Jurnal Riset Kimia

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

Analysis of Total Phenolics, Flavonoids, and Antioxidant Activity of Cashew Leaf Extract (*Anacardium occidentale L.*) with Varying Ethanol Concentrations

Risma Kurniasari[1], Meiny Suzery[1], Bambang Cahyono[1*]
[1]*Department of Chemistry, Diponegoro University, Tembalang, Semarang, Indonesia*

Corresponding Author:
Bambang Cahyono
cahyono@live.undip.ac.id

Received: July 2024
Accepted: September 2024
Published: September 2024

Abstract

This study evaluated the effectiveness of various ethanol concentrations (50%, 70%, and 96%) in extracting bioactive compounds from cashew leaves *(Anacardium occidentale).* Parameters analyzed included total phenolics, total flavonoids, and antioxidant activity using the DPPH and H_2O_2 methods. The results showed that the 70% ethanol extract produced the highest total phenolic and flavonoid content as well as significant antioxidant activity compared to other ethanol concentrations. This study offers practical insights for pharmaceutical, cosmetic, and nutraceutical industries in selecting the optimal solvents for cashew leaf extraction. Additionally, the analysis showed that the 70% ethanol extract offered an optimal balance between extraction efficiency and antioxidant activity, making it the most efficient solvent for commercial use.

Keywords: *Anacardium occidentale, total phenolics, total flavonoids, antioxidant activity, ethanol extraction*

Introduction

The pharmaceutical and natural medicine industry relies heavily on the quality of extracts produced from herbal ingredients[1]. This extract contains various bioactive compounds such as phenolics, flavonoids and antioxidants which have significant health benefits. The quality of this extract is greatly influenced by the type of solvent used in the extraction process [2]. Therefore, research that focuses on the effect of solvents on the quality of herbal medicine extracts is very important for this industry.

Several studies have compared various solvents for the extraction of phenolic compounds, flavonoids, and antioxidants. The results show that aqueous ethanol is an effective solvent. For example, research on the hairy roots of *Scutellaria baicalensis* showed that aqueous ethanol extract had the highest total phenolic content, total flavonoid content and antioxidant activity compared with other solvents [3]. Other research on winemaking has also shown that aqueous ethanol is effective in extracting flavonoids, phenolics and antioxidant compounds [4]. Research on *Opuntia stricta* fruit found that aqueous ethanol is one of the most effective solvents for the extraction of phenolic and flavonoid compounds as well as antioxidant activity [5]. The main reasons for the widespread use of ethanol in extraction are that it is realtively non-toxic compared to other solvents such as acetone and methanol, readily available, and applicable in various extraction methods [6], [7].

Apart from the solvent used, the phenolic content, flavonoids and antioxidant activity are also influenced by the plant species used as raw materials. Each plant species has a unique

DOI: https://doi.org/ 10.25077/jrk.v15i2.735

chemical composition that influences its phenolic and flavonoid profile and antioxidant activity [8]. For example, research shows that the phenolic content and antioxidant activity of *Scutellaria baicalensis, Boletus edulis,* and *Opuntia stricta* vary greatly depending on the species. This variation is caused by genetic differences and the specific metabolism of each species which results in different bioactive compound profiles[9].

Cashew leaves *(Anacardium occidentale)* are one of the herbal plant species listed in the Indonesian herbal pharmacopoeia (herbal no. 36, FHI) with quality standards through physical properties and total flavonoids of at least 46 mg rutin equivalent per gram of extract[10]. The selection of cashew leaves for this research was based on its high content of bioactive compounds, such as phenolics and flavonoids, which have the potential to act as natural antioxidants. Previous research shows that cashew leaf extract has significant antioxidant activity, is able to ward off free radicals, and reduces the risk of chronic diseases such as cancer and cardiovascular disease [11]. The flavonoids in cashew leaves have also been shown to have biological activities such as antidiabetic, anticycling, and antibacterial.[12].

Basically, cashew nuts are just one of Indonesian herbal commodities whose quality is tested through total flavonoids [10]. Of the 253 monographs (127 types of Indonesian herbal plants) recorded, there are only 116 monographs (58 plants) whose quantitative bioactive testing is standardized using this parameter. However, in this document, each commodity has a different reference standard. For example, standard flavonoids in the form of apigenin are used for *Apium graveolens* leaves, quercetin for *Moringa oleifera* leaves and *Carthamus tinctorius* flowers, and rutin for *Chromolaena odorata* leaves and *Anacardium occidentale*. Although the use of these standards indicates the activity of the main compounds in each plant, there are weaknesses in its implementation. The use of different reference standards causes difficulties in comparing total phenolics from one plant to another. Therefore, in this research two standards are used, and a conversion formula will be made from one standard to another. The same thing will also be done for the total phenolic parameters. The majority of studies on total phenolics and all analyzes in the Indonesian Herbal Pharmacopoeia used gallic acid as a standard, while several other studies used catechins as a standard [13].

The majority of research on total phenolics and total flavonoids is always related to antioxidant activity which is usually measured through DPPH reduction. However, many reports show that DPPH cannot interpret the situation in the body because these radicals do not exist in the body[14],[15],[16]. Therefore, antioxidant research with H_2O_2 is considered more relevant because the hydroxyl radicals (HO·) produced are closely related to reactive oxygen species (ROS) in the human body. [17]In the final part of this research, comparative data between H_2O_2 and DPPH as well as the possibility of data conversion between the antioxidant activity of DPPH and the antioxidant H_2O_2 will be presented.

Experimental

Materials

Simplisia cashew leaves were obtained from suppliers in Semarang. The chemicals used have pro analytical grade from Sigma Aldrich, including $AlCl_3$, DPPH, CH_3COONa, rutin, quercetin, catechin, and gallic acid. Ascorbic acid, Folin-Ciocalteau, H_2O_2, phosphate buffer pH 7.4, chloroform, HCl, Na_2CO_3, $FeCl_3$, $CHCl_3$, and Mg.

Equipment/Instruments

The equipment used includes analytical scales (Ohaus 221g/0.0001 g), evaporator (RE100-Pro), UV-Vis spectrophotometer (Genesys 10S UV-Vis), 200 µg and 1000 µg micropipettes (BioRad), grinder blender (MKS- ML500).

Methods

Preparation and Characterization of Extract Physical Properties

A total of 50 grams of simplicia powder was macerated using 50%, 70% and 96% ethanol solvent with a simplicia to solvent ratio (1:5). The solvent was changed every 24 hours five times. The resulting filtrate was evaporated using a rotary evaporator at a temperature of 60° C. The extract obtained was then weighed and its physical properties were characterized[10].

Phytochemical Screening

Phytochemical screening was carried out according to the method described. [18] The analysis included tests for phenolics, steroids, saponins, quinones, flavonoids and tannins.

Total Phenolic Analysis

Quantitative analysis of total phenolics. The total phenolics of the three samples were analyzed using the Folin-Ciocalteu reagent method with several modifications [19]. The standards used were catechin and gallic acid (30-70 ppm) and extract (500 ppm). A total of 0.5 mL of sample was added with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Then, 1 mL of 7,5% Na_2CO_3 was added and incubated again for 30 minutes. The absorbance of each solution was measured using a UV-Vis spectrophotometer at 740 nm for catechin and 765 nm for gallic acid. Results were expressed as mg catechin equivalent to grams of extract (mg CAE/g) and mg gallic acid equivalent to grams of extract (mg GAE/g).

How to convert total phenolics (mg GAE/g) to (mg CAE/g). The concentrations of gallic acid and catechin in the five samples were calculated at the same absorbance values (0.2; 0.4; 0.6; 0.8; 1) *. Next, a graph was made between the concentrations of catechin (x) and gallic acid (y) at various equivalent absorbance values until an equation was obtained. From this equation, the x value (mg CAE/mg) is converted through the known y value (mg GAE/mg), and vice versa.

* Additional eucalyptus and guava data were obtained from one research team to improve the accuracy of the calculation analysis.

TPC suitability analysis of laboratory results and calculations. The correspondence between TPC laboratory results and calculation results was tested using the *Bland-Altman test* and *Paired t-test.*

Total Flavonoid Analysis

Quantitative analysis of total flavonoids. The total flavonoids of the three samples were analyzed using the $AlCl_3$ method with several modifications [20]. The standards used are quercetin and rutin (2-10 ppm) and extract (3000 ppm). A total of 1 mL of sample was added with 3 mL of methanol pa and 0.2 ml of 10% $AlCl_3$. Then added 0.2 ml CH_3COONa 1 M and distilled water to the limit. The solution was incubated for 30 minutes, and absorbance was measured using a UV-Vis spectrophotometer at 420 nm for quercetin and 438 nm for rutin. Results were expressed as mg quercetin equivalent to grams of extract (mg QE/g) and mg rutin equivalent to grams of extract (mg RE/g).

How to convert total flavonoids (mg QE/g) to (mg RE/g). Total flavonoid equivalents of rutin (x) and quercetin (y) of the five extracts were graphed to obtain equation*. From this equation, the x value (mg RE/mg) can be converted via the known y value (mg QE/mg), and vice versa.

* Additional eucalyptus and guava data were obtained from one research team to improve the accuracy of the calculation analysis.

TFC suitability analysis of laboratory results and calculations. The correspondence between TFC laboratory results and calculation results was tested using the *Bland-Altman test* and *Paired t-test.*

Antioxidant Activity Analysis

Analysis of DPPH antioxidant activity. Antioxidant activity was determined using the DPPH assay with several modifications [21]. The

DOI: https://doi.org/ 10.25077/jrk.v15i2.735

test was carried out using quercetin (1-5 ppm) as a positive control and extract (5-25 ppm). A total of 1 ml of sample was added with 3 mL of 40 ppm DPPH and incubated for 30 minutes. Next, absorbance was measured using a UV-Vis spectrophotometer at 517 nm.

Analysis of H_2O_2 Antioxidant Activity. Antioxidant activity was determined using the H_2O_2 test [21]. A total of 2 mL of extract or ascorbic acid (5-25 ppm) was added with 4 mL of 20 mM H_2O_2 solution in phosphate buffer (pH 7.4). The solution was macerated for 10 minutes and absorbance was measured using a UV-Vis spectrophotometer at 227 nm against a phosphate buffer blank.

How to convert IC_{50} DPPH (ppm) to IC_{50} H_2O_2. IC_{50} DPPH (x) with IC_{50} H_2O_2 (y) the six extracts are graphed to obtain the equation*. From this equation, the value of x (IC_{50} DPPH) can be known through the value of y (IC_{50} H_2O_2) which was previously known, and vice versa.

*Data for the six extracts was obtained from one research team to increase the accuracy of the calculation analysis.

Analysis of suitability of IC_{50} laboratory results and calculations. Correspondence between IC_{50} laboratory results and calculation results was carried out using the *Bland-Altman test* and *Paired t-test.*

Results and Discussion

Results of Extract Preparation and Phytochemical Screening

Extraction of cashew leaf samples *(Anacardium occidentale)* using the maceration method with three types of solvents, namely 50%, 70% and 96% ethanol, produces physical properties and yields that are in accordance with those listed in the Indonesian Herbal Pharmacopoeia (FHI). The three ethanol extracts also produced good yields because they exceeded the FHI standard, namely a minimum of 7.8% (Table 1).

Table 1. Results of extraction and phytochemical screening of simplicia and cashew leaf extract

Parameter	Extract 50%	Extract 70%	Extract 96%	FHI
Yield (%)	12,13	9.49	8.4	7.8
Water content (%)	10.66	8.38	12.06	19
Physical Properties				
Aroma	No smell	No smell	No smell	No smell
Texture	Thick	Thick	Thick	Thick
Color	Chocolate	Dark chocolate	Blackish green	Tanned
Phytochemical screening				**Notes**
Phenolics	+	+	+	Blackish blue
Steroids	-	+	+	Green-blue
Saponin	+	+	+	2 cm of foam for 10 minutes
Quinones	+	+	+	Redness
Flavonoids	+	+	+	Yellow
Tannin	+	+	+	Black

FHI: Indonesian Herbal Pharmacopoeia

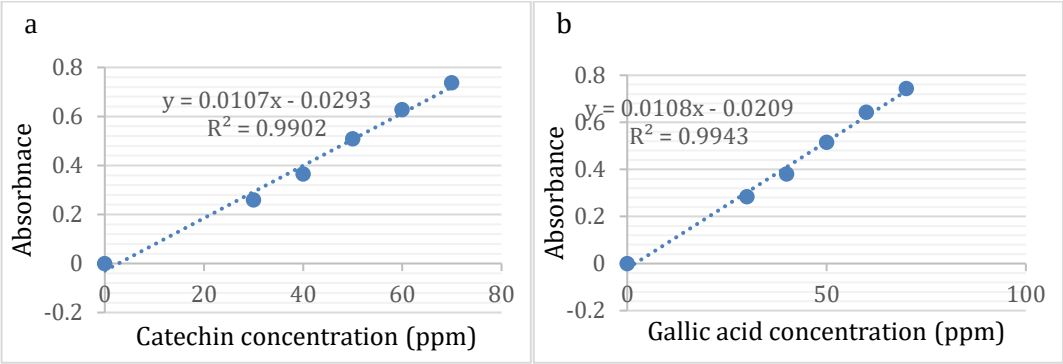

Figure 1. Calibration curve of a) catechin standard, b) gallic acid standard

Table 2. Results of Total Phenolics of Cashew Leaf Extract

Solvent	TPC	
	mg CE/g extract	**mg GAE/g extract**
Ethanol 50%	101.16 ± 1.189	101.13 ± 0.786
Ethanol 70%	126.17 ± 0.925	126.31 ± 0.786
Ethanol 96%	116.39 ± 0.793	115.76 ± 0.262

TPC: Total Phenolic Compound, mg CE/g extract: milligram Catechin Equivalent per gram extract, mg GAE/g extract: milligram Gallic Acid Equivalent per gram extract

The order of yield obtained by the three extracts was 50% ethanol extract > 70% ethanol > 96% ethanol, which means that the lower the ethanol concentration used, the higher the extraction yield obtained. This indicates that a more polar solvent produces a higher extract yield, possibly due to the high content of primary metabolites such as protein and carbohydrates which are also extracted, thus increasing the yield obtained [22] Simplicia and the three ethanol extracts of cashew leaves *(Anacardium occidentale)* showed positive results for six types of phytochemicals, except for the 50% ethanol extract which did not show the presence of steroid compounds. This shows that both simplicia and 50%, 70% and 96% ethanol extracts of cashew leaves contain phenolic and flavonoid compounds.

Determination of Total Phenolic Content

Phenol is a secondary metabolite in plants that has antioxidant activity by reducing free radicals using hydroxyl groups. Analysis of total phenolic content in three extracts using Folin-Ciocalteu reagent was carried out with two standards: catechin and gallic acid. The aim of using these two standards was to compare the total phenolic content of these three extract samples with other samples in the literature. The total phenolic content was obtained from a calibration curve with the equation y=0.0107x+0.0293 (R^2=0.9902) at a wavelength of 740 nm for catechin, and y=0.0108x+0.0209 (R^2=0.9945) at a wavelength of 765 nm for gallic acid (Figure 1).

Ethanol 70% was the most effective solvent for extracting phenolics from cashew leaves (Table 2). This can be attributed to the ideal balance between ethanol and water that increases the solubility and optimal extraction of phenolics. Meanwhile, 50% ethanol was less effective, possibly because the higher water content reduced extraction efficiency. The 96% ethanol solvent was also effective, but slightly less so than 70% ethanol, indicating that a small amount of water in the solvent may aid in the extraction process. These results are in line with several studies showing that ethanol

DOI: https://doi.org/ 10.25077/jrk.v15i2.735

concentrations of around 70% are generally optimal for extracting phenolics from various types of plant materials. 70% ethanol increases extraction efficiency because it can balance the polarity of water and ethanol, thereby maximizing the release of phenolic compounds from the plant matrix [23] [24] [25] [26]. Higher or lower concentrations of ethanol may be less effective because they cannot properly extract phenolic compounds from plant tissue or cause loss of compounds due to evaporation or degradation at high concentrations.

We have created a conversion formula based on the standard concentration of gallic acid (y) with the concentration of catechin (x) of some of the same absorbance values (Figure 2). This equation was built to predict the price of total phenolic equivalents of catechin (mg CE/g extract) using the total phenolic equivalents of gallic acid (mg GAE/g extract) which had been known previously and vice versa.

Based on the equation y=0.9848x-0.3551 with a correlation coefficient of 1 (Figure 2), the total price of phenolic equivalents of gallic acid (y) can be converted into catechin equivalents (x) well. A comparison of the total price of catechin equivalent phenolics both from the laboratory and those obtained from calculations can be seen in Table 3. Based on the *Bland-Altman analysis* , it is known that the two sets of catechin equivalent TPC data, namely laboratory results and conversion calculation results, have an average difference of 0.5401 with the limit The upper limit is 7.423 and the lower limit is -6.342, which means that there is no significant and consistent bias in these two data. Based on *the Paired t-test*, it is known that the calculated t value is (0.344) < t table (2.776) which shows that Ho is accepted and means that there is no significant difference between the total phenolic equivalents of catechin from the laboratory results and the conversion calculation results.

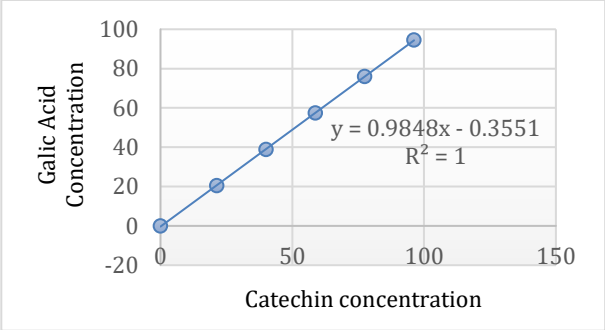

Figure 2. Relationship between gallic acid vs catechin concentrations at various absorbance values

Table 3. Comparison of laboratory and calculated catechin TPC

Sample	TPC Gallic acid equivalent (laboratory)	TPC Catechin equivalents (laboratory)	TPC Catechin equivalents (calculation)	Difference
Ethanolate 50%	101.13	101.16	103.051	-1.89
Ethanolate 70%	126.31	126.17	128.620	-2.45
Ethanolate 96%	115.76	116,39	117.907	-1,52
Eucalyptus	207.41	215.27	210.972	4.29
Cashew	186.45	193.95	189.688	4.26

Note: TPC (Total Phenolic Content), additional data for eucalyptus and cashew nuts obtained from a research team to increase the accuracy of the calculation analysis

Table 4. Application of conversion equations to total phenolics in other studies

Literature	Sample	TPC (mg GAE/g extract) *	TPC (mg CE/g extract) *	Order
[21]	*Leucas cephalotes*	164.96	**167.87**	1
[27]	*Zanthoxylum armatum*	137.72	**140.21**	3
[28]	*Garcinia forbesi*	127.83	**130.16**	4
[29]	*Corrylus avellana*	**153.27**	156	2
[30]	*Vigna angularis*	**87.98**	89.7	5
[31]	*Lens culinaris*	**66.61**	68	6

* Bold print is the converted TPC, TPC (GAE): Total phenolic equivalents of gallic acid, TFC (CE): Total phenolic equivalents of catechins

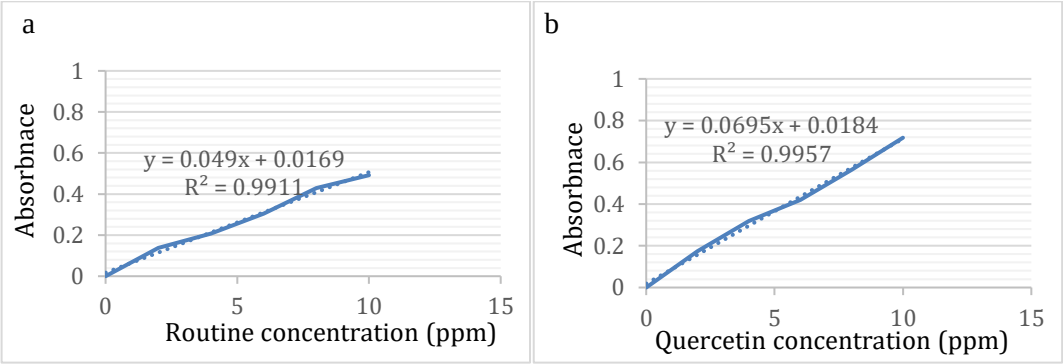

Figure 3. Calibration curve of a) rutin standard, b) quercetin standard

This finding has been applied to several total phenolic results from other researchers who only used one standard, either gallic acid or catechin, so that total phenolics cannot be compared between one sample and another sample using a different standard (table 4). From the results of this study, researchers have succeeded in ranking the total phenolic content of the six samples obtained from the literature.

Determination of Total Flavonoid Content

Flavonoids are secondary metabolites that have antioxidant activity depending on the amount and position of free OH. Analysis of total flavonoid content was carried out using standards in the form of quercetin and rutin because both are often reported as reference standards in the analysis of total flavonoids. [32]The total flavonoid content was obtained from a calibration curve with the equation y=0.049x+0.0169 at a wavelength of 420 nm for rutin and y=0 .0695x+0.0184 at a wavelength of 438 nm for quercetin. The x-axis shows the absorbance and the y-axis shows the concentration of the standard solution (µg/mL) equivalent to mg RE/g extract and mg QE/g extract (Figure 3).

Table 5 shows that the 70% ethanolic extract obtained the highest yield, followed by the 96% ethanolic extract, and the lowest was the 50% ethanolic extract. This result is higher than the total flavonoids of cashew leaf extract with 96% ethanol solvent listed in the Indonesian Herbal Pharmacopoeia, which is a minimum of 4.6% against rutin standards. The high total flavonoids in the 70% ethanol extract compared to the 50% and 96% ethanolic extracts can be attributed to the ideal balance between ethanol and water in dissolving flavonoids optimally. These results are in accordance with several

DOI: https://doi.org/ 10.25077/jrk.v15i2.735

studies showing that an ethanol concentration of around 70% is generally optimal for extracting flavonoids from various types of plant material [22].

We have created a conversion formula based on the equation of results between the concentrations rutin and quercetin at several absorbance values which are equivalent to the graph presented in Figure 6. This equation is intended to predict the price of total flavonoid equivalents rutin (mg RE/g extract) using total flavonoid equivalents of quercetin (mg QE/g extract) are known.

In contrast to the total phenolic conversion results which show a small difference between the total phenolic equivalents of catechins from laboratory results and calculation results, based on the equation y=0.7043x-0.0105, the total flavonoid equivalents from rutin laboratory results and calculation results show large differences (Table 6). This is thought to be because each extract has a non-uniform flavonoid content.

Table 5. Results of Total Flavonoids from Cashew Leaf Extract

Solvent	TFC	
	mg RE/g	mg QE/g
Ethanol 50%	40.82 ± 0.289	24.27 ± 0.712
Ethanol 70%	52.08 ± 0.433	30.39 ± 0.068
Ethanol 96%	50.07 ± 1.539	28.01 ± 0.577

TFC: Total Flavonoid Compound, mg RE/g extract: milligram Rutin Equivalent per gram extract, mg QE/g extract: milligram Quercetin Equivalent per gram extract

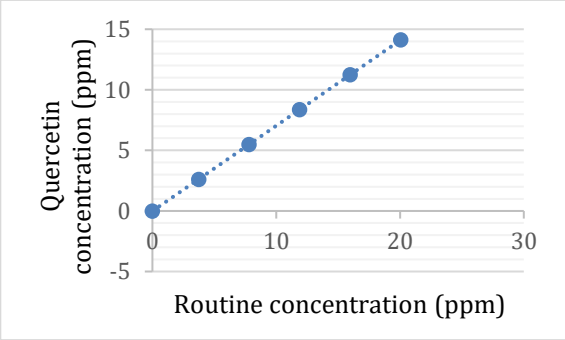

Figure 4. Relationship between concentrations of quercetin vs. rutin at various equivalent absorbance values

Table 6. Comparison of rutin TFC laboratory results and calculations

Sample	Quercetin equivalent TFC (laboratory)	Rutin equivalent TFC (laboratory)	Quercetin equivalent TFC (calculation)	Difference
Ethanolate 50%	24.273	40.823	34.479	6.344
Ethanolate 70%	30.388	52.082	43.161	8.920
Ethanolate 96%	28.014	50.075	39.790	10.284
Eucalyptus	15.875	29.908	22.555	7.352
Cashew	13.625	25.47	19.360	6.109

TFC: Total Flavonoid Compound, TFC quercetin (mg QE/g extract), TFC rutin (mg RE/g extract)

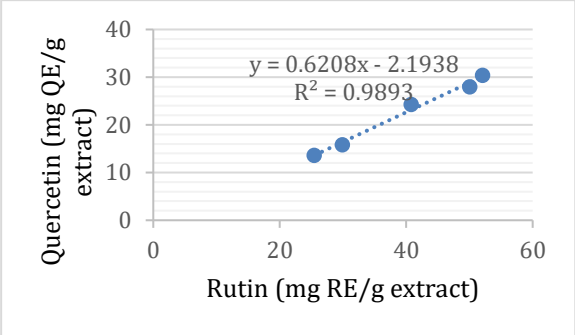

Figure 5. Relationship between total flavonoid equivalents of quercetin vs total flavonoid equivalents rutin

Table 7. Comparison of rutin TFC laboratory results and calculations

Sample	Quercetin equivalent TFC (laboratory)	Rutin equivalent TFC (laboratory)	Rutin equivalent TFC (calculation)	Difference
Ethanolate 50%	24.273	40.823	42.6333	-1.810
Ethanolate 70%	30.388	52.082	52.484	-0.402
Ethanolate 96%	28.014	50.075	48.659	1.416
Eucalyptus	15.875	29.908	29.106	0.802
Cashew	13.625	25.47	25.481	0.011

TFC: Total Flavonoid Compound, TFC quercetin (mg QE/g extract), TFC rutin (mg RE/g extract), additional data on eucalyptus and cashew nuts were obtained from one research team to improve the accuracy of the calculation analysis

Furthermore, we made a relationship between the price of total flavonoid equivalents of quercetin and the price of total flavonoid equivalents rutin (Figure 5). This graph will then be used to determine total flavonoids from mg QE/g extract to mg RE/g extract, or vice versa.

Based on the equation y=0.6208x – 2.1938 with a correlation coefficient of 0.9946, the total price of flavonoid equivalents of quercetin (y) can be converted into rutin equivalents (x) and vice versa. The results of a comparison of the total prices of rutin flavonoid equivalents, both from the laboratory and calculations, can be seen in (Table 7).

Bland-Altman analysis , it is known that the two rutin equivalent TFC data sets, namely laboratory results and conversion calculation results, have an average difference of -0.0011 with an upper limit of 2.417 and a lower limit of -2.419, which means that these two data do not have significant bias and consistent. Based on *the Paired t-test* , it is known that the calculated t value is (0.00192) < t table (2.776) which shows that Ho is accepted and means that there is no significant difference between the total flavonoid equivalents of rutin laboratory results and the results of conversion calculations. This finding has been applied to several total flavonoid results from other researchers who used only one standard, either rutin or

 DOI: https://doi.org/ 10.25077/jrk.v15i2.735

quercetin, which prevented direct comparison of total flavonoids between one sample and another sample that used different standard (table 8).

H_2O_2 Antioxidant Activity Test

This research used two different methods, namely the DPPH and H_2O_2 methods to determine the antioxidant activity of cashew leaf extract. The IC_{50} results of the three extracts obtained from both methods show that the IC_{50} results from the DPPH method tend to be lower than the IC_{50} from the H_2O_2 method. This shows that the DPPH method is more sensitive in evaluating the antioxidant activity of these three samples [36][37]. Comparison of antioxidant activity using DPPH and H_2O_2 is presented in table 9.

The three samples using both the DPPH and H_2O_2 methods show that the higher the sample concentration, the higher its ability to ward off free radicals (Figure 6). Based on the % inhibition, both methods have the same order of results, namely 70% ethanol extract has the highest radical scavenging activity at concentrations between 5 µg/mL to 25 µg/mL, followed by 96% ethanolate, and 50% ethanolate is in the lowest position. However, the three extracts have very strong antioxidant activity because they have an IC_{50} < 50 ppm. According to [38] The smaller the IC_{50}, the stronger the antioxidant activity, where IC_{50} < 50 ppm means very active, IC_{50} 50-100 ppm means active, IC_{50} 101-250 ppm means medium, and IC_{50} 250-500 ppm means inactive.

Table 8. Application of conversion equations to total flavonoids in other studies

Literature	Sample	TFC (mg QE/g extract) *	TFC (mg RE/g extract) *	Order
[21]	*Leucas cephalotes*	36.95	**63,054**	2
[27]	*Zanthoxylum armatum*	76.58	**126.89**	1
[28]	*Garcinia forbesii*	35.97	**61,475**	3
[33]	*Epilobium angustifolium*	**34,309**	58.8	4
[34]	*Ximenia americana*	**4.4**	10.64	6
[35]	*Buckwheat*	**12,215**	23,21	5

*Bold print is the conversion result, TFC (QE): total flavonoid equivalent to quercetin, TFC (RE): total flavonoid equivalent rutin

Table 9. Antioxidant activity of cashew extract

Solvent	IC_{50} (ppm)	
	DPPH	H_2O_2
Ethanol 50%	16.54	27.47
Ethanol 70%	14.31	25.14
Ethanol 96%	15.92	26.13

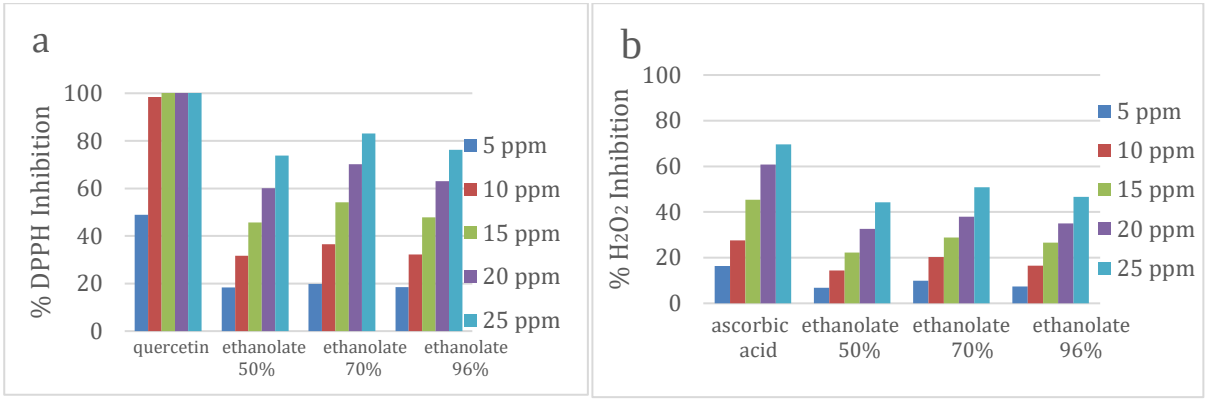

Figure 6. Comparison of the antioxidant activity of ethanolic extracts of cashew leaves a) % inhibition of DPPH, b) % inhibition of H_2O_2

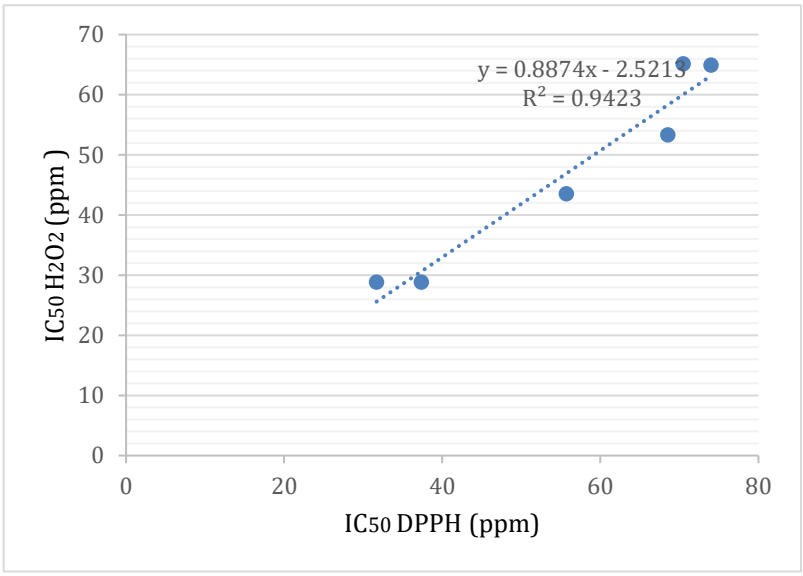

Figure 7. Relationship between IC50 DPPH vs IC50 H_2O_2 values

As with total phenolics and flavonoids, researchers also made a conversion formula from the relationship between the IC_{50} H_2O_2 value (y) and the IC_{50} DPPH value (x) to predict the IC_{50} H_2O_2 using the known or known IC_{50} DPPH. instead it uses six data sets obtained from one research team (Figure 7).

Based on the equation y = 0.8874x - 2.5219 with a correlation coefficient of 0.9707, the comparison of the IC_{50} H_2O_2 laboratory results with the calculated IC_{50} H_2O_2 results has a very large difference (Table 10). Based on the *Bland-Altman* analysis, it is known that the two sets of IC_{50} H_2O_2 data, namely laboratory results and conversion calculation results, have an average difference of 14.933 which is between the upper limit of 15.24 and the lower limit of 14.17, which means that these two data are consistent. However, it has a significant bias, which shows that the laboratory IC_{50} value is generally around 14,933 units higher than the calculated IC_{50} value. Based on *the Paired t-test*, it is known that the calculated t value is (66,384) > t table (4.303) which shows that Ho is rejected and means that there is a significant difference between IC_{50} H_2O_2 laboratory results and IC_{50} H_2O_2 results from conversion calculations.

DOI: https://doi.org/ 10.25077/jrk.v15i2.735

Table 10. Comparison of IC_{50} laboratory results and calculations

Sample	IC_{50} DPPH (laboratory)	IC_{50} H_2O_2 (laboratory)	IC_{50} H_2O_2 (calculation)	Difference
Ethanolate 50%	16.54	27.47	12.1563	15.314
Ethanolate 70%	14.31	25.14	10.1774	14,963
Ethanolate 96%	15.92	26.13	11.6061	14,524

Therefore, the IC_{50} results of the DPPH and H_2O_2 methods cannot be compared and converted using this equation because both laboratory data and conversion calculations have significant differences and are considered not the same. This is thought to be because these two methods have different reaction mechanisms, DPPH directly measures the donation of electrons by antioxidant compounds to DPPH free radicals, while H_2O_2 focuses on the decomposition into water and oxygen [39].

This research provides important insights into the extraction of bioactive compounds from cashew leaves *(Anacardium occidentale).* By comparing the effectiveness of various concentrations of ethanol as a solvent, this research can be a practical guide for the pharmaceutical, cosmetic and nutraceutical industries in choosing the most effective solvent for extracting cashew leaves, so that standards and quality are better. This research also enriches the scientific literature on the chemical composition and antioxidant potential of cashew leaves. However, there are several limitations that need to be noted. First, this study was conducted under in vitro conditions and may not fully reflect its effectiveness under in vivo conditions. Second, this study only used three ethanol concentrations, so other variations have not been tested. Therefore, further research is recommended to conduct in vivo tests of the antioxidant activity of cashew leaves to ensure their effectiveness and safety in clinical applications. Exploration of the concentration of ethanol and other solvents is also recommended to increase extraction efficiency and obtain more optimal methods.

Conclusions

This research shows that 70% ethanol extract from cashew leaves produces the highest total phenolic and flavonoid content as well as significant antioxidant activity compared to 50% and 96% ethanol extracts. These results indicate that 70% ethanol is the most effective solvent for extracting bioactive compounds from cashew leaves. Data analysis also shows that the use of 70% ethanol provides the best balance between solvent polarity and extraction efficiency, and is able to increase the antioxidant activity of the extract. These findings can be the basis for the development of more effective and high quality cashew leaf-based products.

Acknowledgments

This research was supported by Fundamental Research DRTPM–Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology, Indonesia, in 2024.

References

1. H. Wang, Y. Chen, L. Wang, Q. Liu, S. Yang, and C. Wang, "Advancing herbal medicine: enhancing product quality and safety through robust quality control practices," *Front Pharmacol*, vol. 14, p. 1265178, 2023.
2. S. S. Belokurov, I. A. Narkevich, E. V Flisyuk, I. E. Kaukhova, and M. V Aroyan, "Modern extraction methods for medicinal plant raw material," *Pharm Chem J*, vol. 53, no. 6, pp. 559–563, 2019.
3. J. Lim, K. Kim, D. Y. Kwon, J. K. Kim, R.

Sathasivam, and S. U. Park, "Effects of Different Solvents on the Extraction of Phenolic and Flavonoid Compounds, and Antioxidant Activities, in Scutellaria baicalensis Hairy Roots," *Horticulturae*, vol. 10, no. 2, p. 160, 2024.

4. M. J. Jara-Palacios, S. Gonçalves, F. J. Heredia, D. Hernanz, and A. Romano, "Extraction of antioxidants from winemaking byproducts: Effect of the solvent on phenolic composition, antioxidant and anti-cholinesterase activities, and electrochemical behaviour," *Antioxidants*, vol. 9, no. 8, p. 675, 2020.
5. I. El Mannoubi, "Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded Opuntia stricta fruit," *Journal of Umm Al-Qura University for Applied Sciences*, vol. 9, no. 2, pp. 176–184, 2023.
6. H. Chen, H. Xiao, and J. Pang, "Parameter optimization and potential bioactivity evaluation of a betulin extract from white birch bark," *Plants*, vol. 9, no. 3, p. 392, 2020.
7. A. R. Hakim and R. Saputri, "Narrative Review: Optimasi Etanol sebagai Pelarut Senyawa Flavonoid dan Fenolik: Narrative Review: Optimization of Ethanol as a Solvent for Flavonoids and Phenolic Compounds," *Jurnal Surya Medika (JSM)*, vol. 6, no. 1, pp. 177–180, 2020.
8. M. C. Dias, D. C. G. A. Pinto, and A. M. S. Silva, "Plant flavonoids: Chemical characteristics and biological activity," *Molecules*, vol. 26, no. 17, p. 5377, 2021.
9. A. Tripathi, N. Chauhan, and P. Mukhopadhyay, "Recent advances in understanding the regulation of plant secondary metabolite biosynthesis by ethylene-mediated pathways," *Physiology and Molecular Biology of Plants*, vol. 30, no. 4, pp. 543–557, 2024.
10. Kementerian Kesehatan RI, "Farmakope Herbal Indonesia (Edisi II)," *Direktorat Jendereal Kementerian Kesehatan RI*, 2017.
11. R. Fusco *et al.*, "The role of cashew (Anacardium occidentale L.) nuts on an experimental model of painful degenerative joint disease," *Antioxidants*, vol. 9, no. 6, p. 511, 2020.
12. C. Tshiama, D. Tshilanda, D. Tshibangu, N. Ngombe, T. Mbemba, and P. Mpiana, "antidiabetic, antisickling and antibacterial activities of Anacardium occidentale L.(Anacardiaceae) and Zanthoxylum rubescens Planch. Ex Hook (Rutaceae) from DRC," *Int J Diabet Endocrinol*, vol. 3, pp. 7–14, 2018.
13. R. Amarowicz *et al.*, "Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (Lens culinaris)," *Food Chem*, vol. 121, no. 3, pp. 705–711, 2010.
14. R. L. Prior, X. Wu, and K. Schaich, "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements," *J Agric Food Chem*, vol. 53, no. 10, pp. 4290–4302, 2005.
15. R. Apak *et al.*, "Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay," *Molecules*, vol. 12, no. 7, pp. 1496–1547, 2007.
16. J.-K. Moon and T. Shibamoto, "Antioxidant assays for plant and food components," *J Agric Food Chem*, vol. 57, no. 5, pp. 1655–1666, 2009.
17. B. Halliwell and J. M. C. Gutteridge, *Free radicals in biology and medicine*. Oxford university press, USA, 2015.
18. J. B. Harborne, "Metode Fitokimia, penuntun dan cara modern menganalisis Tumbuhan," *Penerjemah: Padmawinata K dan Soediro I. Penerbit ITB Bandung*, 1996.
19. M. Zugazua-Ganado, A. Bordagaray, J. Ezenarro, R. Garcia-Arrona, M. Ostra, and M. Vidal, "Adaptation of the Folin-Ciocalteu and Fast Blue BB spectrophotometric methods to digital image analysis for the determination of total phenolic content: Reduction of reaction time, interferences and sample analysis," *LWT*, vol. 193, p. 115756, 2024.

 DOI: https://doi.org/ 10.25077/jrk.v15i2.735

20. L. T. T. Nguyen, T. T. Nguyen, H. N. Nguyen, and Q. T. P. Bui, "Simultaneous determination of active compounds in Piper betle Linn. leaf extract and effect of extracting solvents on bioactivity," *Engineering Reports*, vol. 2, no. 10, p. e12246, 2020.
21. S. Aryal, M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, and N. Koirala, "Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal," *Plants*, vol. 8, no. 4, p. 96, 2019.
22. Q. D. Do *et al.*, "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica," *J Food Drug Anal*, vol. 22, no. 3, pp. 296–302, 2014.
23. S. Sai-Ut, P. Kingwascharapong, M. A. R. Mazumder, and S. Rawdkuen, "Optimization of ethanolic extraction of phenolic antioxidants from lychee and longan seeds using response surface methodology," *Foods*, vol. 12, no. 15, p. 2827, 2023.
24. P. Sezer Okur and I. Okur, "Recent Advances in the Extraction of Phenolic Compounds from Food Wastes by Emerging Technologies," *Food Bioproc Tech*, pp. 1–22, 2024.
25. A. Biswas *et al.*, "Ultrasound-assisted extraction (UAE) of antioxidant phenolics from Corchorus olitorius leaves: a response surface optimization," *Chemical and Biological Technologies in Agriculture*, vol. 10, no. 1, p. 64, 2023.
26. H. Lohvina, M. Sándor, and M. Wink, "Effect of Ethanol Solvents on Total Phenolic Content and Antioxidant Properties of Seed Extracts of Fenugreek (Trigonella foenum-graecum L.) varieties and determination of phenolic Composition by HPLC-ESI-MS," *Diversity (Basel)*, vol. 14, no. 1, p. 7, 2021.
27. N. Phuyal, P. K. Jha, P. P. Raturi, and S. Rajbhandary, "Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC," *The Scientific World Journal*, vol. 2020, no. 1, p. 8780704, 2020.
28. J. Wairata, A. Fadlan, A. S. Purnomo, M. Taher, and T. Ersam, "Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of Garcinia forbesii King: A correlation study," *Arabian Journal of Chemistry*, vol. 15, no. 2, p. 103541, 2022.
29. C. Alasalvar, M. Karamać, R. Amarowicz, and F. Shahidi, "Antioxidant and antiradical activities in extracts of hazelnut kernel (Corylus avellana L.) and hazelnut green leafy cover," *J Agric Food Chem*, vol. 54, no. 13, pp. 4826–4832, 2006.
30. R. Amarowicz, A. Troszynska, N. Baryłko-Pikielna, and F. Shahidi, "Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency," *Journal of Food Lipids*, vol. 11, no. 4, pp. 278–286, 2004.
31. R. Amarowicz *et al.*, "Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (Lens culinaris)," *Food Chem*, vol. 121, no. 3, pp. 705–711, 2010.
32. A. M. Shraim, T. A. Ahmed, M. M. Rahman, and Y. M. Hijji, "Determination of total flavonoid content by aluminum chloride assay: A critical evaluation," *Lwt*, vol. 150, p. 111932, 2021.
33. J. Bliumaitė, A.-S. Maruška, D. Armonavičius, M. Stankevičius, A. Kanopka, and O. Ragažinskienė, "Evaluation of total phenolic content, total flavonoid content and radical scavenging activity in fractions of Epilobium angustifolium L. extracts and their in vitro anticancer activity," in *18 th international scientific conference" The vital nature sign", May 16 th-17 th, 2024 Kaunas, Lithuania: abstract book/editors A. Maruška, N. Tiso, V. Kaškonienė, M. Stankevičius*, Kaunas: Vytauto Didžiojo universitetas, 2024, p. 57.
34. M. O. Nguru, S. S. Gyang, C. J. Joseph, W. M. Iorjiim, S. Omale, and B. G. David, "Estimation of Total Phenolic and Total Flavonoid Contents, In-Vitro Antioxidant Activity and Median Inhibitory

Concentration of Methanol Root Extract of Ximenia americana, L. Olacaceae," *J Adv Biol Biotechnol*, vol. 24, no. 12, pp. 52–59, 2021.

35. M. Amiri, M. Arab, E. K. Sadrabad, N. Mollakhalili-Meybodi, and H. Fallahzadeh, "Effect of gamma irradiation treatment on the antioxidant activity, phenolic compounds and flavonoid content of common buckwheat," *Radiation Physics and Chemistry*, vol. 212, p. 111127, 2023.
36. D. Huang, B. Ou, and R. L. Prior, "The chemistry behind antioxidant capacity assays," *J Agric Food Chem*, vol. 53, no. 6, pp. 1841–1856, 2005.
37. W. Brand-Williams, M.-E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT-Food science and Technology*, vol. 28, no. 1, pp. 25–30, 1995.
38. P. Molyneux, "The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity," *Songklanakarin J. sci. technol*, vol. 26, no. 2, pp. 211–219, 2004.
39. H. D. T. Madhuranga and D. N. A. W. Samarakoon, "Advancing In vitro Antioxidant Activity Assessment: A Comprehensive Methodological Review and Improved Approaches for DPPH, FRAP and H_2O_2 Assays," *Journal of Natural & Ayurvedic Medicine*, vol. 7, no. 4, pp. 1–7, Oct. 2023, doi: 10.23880/jonam-16000431.

 DOI: https://doi.org/ 10.25077/jrk.v15i2.735