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Synthesis of Deep Eutectic Solvent (DES) Based on Glycerol and Propylene Glycol as a Medium for Anthocyanin Extraction from Senduduk Flowers (*Melastoma* sp.)

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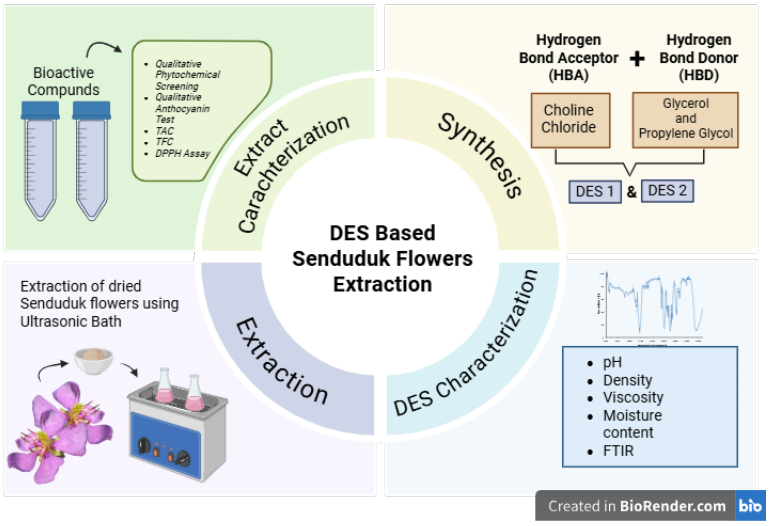
GRAPHICAL ABSTRACT



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ABSTRACT

Environmental concerns about the extensive use of conventional organic solvents have driven the development of greener extraction technologies, including the use of deep eutectic solvents (DES) as environmentally friendly alternatives. This study aimed to evaluate the effectiveness of choline chloride–based DES combined with glycerol and propylene glycol as green solvents for anthocyanin extraction from Senduduk flowers (*Melastoma* sp.) using the ultrasound-assisted extraction (UAE) method. DES were synthesized at a 1:2 molar ratio and characterized by density, viscosity, pH, moisture content, and functional group analysis via FTIR spectroscopy. The results confirmed the successful formation of DES with suitable physicochemical properties for extraction applications. Both DES systems were able to extract a range of bioactive compounds, including flavonoids, alkaloids, terpenoids, saponins, and tannins. The highest total anthocyanin content was obtained using choline chloride/glycerol DES (93.29 mg/L), followed by choline chloride/propylene glycol DES (89.06 mg/L). Total flavonoid contents were 86.97 mg QE/g and 85.78 mg QE/g, respectively. Antioxidant activity analysis indicated strong radical-scavenging capacity, with IC_{50} values of 70.82 ppm

and 72.61 ppm. These findings demonstrate that glycerol- and propylene glycol-based DES are effective green solvents for anthocyanin extraction and show potential for sustainable applications in food, pharmaceutical, and cosmetic industries.

1. INTRODUCTION

The widespread use of conventional organic solvents in the extraction of natural compounds has caused various environmental and health problems due to their toxic, volatile, and low biodegradability properties [1]. In the context of sustainable development, these limitations have prompted increased attention to the application of green chemistry principles, particularly in the development of environmentally friendly solvent systems [2,3]. One promising alternative is the use of Deep Eutectic Solvents (DES), which form through hydrogen-bond interactions between hydrogen-bond acceptors and donors, resulting in a liquid system with a much lower melting point than its constituent components [1,4]. DES are attractive because of their low toxicity, biodegradability, ease of synthesis, and high solubility of polar bioactive compounds [5].

Previous studies have reported the effectiveness of DES as an extraction medium for various natural compounds, including phenolic compounds, flavonoids, and anthocyanins, making it a potential alternative solvent in the extraction of natural materials, particularly in the food, pharmaceutical, and cosmetic industries [6,7]. Additionally, the efficiency of DES-based extraction can be enhanced by applying modern extraction methods, such as Ultrasound-Assisted Extraction (UAE). The UAE method uses ultrasonic waves to disrupt plant cell structures, thereby accelerating mass transfer, increasing extraction efficiency, and reducing process time and solvent consumption [8,9]. Therefore, the combination of DES and UAE represents an effective and sustainable approach for recovering high-value bioactive compounds from biological materials [3,10]. The UAE is recognized as a clean, innovative extraction technology that offers advantages over conventional methods, including reduced solvent consumption, shorter extraction times, minimal equipment requirements, and lower economic and environmental impacts [8-10]. The synergy between DES and UAE arises from DES's high solvation capacity for polar compounds and ultrasonic cavitation's ability to enhance mass transfer and disrupt plant cell walls, thereby improving extraction efficiency while preserving the stability of sensitive compounds such as anthocyanins [11]. Anthocyanins are water-soluble natural pigments that belong to the flavonoid group, which are responsible for the red, purple, and blue colors in various plants [12,13]. In addition to functioning as natural colorants, anthocyanins exhibit various beneficial biological activities, including antioxidant, anticancer, and anti-inflammatory effects, making them potentially applicable in the food, pharmaceutical, and cosmetic industries [14-16]. However, anthocyanins are highly polar and sensitive to environmental factors such as pH, temperature, and light. Hence, selecting the appropriate extraction medium is an important factor in maintaining their stability and biological activity [17-18].

The senduduk flower (*Melastoma* sp.) is known as a natural source of anthocyanins and has long been used in traditional medicine [19]. Despite its high anthocyanin content and broad industrial application potential, systematic studies on the extraction of anthocyanins from senduduk flowers using environmentally friendly solvents remain limited. In particular, the use of glycerol- and propylene glycol-based DES as a medium for the extraction of anthocyanins from senduduk flowers has not been widely reported. Glycerol and propylene glycol are attractive hydrogen bond donors because they are non-toxic, biodegradable, and compatible for applications in the food and pharmaceutical industries [20-23].

Although DES has been increasingly investigated for the extraction of anthocyanin from various plant materials [24], most previous studies have focused on other botanical sources, such as edible flowers [25], using different DES compositions [26-28]. Information on the extraction of anthocyanins from Senduduk Flower using polyol-based DES is still limited. In particular, a direct comparison between choline chloride-based DES containing glycerol and propylene glycol as hydrogen-bond donors has not been reported clearly. This is important because both glycerol and propylene glycol are polyols with multiple hydroxyl groups that may promote hydrogen-bond interactions with anthocyanins. Nevertheless, they differ in viscosity, polarity, and molecular structure, which may influence solvation behavior and mass transfer during UAE [29]. Therefore,

the effect of these two hydrogen bond donors on anthocyanin recovery from Senduduk Flower remains insufficiently understood.

Here, in the present study, a choline chloride-based DES containing glycerol and propylene glycol as hydrogen bond donors was applied in combination with UAE for the extraction of anthocyanins from Senduduk Flower. The objective of this study was to systematically compare the extraction performance of these polyol-based DES systems as solvents and to investigate how differences in hydrogen-bond donor characterization affect anthocyanin, flavonoid, and IC_{50} content. Through this approach, this study is expected to provide new insight into the role of DES composition in anthocyanin extraction and to offer a green and effective alternative method for the recovery of bioactive compounds from plants.

2. EXPERIMENTAL METHODS

2.1. Materials

Senduduk flowers (*Melastoma* sp.) were collected from Sigaol Village, North Sumatra, Indonesia. The flowers were transported to the laboratory, washed with distilled water to remove impurities, and dried at room temperature prior to use. Choline chloride (Himedia), glycerol, propylene glycol, sodium hydroxide, hydrochloric acid, methanol, and all other chemicals used were of analytical grade. Quercetin and ascorbic acid were used as standard compounds, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was employed for antioxidant activity analysis. Aluminum chloride ($AlCl_3$) and sodium acetate (CH_3COONa) were used for total flavonoid determination. Phytochemical screening reagents were used for qualitative analysis. Potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5) were prepared for the determination of total anthocyanins. Distilled water was used throughout the experiments, and all chemicals were used without further purification.

2.2. Instrumentation

The experimental procedures utilized standard laboratory glassware and equipment, including a magnetic stirrer, hot plate (Thermo Scientific), sonicator, centrifuge, drying oven (Memmert 30–1060), pycnometer, and viscometer. Sample mass measurements were performed using an analytical balance (Ohaus AX224). UV-Vis spectrophotometric analyses were performed using a Thermo Scientific Genesys 150 to determine total anthocyanin and total flavonoid content, and antioxidant activity. Functional group analysis and confirmation of deep eutectic solvent formation were conducted using Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu Prestige 21) at the Integrated Laboratory and Technology Innovation Center, Universitas Lampung, Indonesia. All other experimental procedures were conducted under standard laboratory conditions.

2.3. Procedure

Preparation of Senduduk Flower Sample

Dried senduduk flowers are ground into a fine powder using a blender and filtered to obtain a uniform particle size. Powder samples are stored in tightly sealed containers at room temperature, protected from light, until further use.

Synthesis of Deep Eutectic Solvent (DES)

Deep eutectic solutions (DES) are synthesized by mixing choline chloride as a hydrogen bond acceptor with glycerol and propylene glycol as hydrogen bond donors in a molar ratio of 1:2 (0.5 mol:1 mol). The mixture was heated at 80 °C under continuous magnetic stirring until a clear, colorless, and homogeneous liquid was obtained. The resulting DES is allowed to settle at room temperature for 24 hours in a desiccator to prevent moisture absorption prior to physicochemical characterization, including pH, density, viscosity, moisture content, and Fourier Transform Infrared (FTIR) analysis, as well as subsequent extraction experiments.

Extraction of Senduduk Flowers Using Ultrasound-Assisted Extraction

Anthocyanin extraction from senduduk flowers was performed using ultrasound-assisted extraction (UAE). A total of 3 g of dried senduduk flower powder was mixed with a deep eutectic solvent (DES) and water mixture at a volume ratio of 70:30 (v/v), consisting of 21 mL of DES and 9 mL of distilled water, in a 100 mL beaker. The mixture was subjected to ultrasonic irradiation using a sonicator operating at 40 kHz for 30 min at 50 °C [29,30]. After extraction, the resulting mixture was centrifuged at 6000 rpm for 15 min to separate the liquid extract from the solid residue. The supernatant was subsequently filtered and stored at −2 °C prior to further analysis [31]. All extraction experiments were performed in triplicate.

Qualitative Phytochemical Screening of Senduduk Flower

Qualitative phytochemical screening of Senduduk flower crude extracts was performed using standard methods to detect the presence of major secondary metabolites. Flavonoids were identified by adding magnesium powder and concentrated hydrochloric acid, as indicated by the formation of a red color and a precipitate. Alkaloids were tested using ammonia, sulfuric acid, and Mayer's reagent, with the appearance of a white to yellowish precipitate indicating a positive result. Terpenoids and steroids were detected using chloroform and Liebermann–Burchard reagent; the formation of a reddish-brown or purple ring indicated terpenoids, whereas a blue-green ring indicated steroids. Tannins were identified by reaction with ferric chloride solution, which produced a dark blue or dark green color. Saponins were determined by the foam test, in which the formation of stable froth after shaking and acid addition indicated a positive result [32-36].

Qualitative Anthocyanin Test

A total of 5 mL of anthocyanin extract from senduduk flowers was placed into two separate test tubes. In the first tube, a 2 M NaOH solution was added dropwise, and a dark brown color change indicated the presence of anthocyanin. In the second tube, 2 M HCl was added dropwise, and the appearance of a red color confirmed the presence of anthocyanin.

Determination of Total Anthocyanin Content (TAC)

The total anthocyanin content was determined using the pH difference method. The extract was dissolved by mixing 200 µL of the sample with 1,800 µL of distilled water. A total of 200 µL of the dissolved sample was added to 3,800 µL of potassium chloride buffer solution (pH 1.0; 25 mM) and sodium acetate buffer solution (pH 4.5; 400 mM) in separate tubes. The mixture was shaken and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 510 and 700 nm using a UV–Vis spectrophotometer with distilled water as a blank. TAC was calculated as cyanidin-3-glucoside equivalents using a molecular weight of 449.2 g/mol, a molar extinction coefficient of 26,900 L·mol^{-1}·cm^{-1}, a dilution factor of 10, and a path length of 1 cm [37,38].

$$\text{TAC} = \frac{(A510\ nm.pH\ 1.0 - A700\ nm.pH\ 1.0) - (A510\ nm.pH\ 4.5 - A700\ nm.pH\ 4.5) \times Mw \times Df \times 1000}{\varepsilon \times L}$$

Where (A_{510}, $pH_{1.0}$ – A_{700}, $pH_{1.0}$) – (A_{510}, pH $_{4.5}$ – A_{700}, pH $_{4.5}$) represents the corrected absorbance difference of the sample; M_w is the molecular weight of cyanidin-3-glucoside ($449.2\ g/mol$), $DF$is the dilution factor, ε is the molar absorptivity coefficient of cyanidin-3-glucoside ($26{,}900\ L\,mol^{-1}\,cm^{-1}$), and $L$is the path length of the cuvette ($1\ cm$). The TAC values were expressed as mg cyanidin-3-glucoside equivalents (C3G)/L extract.

Total Flavonoid Content (TFC)

The total flavonoid content was determined using the $AlCl_3$ colorimetric method with quercetin as the standard. The sample solution (1000 ppm) was prepared by dissolving 10 mg of extract in 10 mL of distilled water, while the quercetin stock solution (1000 ppm) was prepared in

methanol. Quercetin standard solutions (20–100 ppm) were used to prepare a calibration curve. For analysis, 0.5 mL of sample or standard solution was mixed with 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.8 mL of distilled water, then incubated in the dark for 30 minutes. Absorbance was measured at 430 nm using a UV–Vis spectrophotometer. TFC was calculated from the calibration curve and expressed as mg quercetin equivalent per gram of extract (mg QE/g). All measurements were performed in duplicate [19,39,40].

Antioxidant Activity Assay

Antioxidant activity was evaluated using the DPPH radical scavenging method. A DPPH solution (160 mg/L) was prepared by dissolving 4 mg of DPPH in 25 mL of methanol and stored in a dark vial. A sample stock solution (1000 ppm) was prepared by dissolving 7 mg of extract in 7 mL of methanol. Sample solutions with concentrations of 25, 50, 75, 100, and 125 ppm were prepared by appropriate dilution with methanol.

For analysis, 1 mL of each sample solution was mixed with 1 mL of DPPH solution, then methanol was added to a final volume of 5 mL. The mixtures were incubated for 30 min in the dark at room temperature. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Methanol–DPPH solution without sample was used as the control [41]. The percentage of radical scavenging activity was calculated using the equation [42]:

$$\%\text{Inhibition} = \frac{A0 - A1}{A0} \times 100\%$$

Where A_0 is the control absorbance, and A_1 is the sample absorbance. The IC_{50} value is determined by linear regression of the percentage of inhibition versus sample concentration. All measurements are performed in triplicate.

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical Characterization of Deep Eutectic Solvents

The physicochemical characterization of DES is a crucial step in evaluating their suitability as extraction media, since solvent performance is governed not only by chemical composition but also by density, viscosity, pH, and water content. These properties directly influence diffusivity, matrix penetration, solvent handling, and solvation behavior, thereby affecting the overall extraction efficiency of target bioactive compounds [43]. The physicochemical properties of choline chloride-based DES are stated in Table I.

TABLE I. Physicochemical properties of choline chloride-based DES characterized at room temperature.

DES system	Molar ratio	Density (g/mL)	pH	Water content (%)	Viscosity (mPa·s)	TORQ (%)
Choline chloride/glycerol	1:2	1.1875	4	0.0848	152.0	30.5
Choline chloride/propylene glycol	1:2	1.1121	5	0.0217	34.7	6.9

In the present study, the DES based on choline chloride/glycerol (1:2) exhibited a density of 1.1875 g/mL. In comparison, the choline chloride/propylene glycol (1:2) system showed a density of 1.1121 g/mL at room temperature. Density plays a significant role in determining solvent diffusivity and its miscibility behavior with other liquid systems [44]. These values are consistent with the density range commonly reported for hydrophilic choline chloride-based DESs [45-47], and indicate that both solvents possess relatively compact molecular organization, with the glycerol-based DES showing a more densely packed liquid structure than the propylene glycol-based system. Such

molecular compactness may create a favorable solvent environment for solubilizing target compounds, though extraction efficiency should not be inferred from density alone.

The measured pH values were 4 for choline chloride/glycerol and 5 for choline chloride/propylene glycol, indicating mildly acidic conditions. This acidity is relevant for anthocyanin-containing extraction systems [48]. The stability of anthocyanins is strongly pH-dependent: acidic conditions favor the predominance of the flavylium cation, considered the most stable form, while increasing pH promotes the formation of less stable, colorless species, which can degrade under alkaline conditions [49].

Among the characterized properties, viscosity showed the most pronounced difference between the two systems: the viscosity of choline ChCl/Gly DES was 152 mPa·s, whereas that of ChCl/PG DES was only 34.7 mPa·s. This result is highly relevant to extraction performance because highly viscous DESs generally exhibit lower fluidity, slower diffusion, and reduced penetration into plant matrices, which may hinder the release and transfer of target compounds. By contrast, lower-viscosity DESs tend to promote better mixing, faster mass transfer, and improved contact between the solvent and the plant material, thereby potentially enhancing extraction kinetics. Accordingly, the lower viscosity of the choline ChCl/PG DES may provide a kinetic advantage during extraction, while the higher viscosity of the choline ChCl/Gly DES may reflect a stronger and more extensive hydrogen-bonding network within the solvent system.

Importantly, the physicochemical behavior of DESs arises from intermolecular interactions among their constituents, particularly hydrogen bonding between the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) [50]. Since these interactions are fundamental to DES formation and stability, molecular-level characterization is required to complement macroscopic property measurements. Fourier Transform Infrared (FTIR) spectroscopy is particularly useful in this regard, as it enables the identification of changes in characteristic functional group vibrations associated with hydrogen-bond formation and structural rearrangements. Therefore, FTIR analysis is an important approach for confirming DES formation and elucidating the molecular interactions underlying the observed physicochemical properties. As shown in Figures 1 and 2, the FTIR spectra of choline chloride/glycerol and choline chloride/propylene glycol DESs were analyzed to evaluate the molecular interactions within each solvent system.

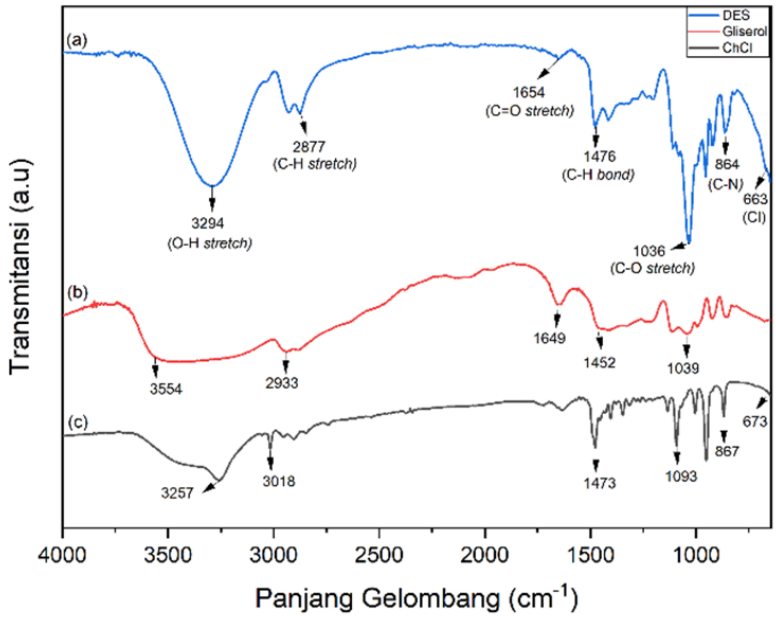

Figure 1. FTIR spectra of synthesized choline chloride-glycerol (ChCl-Gly).

TABLE II. Wavelength vibration of a function groups of ChCl/Glycerol DES.

	Wavelength (cm^{-1})

Functional group	Choline Chloride	Glycerol	ChCl/Gly (1:2)
O─H stretch	3257	3554	3294
C─H stretch	3018	2933	2887
C═O stretch	-	-	1654
C─H bond	1473	1452	1476
C─O stretch	1093	1039	1036
C─N stretch	867	-	864
Cl strech	673	-	663

Figure 1 and Table II indicate that the functional groups identified in the DES were derived from the parent components, choline chloride and glycerol. In the DES spectrum, the absorption band observed in the range of 800–600 cm^{-1} suggests the contribution of the salt component, particularly the presence of halide ions (Cl^-), to the DES structure [51]. The O–H stretching vibration appeared at 3257 cm^{-1} for choline chloride and at 3554 cm^{-1} for glycerol [52]. After DES formation, however, the O–H stretching band was observed at 3294 cm^{-1}, indicating a shift in the O–H vibration and providing evidence for hydrogen-bond interactions between the hydrogen-bond donor and choline chloride [53]. This phenomenon may be attributed to the abundance of chloride ions (Cl^-) in choline chloride, which promotes stronger hydrogen-bond interactions with the hydroxyl groups of glycerol, resulting in the formation of OH···Cl interactions [54]. Therefore, the FTIR analysis confirms that DES formation does not generate new functional groups in the mixture, but rather involves intermolecular interactions between the original functional groups of the constituent components.

The spectrum of choline chloride, propylene glycol, and ChCl/PG DES, as shown in Figure 2 and Table III, exhibited the main characteristic functional groups, including O–H stretching and C=O stretch, at 3294 and 1654 cm^{-1}, respectively. The presence of hydroxyl (-OH) groups from propylene glycol produced a broad O–H stretching band within the range of 3200–3600 cm^{-1}. In pure propylene glycol, the O–H stretching vibration appeared at 3491 cm^{-1}, whereas pure choline chloride showed an absorption band at 3257 cm^{-1} [55]. Upon DES formation, however, the corresponding absorption band shifted to 3302 cm^{-1}, indicating a change in the O–H stretching vibration and suggesting the presence of strong hydrogen-bond interactions between the hydroxyl groups of the HBD and the chloride ions (Cl^-) of choline chloride. These interactions reduce the vibrational energy of the O–H bond, thereby shifting the absorption band to lower wavenumbers. This interpretation is consistent with the findings of Vorobyova and Skiba (2021), who reported that hydrogen bonding between DES components causes a significant spectral shift in the O–H region. The formation of hydrogen bonds lowers the vibrational energy of the O–H group, thereby shifting the absorption band to lower wavenumbers [56].

TABLE III. Wavelength vibration of a function groups of ChCl/PG DES.

		Wavelength (cm^{-1})	
Functional group	**Choline Chloride**	**Propylene Glycol**	**ChCl/PG (1:2)**
O─H stretch	3257	3491	3302
C─H stretch	3018	2931	2992
C═O stretch	-	1652	1654
C─H bond	1473	1377	1476
C─O stretch	1093	1045	1043
C─N stretch	867	-	954
Cl strech	673	-	663

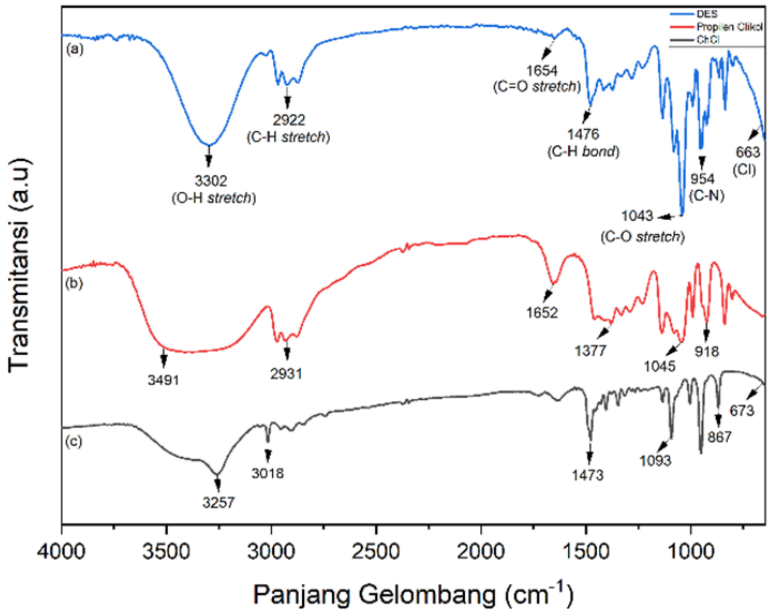

Figure 2. FTIR spectra of synthesized choline chloride-propylene glycol (ChCl/PG).

The FTIR analysis confirmed the formation of DES by the presence of broad O–H stretching bands and shifts in characteristic functional group vibrations, indicating hydrogen-bond interactions between choline chloride and glycerol or propylene glycol. These interactions play a crucial role in enhancing solvent polarity and solubilization capacity toward polar bioactive compounds such as anthocyanins.

3.2. Qualitative Phytochemical Screening of Senduduk Flower and Anthocyanin Test

Qualitative phytochemical screening research shows the presence of flavonoids, alkaloids, terpenoids, saponins, and tannins in senduduk flower extracts obtained using both DES systems (Table IV). These results indicate that DES-based extraction effectively dissolves a range of secondary metabolites. The presence of flavonoids and phenolic compounds supports the antioxidant activity potential of these extracts, as these compounds are known as hydrogen or electron donors capable of neutralizing free radicals.

TABLE IV. Secondary metabolites contain of the *Senduduk Flower*.

Secondary metabolites	Test reagent	Positive Result	Result	
			DES 1	DES 2
Saponins	Distilled water	Stable foam after 10 minutes	+	+
Tannins	$FeCl_3$	Greenish – black solution	+	+
Flavonoids	Mg + HCl	Reddish – brown solution	+	+
Terpenoids	Liebermann-Burchard	Reddish-brown or purple ring at the interface	+	+
Alkaloids	Mayer	Yellowish – brown solution	-	+

Under acidic conditions, anthocyanins predominantly exist in the form of the flavylium cation, which is responsible for the bright red coloration of anthocyanin-containing extracts. In contrast, under alkaline conditions, deprotonation occurs, leading to the formation of carbinol or pseudobase structures, which are colorless forms of anthocyanins produced when the flavylium ring opens in the presence of a base, resulting in a dark green to brownish-black coloration [57]. Based on the qualitative anthocyanin test, the Senduduk flower DES extracts both showed positive results, as

indicated by the observed color changes in both test samples (Table V). These findings suggest that both DES systems were capable of extracting anthocyanin compounds from Senduduk flowers.

TABLE V. Result of the qualitative test of anthocyanin content

Sample	Reagen	Result	
		ChCl/Gly	ChCl/PG
Senduduk Flower DES Extract	HCl 2 M	+	+
	NaOH 2 M	+	+

3.3. Total Anthocyanin Content (TAC)

The pH differential method is based on differences in anthocyanin structure at pH 1 and pH 4.5 [58]. At pH 1, anthocyanin forms a red flavilium cation, while at pH 4.5, anthocyanin forms a colorless hemiketal, as shown in Figure 3.

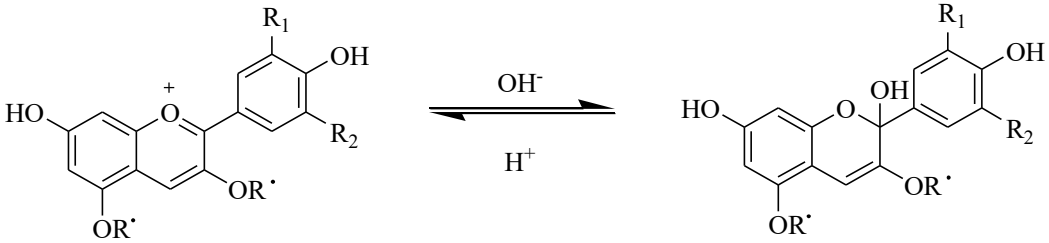

Figure 3. The red flavilium cation structure formed at pH 1 and the colorless hemiketal formed at pH 4.5.

Prior to quantifying anthocyanin content, a wavelength scan was performed to identify the maximum absorption wavelength of anthocyanins. The scan was conducted over the range of 400–800 nm. Based on the anthocyanin analysis of Senduduk flower extract, the maximum absorption wavelength for cyanidin was found to be 430 nm. Therefore, quantitative measurements were conducted at 430 nm, the anthocyanin λmax, and at 700 nm to correct for turbidity, sediment, or residual fine particles in the sample. Ideally, the absorbance value at 700 nm should be 0 if the sample is completely clear. However, in this study, the absorbance value was not exactly 0, indicating that fine particles remained in the sample [58]. Quantitative analysis showed that both DES systems were effective in extracting anthocyanins from Senduduk flowers. The highest total anthocyanin content was obtained using ChCl/Gly DES, reaching 93.29 mg/L, while ChCl/PG DES yielded 89.06 mg/L. The higher extraction efficiency of ChCl/Gly DES may be due to its greater hydrogen-bonding capacity and polarity, which strengthen interactions with hydroxyl-rich anthocyanin molecules [59].

The mild acidic environment of DES, combined with ultrasonic cavitation during UAE, facilitates cell wall disruption and enhances mass transfer, allowing anthocyanins to be released more efficiently from plant tissues. These findings demonstrate the suitability of DES-UAE as an alternative to conventional organic solvents in anthocyanin recovery. The test results showed differences in anthocyanin levels among the DES extracts. This effectiveness was influenced by hydrogen bonding ability and solvent viscosity. Choline chloride/glycerol-based DES had more stable hydrogen bonds with anthocyanin hydroxyl groups and appropriate polarity, thereby increasing the solubility of polar compounds. Lower viscosity also accelerates the diffusion of anthocyanins from flower tissue into the solvent [59,60].

3.4. Total Flavonoid Content (TFC)

Flavonoids are secondary metabolites of the polyphenol group with a C6-C3-C6 skeleton composed of two aromatic rings and one heterocyclic ring. These compounds are known to have important biological activities, particularly as natural antioxidants that help scavenge free radicals and prevent the development of degenerative diseases. Therefore, the determination of total flavonoid content is often used as an indicator of plant extract quality [39].

Total flavonoid analysis was performed using UV-Vis spectrophotometry at a wavelength of 430 nm. Senduduk flower extracts obtained with DES choline chloride/glycerol and choline

chloride/propylene glycol were reacted with $AlCl_3$ and sodium acetate. This reaction produced yellow flavonoid complexes due to the interaction between $AlCl_3$ and flavonoid hydroxyl groups, while sodium acetate maintained the reaction in the visible region. The color intensity of the complex is proportional to the flavonoid concentration in the sample. Quercetin was used as a standard solution because it forms a stable complex with $AlCl_3$ [40]. The formation of a complex between $AlCl_3$ and quercetin is shown in Figure 4.

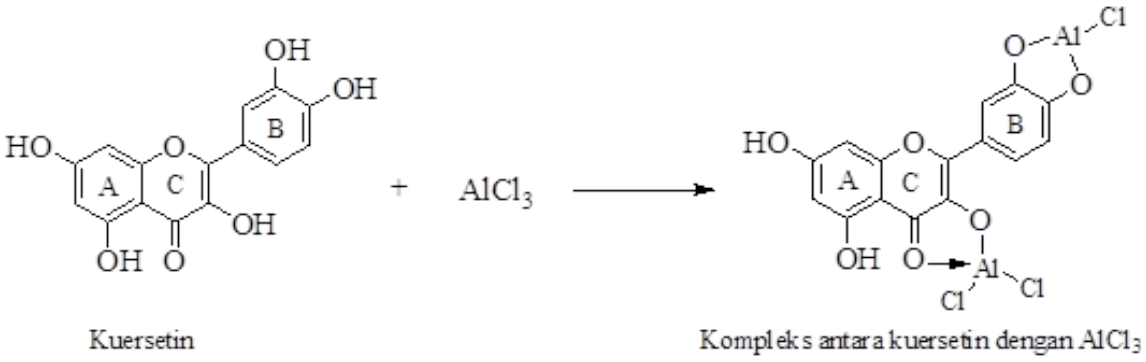

Figure 4. Complex compound between $AlCl_3$ and quercetin.

The total flavonoid content of the extract showed a similar trend to the anthocyanin results. The DES choline chloride–glycerol extract showed a slightly higher flavonoid content (86.969 mg QE/g) compared to the DES choline chloride–propylene glycol extract (85.784 mg QE/g). These results indicate that glycerol-based DES provides a more favorable solvent environment for flavonoids, which are generally polar and contain multiple hydroxyl groups. The effectiveness of DES in flavonoid extraction highlights the strong interaction between the solvent and the solute, driven by hydrogen-bonding networks, which increases the compound's solubility while reducing the need for toxic organic solvents.

3.5. Antioxidant Activity Test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay, and the results are presented in Table VI and Figure 5.

TABLE VI. DPPH radical scavenging activity of DES Extract.

Extract Concentration (ppm)	ChCl/Gly DES		ChCl/PG	
	Absorbance	Percent Inhibition (%)	Absorbance	Percent Inhibition (%)
0	0.995	0	0.951	0
25	0.843	15.209	0.815	14.301
50	0.636	36.047	0.622	34.525
75	0.481	51.624	0.443	53.347
100	0.267	73.098	0.288	69.716
125	0.104	89.547	0.126	86.680

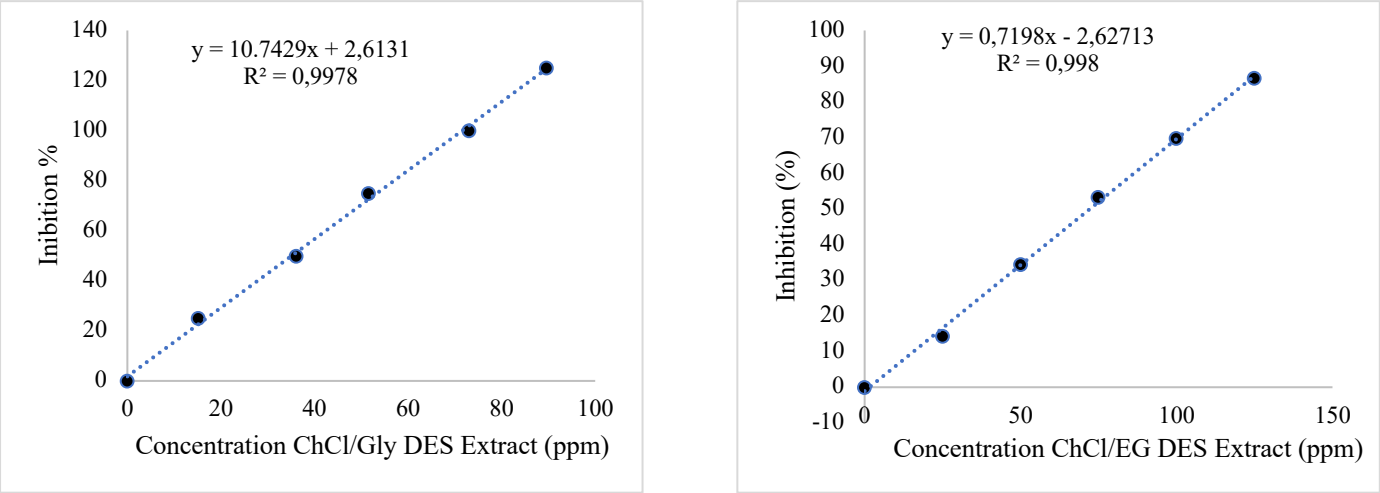

Figure 5. Linear regression plots of DPPH radical scavenging activity (%).

The antioxidant activity of the DES extracts, as presented in Table VI and Figure 5, showed a linear relationship between extract concentration and DPPH radical scavenging activity. Linear regression analysis of the ChCl/Gly DES extract produced the equation y = 0.7429x – 2.6131, whereas the ChCl/PG DES extract yielded y = 0.7198x – 2.2713. These regression equations were subsequently used to determine the IC_{50} values, defined as the concentration of extract required to inhibit 50% of DPPH radicals. The IC_{50} value of the ChCl/Gly DES extract was 70.82, while that of the ChCl/PG DES extract was 72.61. Since a lower IC_{50} value indicates stronger antioxidant activity, the ChCl/Gly DES extract exhibited slightly higher radical scavenging activity than the ChCl/PG DES extract. This result suggests that the glycerol-based DES was marginally more effective in extracting antioxidant constituents from the sample matrix. The small difference between the two IC_{50} values, however, also indicates that both DES systems showed comparable antioxidant extraction performance. Since a lower IC_{50} value indicates stronger antioxidant activity, ChCl/Gly DES extract demonstrated the highest radical scavenging activity [61].

4. CONCLUSIONS

This study confirms that choline chloride-based DESs are effective and sustainable solvents for extracting bioactive compounds from Melastoma sp. flowers. Both choline chloride/glycerol and choline chloride/propylene glycol successfully formed DES systems, as verified by physicochemical characterization and FTIR analysis, and both were capable of extracting anthocyanins, flavonoids, and other secondary metabolites. However, choline chloride/glycerol showed slightly better overall performance, yielding higher total anthocyanin and flavonoid contents and a lower IC_{50} value, indicating stronger antioxidant activity. These results suggest that stronger hydrogen-bonding interactions and more favorable solvent polarity may be more important than viscosity alone in determining extraction efficiency. Despite these promising findings, the study was limited to two DES formulations, one antioxidant assay, and bulk phytochemical measurements without individual compound identification. Future work should include optimization of extraction parameters, detailed phytochemical profiling using chromatographic techniques, comparison with conventional solvents, and evaluation of DES recyclability and extract stability to strengthen the practical applicability of this green extraction approach.

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