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RESEARCH PAPER

Phytochemical Analysis and Antioxidant Activity of Brebes Red Onion (*Allium cepa* L.) Peel Using Deep Eutectic Solvent Based on Green Chemistry

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Abstract: Brebes Regency is the largest red onion (*Allium cepa* L.) production centre in Indonesia, generating large quantities of red onion peel waste that remains underutilised. Red onion peel contains bioactive compounds including flavonoids, phenolics, and tannins with potential as strong antioxidants. This study aimed to analyze the phytochemical content and antioxidant activity of Brebes red onion peel extracted using a green-chemistry-based Deep Eutectic Solvent (DES) compared with conventional ethanol solvent. This laboratory experimental study used a posttest-only control group design and the Ultrasound-Assisted Extraction (UAE) method, with ChCl:glycerol DES (molar ratio 1:2) as the treatment group and 96% ethanol as the control. Qualitative phytochemical tests (flavonoids, phenols, saponins, alkaloids, tannins), Total Flavonoid Content (TFC) analysis using the $AlCl_3$ method, Total Phenolic Content (TPC) analysis using the Folin-Ciocalteu method, and DPPH antioxidant assay were performed. The results showed that the DES extract tested positive for flavonoids, phenols, alkaloids, and tannins, whereas the ethanol extract tested positive only for flavonoids, phenols, and tannins. The TFC of the DES extract (57.94 mg QE/g) was 52.4% higher than that of ethanol (38.02 mg QE/g). The TPC of the DES extract (42.06 mg GAE/g) was 23.1% higher than that of ethanol (34.16 mg GAE/g). The antioxidant activity of the DES extract, with an IC50 of 70.38 ppm (strong category), was better than that of ethanol (IC50 = 76.56 ppm), with vitamin C (IC50 = 6.61 ppm) as the positive control (very strong category). ChCl:glycerol DES (1:2) proved more effective than 96% ethanol in extracting bioactive compounds from Brebes red onion peel, supporting the sustainable utilization of agricultural waste.

Keywords: Antioxidant; Deep Eutectic Solvent; Green Chemistry; Phytochemical; Red Onion (*Allium cepa* L.).

INTRODUCTION

Brebes Regency is the largest red onion (*Allium cepa* L.) production centre in Indonesia, contributing 68.94% of total national production, equivalent to 383.68 thousand tons per year [1], [2]. The high intensity of red onion production in this region not only contributes significantly to the agricultural sector but also generates large quantities of red onion peel waste [3]. In fact, red onion peel waste in Brebes accounts for approximately 10–15% of total production, estimated at 28–57 thousand tons per year [4]. However, this waste is often discarded without further management, posing a potential risk of environmental pollution.

Red onion peel contains various bioactive compounds such as flavonoids (quercetin), anthocyanins, and phenolic compounds, which have higher antioxidant potential than the bulb [5], [6], [7]. Various studies have also shown that red onion peel compounds exhibit biological activities such as cardioprotective, neuroprotective, antiobesity, antidiabetic, and antimicrobial effects [8], [9], [10]. Brebes red onions, with their deeper reddish-purple colour, indicate higher anthocyanin and flavonoid content than other varieties. However, their utilization remains very limited, and comprehensive studies on the specific phytochemical characteristics of the deeper reddish-purple Brebes red onion remain scarce [1], [4]. Therefore, red onion peel can be categorized as a high-value agricultural by-product owing to its strong antioxidant bioactivity potential and possible protective effects against various degenerative diseases [11].

Antioxidants play an important role in neutralizing free radicals that contribute to various degenerative diseases and premature aging [12]. Using red onion peel as a source of natural antioxidants not only adds value to agricultural waste but also aligns with the concept of a circular economy and the *Sustainable Development Goals* (SDGs) [13]. However,

extracting bioactive compounds from natural materials still largely relies on large volumes of conventional organic solvents such as methanol or ethanol [14]. These solvents have several drawbacks, including toxicity, high volatility, and negative environmental impacts, which run counter to the principles of *green chemistry* [15]. Therefore, as awareness of the importance of the *green chemistry* approach in the research and development of natural-based products continues to grow, safer and more environmentally friendly solvent alternatives are needed [16]. One such alternative is *Deep Eutectic Solvent* (DES), a green solvent formed from natural primary metabolites such as organic acids, sugars, and amino acids [17], [18].

Deep Eutectic Solvent (DES) has emerged as an environmentally friendly solvent alternative formed from natural components and characterised by biodegradability, low toxicity, and low volatility [19], [20]. DES is combined with Ultrasound-Assisted Extraction (UAE), which uses the cavitation effect of ultrasonic waves to enhance the release of bioactive compounds from the plant cell matrix [21].

Although DES has been widely studied for various plant matrices, systematic studies on the application of DES-UAE to Brebes red onion peel waste have not previously been reported. This study aimed to analyze the phytochemical content and antioxidant activity of Brebes red onion peel extract obtained using ChCl:glycerol DES (1:2), based on *green chemistry* principles via the UAE method, as well as to compare its effectiveness with 96% ethanol as a conventional solvent.

MATERIALS AND METHODS

Research Design

This study was a laboratory experimental study using a true experimental posttest-only control group design, with ChCl:glycerol DES (1:2) as the treatment group and 96% ethanol as the control group. Before ANOVA, the data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's Test). Data were analyzed using two-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at a significance level of 5% ($\alpha = 0.05$), assisted by IBM SPSS Statistics version 27.

Sample Preparation

The first and second outer layers of Brebes red onion (*Allium cepa* L.) peel were selected, washed, dried at 40–45°C, then ground and sieved to 80 mesh.

Preparation of ChCl:Glycerol DES (1:2)

The DES was prepared by heating and stirring. ChCl and glycerol were weighed in a 1:2 molar ratio (MW ChCl = 139.62 g/mol; MW glycerol = 92.09 g/mol), heated at 60–80°C, and stirred for 1–2 hours until a clear, homogeneous liquid formed [22], [23]. Once formed, 30% (w/w) distilled water was added gradually, and the mixture was placed in a tightly sealed container [17], [24].

Extraction by *Ultrasound-Assisted Extraction* (UAE)

A total of 4 g of powdered sample was added to 80 mL of DES solvent (treatment group) or 96% ethanol (control group), then sonicated at 37–40 kHz and 40–50°C for 25 minutes. The mixture was centrifuged at 4,000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper. Filtrate volumes obtained were 48 mL for DES and 52 mL for ethanol [25], [26].

Qualitative Phytochemical Screening

Flavonoid Test: A 1 mL aliquot of the red onion peel extract solution was treated with Mg powder and 1 mL of HCl. The formation of a red, yellow, or orange colour indicated a positive result for flavonoids [27].

Phenol Test: A 1 mL aliquot of the red onion peel extract solution was treated with 1 mL of $FeCl_3$ 10% solution and then observed. A change to a green or blackish colour indicated the presence of phenols.

Saponin Test: A 1 mL aliquot of the red onion peel extract solution was treated with hydrochloric acid, then shaken vigorously until foam formed. Foam that remained stable for 10 minutes indicated a positive result for saponins.

Alkaloid Test: A 1 mL aliquot of the red onion peel extract solution was treated with 1 mL of 1% HCl and 1 mL of Mayer's reagent, then heated in a water bath for 1 minute. The formation of a white precipitate indicated the presence of alkaloids.

Tannin Test: A 1 mL aliquot of the red onion peel extract solution was treated with 1 mL of $FeCl_3$ 10% solution. The formation of a dark blue or blackish-green colour indicated the presence of tannins [28].

Total Flavonoid Content (TFC) Analysis

A 0.5 mL aliquot of filtrate was treated with 2 mL of distilled water, 0.15 mL of 5% $NaNO_2$ (incubated for 6 minutes), 0.15 mL of 10% $AlCl_3$ (incubated for 6 minutes), 1 mL of 1 M NaOH, and 1.2 mL of distilled water (incubated for 15 minutes). Absorbance was measured at λ 510 nm. The standard curve was prepared using quercetin (10–80 ppm). Results were expressed as mg quercetin equivalents per gram of sample (mg QE/g) [29], [30].

Total Phenolic Content (TPC) Analysis

A 0.5 mL aliquot of filtrate was treated with 2.5 mL of Folin-Ciocalteu reagent (1:10) and 2 mL of Na_2CO_3

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7.5% solution, then incubated for 30 minutes in the dark. Absorbance was measured at λ 765 nm. The standard curve was prepared using gallic acid (10–80 ppm). Results were expressed as mg gallic acid equivalents per gram of sample (mg GAE/g) [29].

DPPH Antioxidant Activity Assay

A 1 mM DPPH solution in analytical-grade (p.a.) methanol (A_0 = 2.246 at λ 516 nm) was used. Extract concentration series of 20, 40, 60, 80, and 100 ppm were tested, with vitamin C (2, 4, 6, 8, 10 ppm) as the positive control. A mixture of 0.2 mL sample and 3.8 mL DPPH was incubated in the dark for 30 minutes. The IC_{50} value, representing the antioxidant concentration of the red onion peel extract required to scavenge 50% of the radicals relative to the vitamin C standard solution, was calculated. The IC_{50} value was obtained from the linear regression equation:

[% inhibition = $(A_0 – A_1)/A_0 \times 100$] [31].

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The results of the qualitative phytochemical screening of the Brebes red onion peel extract are presented in Table 1. The DES extract detected more classes of bioactive compounds (flavonoids, phenols, alkaloids, and tannins) than the ethanol extract (flavonoids, phenols, and tannins).

Table 1. Results of the Qualitative Phytochemical Screening

Test	Reagent	Positive Result	DES Extract	Ethanol Extract
Flavonoid	Mg + HCl	Red	(+)	(+)
Phenol	$FeCl_3$ 10%	Blackish green	(+)	(+)
Saponin	HCl + shaking	Stable foam	(±)	(−)
Alkaloid	HCl + Mayer	White precipitate	(+)	(−)
Tannin	$FeCl_3$ 10%	Deep dark blue	(+)	(+)

(+) = positive; (±) = weakly positive; (−) = negative

Table 1 shows that the DES extract detected a more diverse range of bioactive compound classes than the ethanol extract. The DES extract tested positive for flavonoids, phenols, alkaloids, and tannins, with a weakly positive result for saponins (±). In contrast, the ethanol extract tested positive only for flavonoids, phenols, and tannins, with negative results for alkaloids and saponins. This finding is inconsistent with the study by Suryandari et al., who reported that ethanolic red onion peel extract contained saponins and alkaloids [32]. The detection of alkaloids in the DES extract but not in the ethanol extract can be explained by the molecular interaction mechanism of DES. ChCl: glycerol DES forms a strong hydrogen-bonding network through the hydroxyl groups of glycerol acting as a *hydrogen bond donor* (HBD) and the chloride ion from choline chloride acting as a *hydrogen bond acceptor* (HBA), enabling interaction with nitrogen-containing alkaloid compounds and enhancing their solubility [33][24].

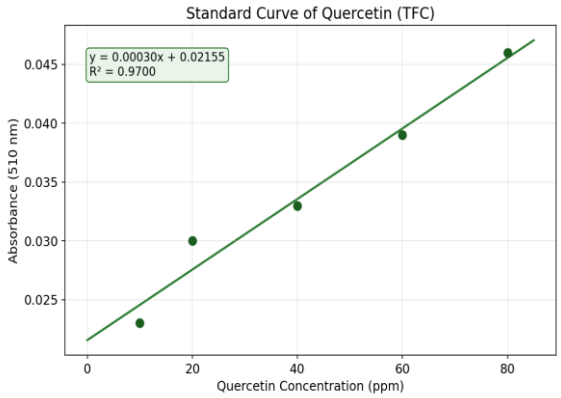


Figure 1. Standard Curve of Quercetin.

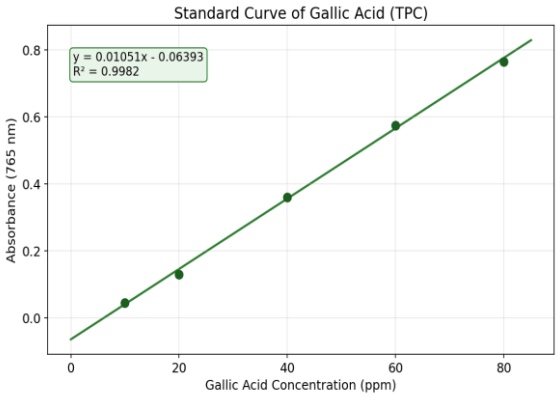


Figure 2. Standard Curve of Gallic Acid.

Quantitative analysis of TFC and TPC was performed based on validated calibration curves. The quercetin standard curve equation (TFC) was y = 0.00030x + 0.02155 (R^2 = 0.9700), while the gallic acid standard curve equation (TPC) was y = 0.01051x − 0.06393 (R^2 = 0.9982). Both curves showed acceptable linearity for quantitative analysis. The TFC and TPC results are presented in Table 2.

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Table 2. Results of TPC and TFC Analysis

Parameter	DES Extract	Ethanol Extract	Unit
TPC	42.06 ± 0.12	34.16 ± 0.09	mg GAE/g
TFC	57.94 ± 0.18	38.02 ± 0.14	mg QE/g

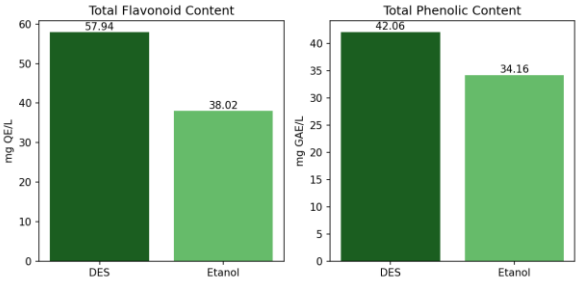


Figure 3. Comparison of TFC and TPC Levels between DES and Ethanol Extracts.

The TPC value of the DES extract (42.06 mg GAE/g) was 23.1% higher than that of the ethanol extract (34.16 mg GAE/g), and the TFC of DES (57.94 mg QE/g) was 52.4% higher than that of ethanol (38.02 mg QE/g). The superiority of DES in extracting phenolic and flavonoid compounds can be explained through molecular interaction mechanisms. ChCl:glycerol DES (1:2) forms a strong hydrogen-bonding network between the hydroxyl groups of glycerol (HBD) and the chloride ion of ChCl (HBA). This network enhances the solvent's ability to dissolve polar phenolic compounds bearing aromatic hydroxyl groups, as well as flavonoids with a polyphenolic backbone containing several –OH groups [19], [20]. In addition, the *non-volatile* nature of DES minimizes the loss of thermolabile compounds during the UAE process at 40–50°C [34].

The TPC and TFC values obtained in this study are consistent with several previous studies. Hikmah & Anggarani reported a TFC of 55.82 mg QE/g for Nganjuk red onion peel using ethanol [35], while Dalimunthe et al. reported a TFC of 18.03 mg QE/g using microwave-assisted extraction [36]. The DES TPC value in this study (42.06 mg GAE/g) was higher than the value reported by Martati & Simamora for ethanolic red onion peel extract (31.24 mg GAE/g), confirming the superiority of DES as an extraction solvent [29].

DPPH Antioxidant Activity

The antioxidant activity of the Brebes red onion peel extract was evaluated using the DPPH radical scavenging method at five concentration levels (20, 40, 60, 80, and 100 ppm for the DES and ethanol extracts), with vitamin C (2, 4, 6, 8, and 10 ppm) as the positive control. The percentage inhibition increased linearly with increasing concentration across all three test groups (Figure 4).

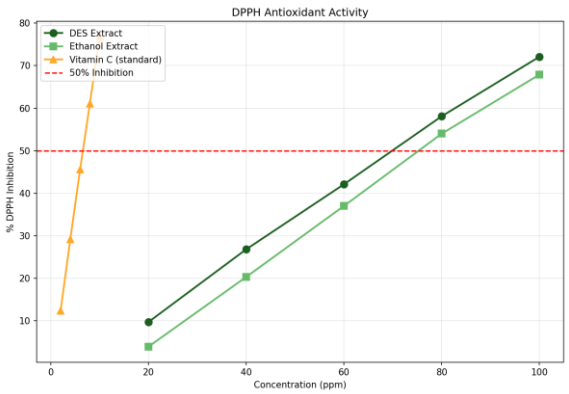


Figure 4. Dose-Response Curve of %DPPH Inhibition for DES Extract, Ethanol Extract, and Vitamin C.

Table 3. Regression Equations and IC50 Values of DES Extract, Ethanol Extract, and Vitamin C.

Group	Regression Equation	R^2	IC50 (ppm)
DES	y = 0.7817x − 5.016	0.9990	70.38
Ethanol	y = 0.8088x − 11.923	0.9988	76.56
Vitamin C	y = 7.9586x − 2.625	0.9991	6.61

All three dose-response curves showed excellent linearity (R^2 > 0.998), and the IC50 values for all groups fell within the tested concentration range. Vitamin C exhibited the highest antioxidant activity (IC50 = 6.61 ppm), followed by the DES extract (IC_{50} = 70.38 ppm) and the ethanol extract (IC_{50} = 76.56 ppm). A lower IC_{50} value indicates stronger antioxidant activity [37], indicating that the DES extract had greater potential for free radical scavenging than the ethanol extract. However, both were still considerably weaker than pure vitamin C as the positive control.

The IC_{50} value of the DES extract, being lower than that of the ethanol extract, is consistent with the higher TFC and TPC levels of the DES extract, supporting the positive correlation commonly reported between phenolic-flavonoid content and free

radical scavenging capacity. The two-way ANOVA and DMRT results statistically reinforced these findings, showing highly significant differences in %inhibition between solvent groups and concentration levels ($p < 0.05$), with distinct subsets. The significant interaction between solvent type and concentration indicates that the relative advantage of DES over ethanol varies across concentration levels, suggesting that using DES at higher working concentrations may yield greater benefits than lower concentrations.

Using vitamin C as a positive control provides important quantitative context for interpreting the antioxidant potential of both extracts. Numerically, the potency of vitamin C was approximately 10.7 times higher than that of the DES extract and 11.6 times higher than that of the ethanol extract. This difference is reasonable given that vitamin C is a pure antioxidant compound (single compound) that efficiently donates hydrogen atoms through the two hydroxyl groups on its enediol structure, forming an ascorbyl radical stabilized by electron resonance, so that its reaction with the DPPH radical proceeds rapidly and is nearly stoichiometric [38][39]. In contrast, red onion peel extract is a heterogeneous mixture of phenolic compounds, flavonoids, and tannins with varying degrees of hydroxylation, aromatic conjugation, and steric hindrance, so that not all components in the extract contribute optimally to DPPH radical scavenging via the Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET) mechanisms [40], [41].

Based on the antioxidant strength categories used in the DPPH assay (IC_{50} <50 ppm: very strong; 50–100 ppm: strong; 100–150 ppm: moderate; >150 ppm: weak) [42], both Brebes red onion peel extracts, whether obtained with DES or ethanol, fell into the strong category, while vitamin C fell into the very strong category. This gap in potency relative to the pure positive control is consistent with what has commonly been reported in various studies on the antioxidant activity of red onion peel extract using the DPPH method [6], [7], [11], and does not diminish the significance of Brebes red onion peel as a promising source of natural antioxidants. The consistency between TFC/TPC levels and the IC_{50} value in this study further supports the structure-activity relationship between phenolic-flavonoid content and free radical scavenging capacity [43]: the more aromatic hydroxyl groups available to donate hydrogen atoms or electrons to the DPPH radical, the lower the resulting IC_{50} value [44]. The DES extract, with higher TFC and TPC, produced a lower IC_{50} than ethanol, reinforcing the dual role of DES as both a green-chemistry-based extraction solvent and a facilitator of bioactive compound release with higher antioxidant activity.

CONCLUSION

This study demonstrated that ChCl:glycerol DES (molar ratio 1:2) combined with UAE was more effective than 96% ethanol for extracting bioactive compounds from Brebes red onion peel. The DES extract contained more bioactive compounds (flavonoids, phenols, alkaloids, tannins, and weakly positive saponins) than the ethanol extract (flavonoids, phenols, tannins). The TFC (57.94 mg QE/g) and TPC (42.06 mg GAE/g) of the DES extract were 52.4% and 23.1% higher, respectively, than those of the ethanol extract (TFC 38.02 mg QE/g; TPC 34.16 mg GAE/g). The antioxidant activity of the DES extract (IC50 = 70.38 ppm) was statistically significantly higher than that of the ethanol extract (IC50 = 76.56 ppm), based on two-way ANOVA ($F(1,20) = 691.14$; $p < 0.001$) and the DMRT ($\alpha = 0.05$), which confirmed that the two groups occupied significantly different subsets; both fell into the strong category but remained considerably weaker than vitamin C as the positive control (IC50 = 6.61 ppm). These findings provide preliminary scientific data supporting the use of DES as a green solvent for the sustainable extraction of bioactive compounds from agricultural waste, in line with circular-economy principles.

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