, n-hexane fraction, ethyl acetate fraction and water fraction of 20, 40, 60, 80 and 100 ppm respectively. Vitamin C 0.5; 1; 1.5; 2 and 2.5 ppm. Vitamin C is used as a comparator with the same treatment as the test solution. Each concentration of the test solution was pipetted as much as 2.0 mL, then added 2.0 ml of 40 ppm DPPH reagent solution in flakon and allowed to stand for operating time. The absorbance was measured with a UV-Vis spectrophotometer at a predetermined wavelength. The absorbance of the carica peel extract and its fractions obtained were compared with the absorbance of DPPH to obtain the % antioxidant activity. Data from the determination of antioxidant activity with DPPH method was calculated IC50 value by using linear regression equation. After obtaining the IC50 value, it was converted into AAI value which was used to determine the antioxidant activity index.

2.6. Thrombolytic testing with clot lysis method

Each 500 µL of blood was transferred to an ependroph tube that had been weighed with an analytical balance first, then the blood was incubated at 370C for 60 minutes, then the weight was weighed as the initial clot weight. After clot formation, the serum was carefully removed and the tube weighed. 100 µl of each test solution of extract, aqueous, n-hexane and ethyl acetate fractions were added into each tube. 100 µl of nattokinase and 100 µl of distilled water were used as positive control and negative control respectively. Another negative control used was n-hexane and ethyl acetate and then the tubes were

incubated at 37°C for 90 minutes, the liquid formed from the clot was removed and the tubes were reweighed to measure clot lysis Prasad et al.[17], Thrombolytic data from the weighing of clot lysis then calculated the percentage of thrombolytic.

2.7. Data analysis

Antioxidant activity test result is IC50 value. The result of thrombolytic activity test is the percentage of clot lysis. If the data distribution is normal and the data variance is the same ($p > 0.05$), the data analysis used is One Way Anova. However, if it is not the same ($p < 0.05$) the author uses the Kruskal Wallis test. Furthermore, the Least Significantly Difference (LSD) test was used as a continuation of One Way Anova and Mann Whitney as a follow-up test to Kruskal Wallis to determine significant differences in each group. The difference between each group was considered significant if the p value was <0.05.

3. Results and Discussion

3.1. Antioxidant activity

Antioxidant activity can be known by several methods, in this study the method used is DPPH free radical capture. Absorbance readings in this study were carried out at a predetermined operating time and then calculated the percent of silencing. The results of absorbance of the test solution can be seen in Table 1.

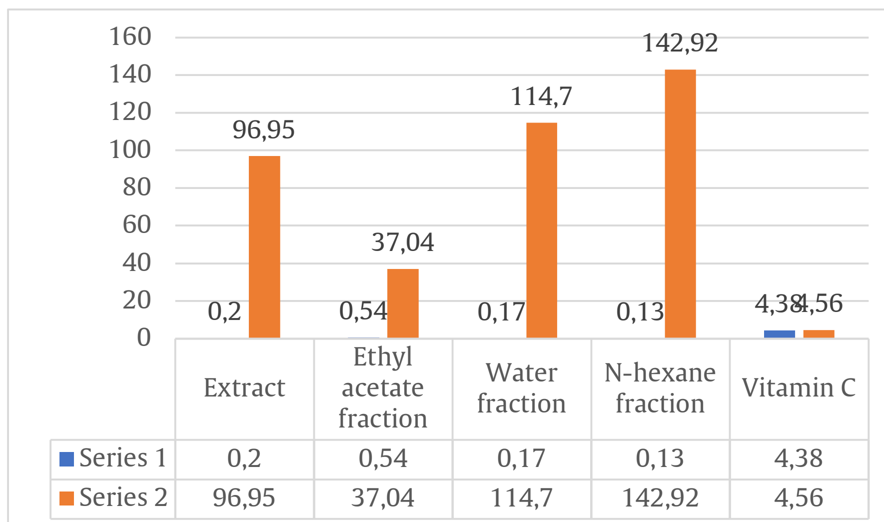
Table 1. The absorbance value of test solution

Test Solution	Concentration (ppm)	Absorbance	% inhibition
Vitamin C	0.5	0.511	11.033
	1	0.483	15.853
	1.5	0.457	20.441
	2	0.428	25.435
	2.5	0.400	30.255
Ethanol extract	20	0.413	27.347
	40	0.373	34.330
	60	0.336	40.727
	80	0.319	43.896
	100	0.277	51.056
N-hexane fraction	20	0.480	15.995
	40	0.443	22.358
	60	0.419	26.678
	80	0.381	33.216
	100	0.354	38.003
Water fraction	20	0.491	14.010
	40	0.455	20.315
	60	0.396	30.389
	80	0.352	38.412
	100	0.326	42.907
Ethyl acetate fraction	20	0.301	47.285
	40	0.286	49.912
	60	0.261	54.290
	80	0.241	57.851
	100	0.221	61.237

Based on table 1, each test solution is able to reduce the free radical levels of 40 ppm DPPH solution which is characterized by a decrease in the abosrbansi value. The concentration is directly proportional to the percent of silencing, the higher the concentration of the test solution, the higher the percent of silencing against DPPH. The mechanism of capture of DPPH radicals, from antioxidant compounds that can cause color attenuation of radicals that are purple to yellow which is nonradical. The process of color change that occurs due to the reduction of conjugated double bonds in DPPH.

The results show that the greater the concentration of the test solution, the absorbance value will decrease. The n-hexane fraction with a concentration of 20 ppm shows an absorbance value of 0.480 then at a concentration of 100 ppm there is a decrease in absorbance value of 0.354, the water fraction with a concentration of 20 ppm has an absorbance value of 0.491 then with an increase in concentration to 100 ppm there is a decrease in absorbance value to 0.326. Ethyl acetate fraction with a concentration of 20 ppm has an absorbance value of 0.301 then with increasing concentration to 100 ppm shows a decrease in absorbance value to 0.221. Vitamin C at a concentration of 0.5 ppm showed an absorbance value of 0.511 with an increase in concentration to 2.5 ppm there was a decrease in absorbance value to 0.400. The potential antioxidant activity of vitamin C, ethanol extract, n-hexane fraction, fraction, water fraction and ethyl acetate fraction can be expressed by IC50 (Inhibition Concentration), IC50 is the concentration of sample needed to capture 50% of DPPH free radicals during operating time.

Data from the n-hexane fraction, water fraction and ethyl acetate fraction of ethanol extract of carica fruit peel and vitamin C were then calculated IC50 values using a linear regression equation based on the equation Y = a + bx. After the IC50 value is known, it is then converted into an AAI value to determine the strength or weakness of the sample that acts as an antioxidant. AAI value is used to classify antioxidant properties. The IC50 value and AAI value of vitamin C test solution, ethanol extract, n-hexane fraction, fraction, water fraction and ethyl acetate fraction can be seen in Figure 1.



	Extract	Ethyl acetate fraction	Water fraction	N-hexane fraction	Vitamin C
Series 1	0,2	0,54	0,17	0,13	4,38
Series 2	96,95	37,04	114,7	142,92	4,56

Figure 1. IC50 and AAI value (Serier 1: AAI value; series 2: IC50 value)

Based on Figure 1, the ability of the samples to reduce 50% of the radicals is based on the IC50 value and the antioxidant potential is grouped into several groups based on the AAI value. Vitamin C was used as a positive control in this study, with an AAI value of 4.38 so

that it is included in a very strong antioxidant. When compared with the AAI value of ethyl acetate fraction which is 0.54, then the activity in capturing DPPH radicals is stronger than Vitamin C, this is because Vitamin C is an effective compound to reduce the action of radicals [18].

The ethyl acetate fraction has the greatest antioxidant activity when compared to the antioxidant activity of the extract, n-hexane fraction, and water fraction. The AAI value of the ethyl acetate fraction is 0.54, meaning that the ethyl acetate fraction has moderate antioxidant activity, this is because the ethyl acetate fraction contains flavonoid compounds that can inhibit oxidation reactions through a radical capture mechanism. The ethyl acetate fraction has the highest antioxidant activity compared to the other fractions because the flavonoid content in semi-polar solvents is attracted to a greater extent because semi-polar solvents can attract polar and non-polar flavonoids, where these compounds play a role in the capture of free radicals, so that the more compounds that play a role the higher the antioxidant activity [19].

The n-hexane fraction has an AAI value of 0.13, meaning that the n-hexane fraction has weak antioxidant activity, this antioxidant activity is the smallest when compared to the antioxidant activity of the ethyl acetate fraction, water fraction and ethanol extract of carica fruit peel. Rale et al.[20], stated that the n-hexane fraction is a weak antioxidant. Side groups that bind to a particular compound cause inhibition of antioxidant compound activity [21], so it is suspected that steroid compounds have side groups that can result in lower antioxidant activity. This resulted in steroids not donating hydrogen and electrons to counteract free radicals. The water fraction has an AAI value of 0.17, which means that the antioxidant activity of the water fraction is greater than the antioxidant activity of the n-hexane fraction. This is because the water fraction contains flavonoid compounds that can capture radicals.

The AAI value of ethanol extract is 0.20, meaning that the antioxidant activity of ethanol extract is smaller when compared to the antioxidant activity of the ethyl acetate fraction, but greater when compared to the water fraction and n-hexane fraction, this is because the ethanol extract has not undergone the process of separating specific compounds that have antioxidant activity, so it still contains various compounds as stated by Rale et al.[20], that ethanol extract of papaya fruit peel has weak antioxidant activity. The AAI value of ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction, and vitamin C was tested using Mann Whitney as a Kruskal Wallis follow-up test to determine significant differences in each group on the AAI value ($p \leq 0.05$). The results of the statistical analysis showed that the antioxidant quality of the ethanol extract, n-hexane fraction, water fraction and vitamin C was significantly different, but in the ethyl acetate fraction and vitamin C there was no significant difference, so it is likely that the ethyl acetate fraction has high potential as an antioxidant like vitamin C.

3.2. Thrombolytic activity

Proper citation of other works should be made to avoid plagiarism. When referring to a reference item, please use the reference number as in [16] or [17] for multiple references. The use of "Ref [18]..." should be employed for any reference citation at the beginning of sentence. For any reference with more than 3 or more authors, only the first author is to be written followed by *et al.* (e.g. in [19]). Examples of reference items of different

categories shown in the References section. Each item in the references section should be typed using 8 pt font size [20]–[25].

In vitro testing of thrombolytic activity was performed using the clot lysis method. The positive control used was nattokinase. The results of clot lysis values can be seen in Table 2.

Table 2. Thrombolytic activity test results

Sample	Volume Concentration (mg/ml)	Mean ± SD
Nattokinase	0,1	52,39 ± 1,73
Aquadest negative control		0,1 ± 0,04
Ethyl acetate negative control		0,1 ± 0,04
Ethyl acetate fraction	0,1	25,77 ± 1,16
	0,2	35,50 ± 1,23
	0,4	46,06 ± 2,02
N-hexane negative control		0 ± 0
N-hexane fraction	0,1	9,23 ± 1,71
	0,2	5,7 ± 1,32
	0,4	18,43 ± 1,83
Water fraction	0,1	10,98 ± 1,16
	0,2	20,46 ± 1,76
	0,4	28,96 ± 1,37
Extract	0,1	5,7 ± 1,32
	0,2	10,63 ± 1,26
	0,4	19,73 ± 0,97

The results of thrombolytic testing used nattokinase as a positive control, the use of nattokinase has a similar mechanism with some of the ingredients in carica fruit peel extract, namely flavonoids, which can degrade fibrin and fibrinogen [22], besides that nattokinase is used because this thromboitic agent is cheaper when compared to other thrombolytic agents such as t-PA streptokinase and urokinase. Negative controls used aquadest, n-hexane and ethyl acetate solvents, these three controls were used as solvents in each test sample. The purpose of using negative control is to determine whether the solvent used has an effect or not on the test substance [23].

The result of the thrombolytic test on the positive control of nattokinase was 52.39%. Aquadest was used as a negative control for nattokinase which had no effect on the test substance in this case rabbit blood. N-hexane and ethyl acetate solvents were also used as negative controls with a clot lysis value of 0%, which means that the three controls had no effect on blood clot lysis. Determination of the concentration used in extracts and fractions of carica fruit peel is obtained from the highest concentration in the antioxidant activity test which is 0.1 mg/ml, variation of concentration using 2N + 1 is 0.1, 0.2, and 0.4 mg/ml, because the higher the concentration, the greater the thrombolytic activity [24]. Measurement of clot lysis in the n-hexane fraction, water fraction, ethyl acetate fraction, and ethanol extract of carica fruit peel is directly proportional to the concentration of the test sample, in extract samples with concentrations of 0.1 mg/ml and 0.2 mg/ml increased by 4.96%, ethyl acetate fraction concentrations of 0.1 mg/ml and 0.2 mg/ml increased by

9.73%, n-hexane fraction at these concentrations increased by 9.20% and water fraction increased by 9.48%.

The increase in clot lysis activity with increasing concentration may be due to the greater the concentration, the greater the amount of secondary metabolites contained in the suspension of test samples that act as thrombolytic agents. The results of the calculation of the percentage of clot lysis of extracts and fractions can be seen in appendices 23 to 28 In the administration of each fraction with the lowest concentration of 0.1 mg/ml and an incubation time of 90 minutes, the ethyl acetate fraction was able to provide a lysing effect of 25.77%, this value is significantly different from the negative control which is only 0, 01% at 90 minutes incubation, at the same incubation time giving 0.2 mg/ml ethyl acetate fraction showed a greater lysis effect than 0.1 mg/ml, and the highest lysis effect was in giving 0.4 mg/ml fraction which was 46.51%, the value was significantly different from the positive control, but the value was close to the positive control which was 52.39%.

The results of the blood clot lysis value show that the ethyl acetate fraction of carica fruit peel has the ability to lyse the highest blood clot of the n-hexane fraction and the water fraction. The ethyl acetate fraction contains compounds capable of degrading fibrin, namely flavonoids and alkaloids where these compounds are able to degrade fibrin [25]. Thrombolytic activity in the ethyl acetate fraction is thought to be due to the content of flavonoids and alkaloids. Clinical studies show that flavonoids can increase tissue plasminogen activator (t-PA), reduce the content of plasminogen activator inhibitor-1 [26]. Based on the test results at the same three test concentrations, the n-hexane fraction, water fraction, ethyl acetate fraction and ethanol extract of carica fruit peel have thrombolytic activity.

The clot lysis value of ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction, and nattokinase was tested using the Oneway Anova test, so that the overall effect between concentrations was known ($p \leq 0.05$). The results of statistical analysis showed that the thrombolytic activity of ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction against the negative control, so it can be concluded that the sample has thrombolytic activity. Significant differences also occur in the positive control of nattokinase, but the ethyl acetate fraction is close to the positive control of nattokinase, these results can be concluded that the ethyl acetate fraction has the highest potential as a thrombolytic.

4. Conclusion

The extract, n-hexane fraction, water fraction, and ethyl acetate fraction of carica fruit peel (*Carica pubescens*) have antioxidant and thrombolytic activities. The ethyl acetate fraction has the strongest antioxidant activity characterized by an IC50 value of 37.06 and an AAI value of 0.54. The ethyl acetate fraction has the strongest thrombolytic activity characterized by a clot lysis value of 46.06%.

REFERENCES

[1] L. . Linda, O. and Rahayu, "Prevensi Awal Dan Lanjutan Penyakit Degeneratif Untuk Usia Dewasa Di Masa Pandemi Covid-19," *J. Arsip Pengabdi. Masy.*, vol. 2, no. 1, hal. 107–115, 2021.

[2] V. P. Reddy, "Oxidative Stress in Health and Disease," *Biomedicines*, vol. 11, no. 11, hal. 1–17, 2023, doi: 10.3390/biomedicines11112925.
[3] A. I. Maharani *et al.*, "Peran Antioksidan Alami Berbahan Dasar Pangan Lokal dalam Mencegah," *Pros. Semin. Nas. Bio*, hal. 390–399, 2021.
[4] S. D. Chandrasekaran *et al.*, "Exploring the In Vitro Thrombolytic Activity of Nattokinase from a New Strain Pseudomonas aeruginosa CMSS," *Jundishapur J. Microbiol.*, vol. 8, no. 10, 2015, doi: 10.5812/jjm.23567.
[5] E. R. Yuslianti, *Pengantar Radikal Bebas dan Antioksidan*. Yogyakarta: Budi Utama, 2018.
[6] M. R. Ali *et al.*, "Aspect of Thrombolytic Therapy: A Review," *Sci. World J.*, vol. 2014, no. 1, 2014, doi: 10.1155/2014/586510.
[7] N. Mackman, W. Bergmeier, G. A. Stouffer, dan J. I. Weitz, "Therapeutic strategies for thrombosis: new targets and approaches," *Nat. Rev. Drug Discov.*, vol. 19, no. 5, hal. 333–352, 2020, doi: 10.1038/s41573-020-0061-0.
[8] A. Sari, "Berbagai Tanaman Rempah sebagai Sumber Antioksidan Alami," *Elkawnie*, vol. 2, hal. 203, Des 2016, doi: 10.22373/ekw.v2i2.2695.
[9] M. Bojić, Z. Males, A. Antolić, I. Babic, dan M. Tomicic, "Antithrombotic Activity of Flavonoids and Polyphenols Rich Plant Species," *Acta Pharm.*, vol. 69, hal. 483–495, Des 2019, doi: 10.2478/acph-2019-0050.
[10] E. Minarno, "Skrining Fitokimia dan Kandungan Total Flavonoid pada Buah Carica pubescens Lenne & K. Koch Di Kawasan Bromo, Cangar, dan Dataran Tinggi Dieng," *el–Hayah*, vol. 5, hal. 73, Mar 2015, doi: 10.18860/elha.v5i2.3022.
[11] H. Sasongko, R. Guswinda, A. Yugatama, Y. Farida, dan S. S, "Antidiabetic and Antioxidant Effect Combination Vasconcellea pubescens A.DC. and Momordica charantia L. Extract in Alloxan- Induced Diabetic Rats," *Pharmacogn. J.*, vol. 12, hal. 311–315, Mar 2020, doi: 10.5530/pj.2020.12.49.
[12] H. R. Yang *et al.*, "Unveiling the Potent Fibrino(geno)lytic, Anticoagulant, and Antithrombotic Effects of Papain, a Cysteine Protease from Carica papaya Latex Using κ-Carrageenan Rat Tail Thrombosis Model," *Int. J. Mol. Sci.*, vol. 24, no. 23, hal. 1–15, 2023, doi: 10.3390/ijms242316770.
[13] A. K. Martial-Didier, K. K. Hubert, K. E. Jean Parfait, dan T. Kablan, "Phytochemical Properties and Proximate Composition of Papaya (Carica papaya L. var solo 8) Peels," *Turkish J. Agric. - Food Sci. Technol.*, vol. 5, no. 6, hal. 676–680, 2017, doi: 10.24925/turjaf.v5i6.676-680.1154.
[14] L. Marliani, R. Velayanti, dan A. Roni, *Aktivitas Antioksidan dan Tabir Surya pada Ekstrak Kulit Buah Pepaya (Carica papaya L.)*. Bandung: JSTF, 2015.
[15] P. Kubatka *et al.*, "Antithrombotic and Antiplatelet Effects of Plant-derived Compounds: a Great Utility Potential for Primary, Secondary, and Tertiary Care in the Framework of 3P Medicine," *EPMA Journal,* vol. 13, Agu 2022, doi: 10.1007/s13167-022-00293-2.
[16] W. S. Tatiana, *Isolasi dan Analisis Tumbuhan Obat*. Pustaka Baru Press, 2021.
[17] S. Prasad, R. S. Kashyap, J. Y. Deopujari, H. J. Purohit, G. M. Taori, dan H. F. Daginawala, "Development of an In Vitro Model to Study Clot Lysis Activity of Thrombolytic Drugs," *Thromb. J.*, vol. 4, hal. 9–12, 2006, doi: 10.1186/1477-9560-4-14.
[18] U. Maria dan A. Mua'wannah, "Uji Aktivitas Antioksidan Fraksi n_heksan Ekstrak Etanol Daun Carica (carica pubescens) dan Identifikasi Senyawa Alkaloid dan Flavonoidnya," Universitas Wahid Hasyim, 2015.
[19] M. Ruoying *et al.*, *Antioxidant Activities And Phenolic Compounds Analysis of Extractions of Carica papaya Seed Residue*. 2016.
[20] S. Rale, H. Hasim, dan H. R. Falah Taj Abadi, "Antioxidant Activity, Inhibition α-Glucosidase of Ethanol Extract of Strychnos nitida G. Don and Identification of Active Compounds," *Curr. Biochem.*, vol. 5, hal. 11–20, Mar 2019, doi: 10.29244/cb.5.3.11-20.
[21] Y. Tao, H. Zhang, dan Y. Wang, "Revealing and Predicting the Relationship Between the Molecular Structure and Antioxidant Activity of Flavonoids," *Lwt - Food Sci. Technol.*, vol. 174, no. September 2022, hal. 114433, 2023, doi: 10.1016/j.lwt.2023.114433.
[22] Y. Weng, J. Yao, S. Sparks, dan K. Y. Wang, "Nattokinase: An oral antithrombotic agent for the prevention of cardiovascular disease," *Int. J. Mol. Sci.*, vol. 18, no. 3, hal. 1–13, 2017, doi: 10.3390/ijms18030523.

[23] L. Sari, S. Sumpono, dan E. Elvinawati, "Uji Efektifitas Asap Cair Cangkang Buah Karet (Hevea braziliensis) sebagai Antibakteri Bacillus subtilis," *Alotrop*, vol. 3, Sep 2019, doi: 10.33369/atp.v3i1.9033.

[24] P. Srinivasan, M. Jayagopal, dan J. Ramachandran, "Evaluation of In-Vitro Antioxidant and Fibrinolytic Activity of Flavonoid-Rich Fraction from the Whole Plant of Wedelia chinensis," *Asian J. Pharm. Clin. Res.*, vol. 9, hal. 234, Des 2016, doi: 10.22159/ajpcr.2016.v9s3.13419.

[25] W. Kasifa, M. Rohmah, D. Fickri, dan K. Wahyuni, "Uji Aktivitas Fibrinolisis Ekstrak Alkaloid Total Rimpang Lengkuas Merah (Alpinia purpurata (Vielli) K.Schum) secara In Vitro," *J. Pharm. Care Anwar Med.*, vol. 2, Jun 2019, doi: 10.36932/j-pham.v2i1.29.

[26] J. P. Quintal Martínez dan M. R. Segura Campos, "Flavonoids as a Therapeutical Option for the Treatment of Thrombotic Complications Associated with COVID-19," *Phyther. Res.*, hal. 1–23, 2022, doi: 10.1002/ptr.7700.