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Green Extraction of Bioactive Compounds from Peronema canescens Jack. *Using Sodium Acetate/Glycerol-Based Natural Deep Eutectic Solvents as a Source of Natural Antioxidants*

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Abstract

Peronema canescens Jack (commonly known as sungkai) is an endemic medicinal plant native to Sumatra and Kalimantan, known for its broad spectrum of pharmacological activities, including antioxidant, antibacterial, antidiabetic, antihyperuricemic, and anti-inflammatory effects. These properties are largely attributed to its high content of bioactive phenolic and flavonoid compounds. Conventional extraction techniques typically utilize organic solvents such as ethanol or methanol, which raise concerns regarding toxicity, cost, and environmental sustainability. This study explores a green extraction strategy employing Natural Deep Eutectic Solvents (NADES), formulated from sodium acetate and glycerol at varying molar ratios, to optimize the recovery of antioxidant compounds from sungkai leaves. The extraction process involved maceration for 2 × 24 hours. FTIR analysis identified key functional group absorptions (O–H, C–H, C=C), with distinct spectral shifts and intensity variations, indicating interactions between NADES components and bioactive molecules. Among the four tested formulations, NADES 4 (sodium acetate:glycerol, 1:3) yielded the highest total phenolic and flavonoid contents, and exhibited the strongest antioxidant activity, with an IC_{50} value of 32.30 ppm—classified as very strong and comparable to ascorbic acid. These results demonstrate that the compositional adjustment of NADES significantly influences solvent polarity and extraction efficiency, underscoring their promise as sustainable alternatives for the extraction of natural antioxidants.

Keywords: Antioxidant, NADES, Sungkai Plant

1 Introduction

Originating from the Sumatra and Kalimantan regions, it has excellent potential as a source of natural medicinal ingredients. Sungkai (*Peronema canescens* Jack) leaves are known to have high antioxidant activity, indicating their ability to participate in various biopharmacological activities [1–4]. Previous studies have shown that sungkai leaf extract contains flavonoid compounds at a concentration of 142.247 mg QE/g and total phenols at 5.64 mg GAE/g when extracted using 96% ethanol [5]. The antioxidant activity of *Peronema canescens* Jack (*P. canescens)* leaf extract can be measured by the IC_{50} value. The lower the IC_{50} value, the stronger the antioxidant activity of a compound. The ethanol extract of *P. canescens* leaves has an IC_{50} value of 50.838 ppm, while extracts using aquadest and methanol show values of 53.979 ppm and 49.709 ppm, respectively [6]. The yield of *P. canescens* leaf extract also showed significant results. The yield of 70% ethanol extract in young and old leaves reached 16.75% and 19.53%, respectively [6,7], while the use of 96% ethanol in old leaves produced a yield of 23.33% [8]. *P. canescens* extract contains various secondary metabolite compounds that have been identified, including steroid, terpenoid, phenolic, flavonoid, and alkaloid compounds [3,7,9,10]. In addition, the ethanol extract from *P. canescens* was also confirmed to contain flavonoids, alkaloids, phenolics, steroids, saponins, and

tannins [11]. Various studies have demonstrated that sungkai possesses various pharmacological benefits, including hyperuricemia, cholesterol-lowering, antidiabetic, and antibacterial activities [7,9,11–13]. The bioactive compounds contained in sungkai leaf extract are believed to contribute to this antibacterial activity [14–16].

The use of organic solvents in the extraction of bioactive compounds is of paramount importance, as it directly influences selectivity, toxicity, viscosity, and cost-effectiveness [17]. Commonly employed organic solvents include *n*-hexane, toluene, chloroform, ethyl acetate, acetone, and various alcohols. While these solvents are effective, they often exhibit high levels of toxicity and are relatively expensive. Moreover, conventional extraction techniques such as maceration require substantial volumes of solvent, presenting challenges related to environmental sustainability and process efficiency [17]. To address these limitations, a range of innovative approaches has been explored, including the utilization of green solvents and the application of ultrasonic-assisted extraction to enhance yield and efficiency. Among these developments, the use of Natural Deep Eutectic Solvents (NADES) has emerged as a particularly promising strategy. NADES are formed by combining two or more naturally derived components—such as organic acids, sugars, or polyalcohols—in specific molar ratios, resulting in eutectic mixtures with melting points lower than those of the individual constituents [18,19].

NADES offer several advantages over traditional organic solvents, including low toxicity, biodegradability, economic feasibility, and biocompatibility [18]. Numerous studies have shown that NADES can significantly improve the solubility, stability, and bioactivity of extracted phytochemicals, particularly phenolic and flavonoid compounds [20,21]. In certain applications, extracts obtained using NADES can be directly incorporated into pharmaceutical formulations or functional food products without necessitating further purification steps.

This study aims to formulate a Natural Deep Eutectic Solvent (NADES) and evaluate its influence on the total phenolic and flavonoid content in *P. canescens* leaves. Previous research on sungkai extracts has demonstrated a wide range of pharmacological activities. The ethanolic extract of sungkai leaves has been reported to exhibit antidiabetic, anti-inflammatory, antihyperuricemic, antioxidant [22], and cytotoxic properties [23]. Additionally, the acetone extract of sungkai has been found to contain seven novel compounds, collectively referred to as Peronemins, which belong to the alkaloid class of secondary metabolites [24,25]. However, most studies to date have focused on either crude extracts or individual compounds, and comprehensive investigations into optimizing extraction conditions—such as solvent formulation—remain limited [12].

2 Method

2.1 Chemicals and Equipments

Sungkai (*Peronema canescens* Jack) leaves were collected from Setiris Village, Muaro Sebo District, Muaro Jambi Regency, Indonesia. The leaves were washed with distilled water, air-dried at room temperature, and subsequently ground into a fine powder using a mechanical grinder (Phillips HR2221/00, Philips, Netherlands) and sieved through a 100-mesh stainless steel sieve (Retsch GmbH, Germany) to obtain a homogenous simplex. The reagents used in this study included distilled water, ethanol 90% (Merck, Darmstadt, Germany), phytochemical reagents such as Mayer's, Wagner's, and Dragendorff's reagents (Sigma-Aldrich, St. Louis, MO, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, USA), sodium carbonate (Na_2CO_3; Merck, Germany), glacial acetic acid (Merck, Germany), lactic acid (Sigma-Aldrich, USA), citric acid (Sigma-Aldrich, USA), glycerol (Merck, Germany), sodium acetate (Merck, Germany), 1,2-propanediol (Sigma-Aldrich, USA), hydrochloric acid (HCl; Merck, Germany), lanolin (Sigma-Aldrich, USA), and paraffin wax (Merck, Germany). The instruments and laboratory apparatus employed included an analytical balance (Shimadzu AUW220, Japan), 20-mesh sieve (Retsch GmbH, Germany), digital pH meter (Hanna Instruments HI2211, Romania), water bath (Memmert WNB7, Germany), hot air oven (Binder ED115, Germany), high-speed blender (Philips HR2223, Netherlands), magnetic stirring rod, glass vials (Duran, Germany), ultrasonic bath (Branson 2510, Branson Ultrasonics Corp., USA), UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), and a hot plate stirrer (IKA C-MAG HS 7, Germany).

2.2 Solvent System Based on Natural Deep Eutectic Solvents (NADES)

The solvent system employed in this study was based on Natural Deep Eutectic Solvents

(NADES), consisting of mixtures of choline chloride and various carboxylic acids in specific molar ratios, as shown in Table 1. The selection of choline chloride-based NADES was informed by their high solubilizing capacity for bioactive compounds, as well as their favorable environmental profile, including low toxicity and biodegradability, as reported by previous studies [17,26]. After preparation, the functional group identification was performed using Fourier-transform infrared (FT-IR) spectroscopy to verify the formation of intermolecular hydrogen bonding between NADES constituents, which is essential for solvent structural stability.

Tabel 1 NADES Formulation

Solvent Codes	Composition	Ratio
NADES1	Sodium Acetate : Glycerin	1:1
NADES2	Sodium Acetate : Glycerin	1:2
NADES3	Sodium Acetate : Glycerin	2:1
NADES4	Sodium Acetate : Glycerin	1:3

2.3 Extraction

The extraction of active compounds was carried out using the maceration method, in which the powdered simplicia was soaked in a suitable solvent for two days at room temperature (approximately 25°C) without the application of heat. This method was chosen to allow the gradual diffusion of secondary metabolites while preserving the thermal stability of heat-sensitive compounds that may degrade under elevated temperatures. Upon completion of the maceration process, the mixture was filtered, and the resulting filtrate was concentrated using a water bath set at 70°C to obtain a thick extract. The extraction efficiency was then evaluated by calculating the yield percentage (% w/w), which reflects the proportion of extract obtained relative to the initial weight of the raw material [27].

2.4 Analysis of Total Phenolic and Flavonoid Content

The total phenolic and flavonoid contents of the extract were determined using UV-Visible spectrophotometry.

2.4.1 Flavonoid Content Determination

To analyze the flavonoid content, the extract was initially dissolved in methanol. Subsequently, aluminum chloride ($AlCl_3$), sodium acetate, and distilled water were added to the solution. The mixture was then subjected to spectrophotometric measurement at a wavelength of 415 nm. Specifically, 2 mL of the extract solution, prepared at a concentration of 50 µg/mL, was mixed with 2 mL of 2% $AlCl_3$ solution in ethanol. The resulting mixture was vortexed for 20 minutes and incubated for an additional 24 minutes. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Each measurement was performed in triplicate, and the flavonoid content was expressed as an equivalent of the standard reference compound [27].

2.4.2 Phenolic Content Determination

For the determination of total phenolic content, 100 mg of the sample was dissolved in 5 mL of 96% ethanol. To achieve a final concentration of 10 mg/mL, 10 mL of distilled water was subsequently added. From this solution, 0.1 mL was pipetted and combined with 0.5 mL of Folin–Ciocalteu reagent. The mixture was shaken until homogeneous and allowed to stand for 8 minutes. Then, 1.5 mL of 10% sodium carbonate (Na_2CO_3) solution was added, followed by the addition of distilled water to bring the total volume to 10 mL. The solution was mixed thoroughly and left to stand at room temperature for 2 hours. The absorbance was then measured at a wavelength of 780 nm using a UV-Vis spectrophotometer (UV-Vis). Measurements were conducted in triplicate, and the phenolic content was calculated with reference to the appropriate standard [27].

2.5 Antioxidant Activity

The antioxidant activity of the extract was evaluated through its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals using a colorimetric assay, based on previously reported methods with slight modifications [8]. A 0.4 mM DPPH solution in ethanol was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of the extract at varying concentrations (5–100 µg/mL). The resulting mixture was incubated at room temperature for 30 minutes in the dark to allow the reaction to proceed. The decrease in absorbance, indicating DPPH radical scavenging, was measured at a wavelength of 518 nm using a UV-Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following **Eq.1.**

Scavenging activity (%) =

(Abs.control−Abs.sample)/Abs.control × 100

(Equation 1)

To determine the concentration of extract required to inhibit 50% of the DPPH radicals (IC_{50}), a graph was constructed by plotting the percentage of scavenging activity against the logarithm of the extract concentration. The IC_{50} value was determined through linear regression analysis of the dose-response curve, and expressed in µg/mL (**Eq. 2 and 3**). A lower IC_{50} value indicates stronger antioxidant activity.

$y = ax + b$ (Equation 2)

$IC_{50} = (50 - b)/a$ (Equation 3)

3 Result and Discussion

3.1. IR-Fingerprint of NADES Solvent

Natural Deep Eutectic Solvents (NADES) represent a class of green solvents that have gained increasing prominence in scientific and industrial applications due to their favorable physicochemical properties and environmental sustainability. These solvents are typically composed of naturally derived compounds—such as organic acids, sugars, amino acids, or polyols—which, despite having high individual melting points, can form eutectic mixtures with significantly lowered melting points when combined in specific molar ratios. Among the various formulations, the combination of sodium acetate and glycerol has shown considerable promise, largely because of its capacity to dissolve both polar and non-polar bioactive compounds, along with its chemical stability, biodegradability, and low toxicity.

The sodium acetate–glycerol NADES system is formed through extensive hydrogen bonding between the acetate anion (CH_3COO^-) and the hydroxyl (–OH) groups of glycerol. This molecular interaction reduces the Gibbs free energy of the mixture, resulting in a eutectic point significantly below the melting points of the individual components. Consequently, the mixture remains in a stable liquid phase at ambient temperature (~25 °C), making it highly suitable for processes requiring mild conditions, such as the extraction of bioactive compounds from plant materials, pharmaceutical formulation, and food applications. These properties are consistent with previous studies, including those by Dai et al. (2013) [17] and Florindo et al. (2016) [28], which highlighted the potential of NADES as safe and efficient alternatives to volatile organic solvents

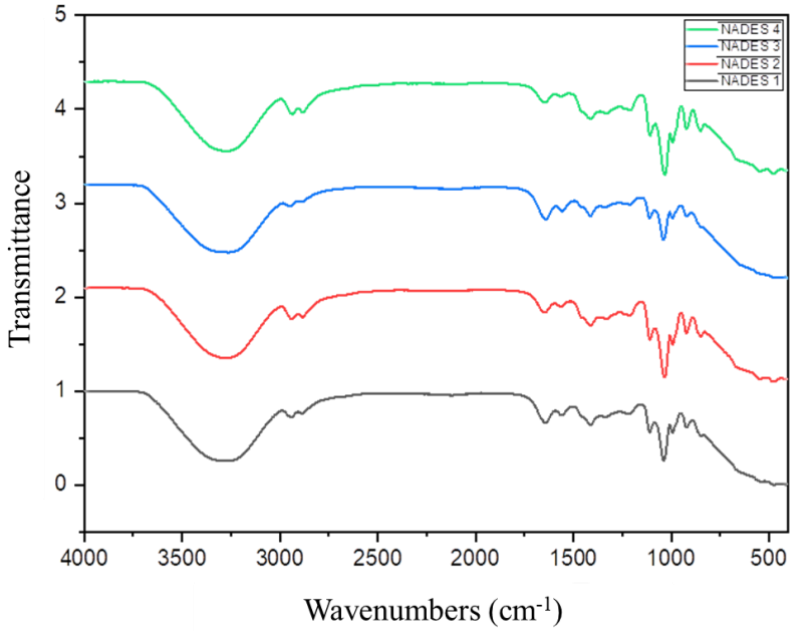

Figure 1 IR spectrum of NADES solvent

To confirm NADES formation and investigate the nature of molecular interactions, Fourier-transform infrared (FT-IR) spectroscopy was employed (**Fig. 1**). Particular attention was given to the fingerprint region of the spectra, which provides insight into the functional groups

present and the nature of hydrogen bonding within the system. Broad O–H stretching bands were observed in the 3,200–3,600 cm^{-1} range across all four formulations (NADES 1 to NADES 4), indicating the establishment of strong hydrogen bond networks. Slight shifts in peak positions among the different formulations suggest variations in hydrogen bond strength and molecular organization due to differing component ratios. Aliphatic C–H stretching vibrations were also detected in the 2850–3000 cm^{-1} region. For example, NADES 1 exhibited peaks at 2,942.56 and 2,886.95 cm^{-1}, while NADES 4 showed bands at 2,936.84 and 2,882.61 cm^{-1}. These variations reflect subtle differences in van der Waals forces and hydrogen bonding distributions that may influence properties like viscosity and solvation behavior. Additionally, bands corresponding to aromatic C=C stretching were found between 1,640–1,650 cm^{-1} and 1,550–1,560 cm^{-1}, possibly arising from residual aromatic compounds or specific carboxylic acid structures. The 1,400–1,600 cm^{-1} region also revealed aromatic C–H bending, further supporting the presence of minor aromatic features in the solvent systems (**Table 2**).

These spectroscopic findings align with those of Zhang et al. (2012) [29], who reported that shifts in IR absorption bands are indicative of hydrogen bond strength and configuration in NADES. Such interactions significantly impact key solvent characteristics, including polarity and solubility, underscoring the value of FT-IR as a tool for both structural verification and rational design of NADES for specific applications.

Tabel 2 IR NADES interpretation

Functional Groups	NADES 1	NADES 2	NADES 3	NADES 4
O-H	3,266.67	3,272.91	3,258.87	3,277.98
C-H_3	2,942.56; 2,886.95	2,937.85; 2,883.33	2,946.06; 2,889.09	2,936.84; 2,882.61
C=C	1,643.56; 1,560.67	1,646.14	1,641.7; 1,556.26	1,649.94
C-H	1,411.9	1,411.44; 1,332.34	1,412.17	1,411.05; 1,331.96
Fingerprint regions	1,212.06; 1,110.27; 1,038.02; 993.23; 847.05; 541.82	1,211.44; 1,109.12; 1,034.31; 992.84	1,110.98; 1,039.98; 993.35; 922.68	1,210.94; 1,108.86, 992.81; 922.26; 849.47

The fingerprint region of an infrared (IR) spectrum—typically found below 1,500 cm^{-1}—is highly specific to the molecular structure of individual compounds and is widely used for structural identification. In this study, notable variations were observed in the fingerprint region of the four synthesized NADES formulations, reflecting subtle differences in their molecular structures and intermolecular interactions. For example, NADES 1 exhibited characteristic absorption bands at 1,212.06 cm^{-1}, 1,110.27 cm^{-1}, 1,038.02 cm^{-1}, 993.23 cm^{-1}, 847.05 cm^{-1}, and 541.82 cm^{-1}. NADES 2 showed peaks at 1,211.44 cm^{-1}, 1,109.12 cm^{-1}, and 1,034.31 cm^{-1}, while NADES 3 presented similar peaks at 1,110.98 cm^{-1}, 1,039.98 cm^{-1}, and 993.35 cm^{-1}. NADES 4 demonstrated distinct absorption bands at 1,210.94 cm^{-1}, 1,108.86 cm^{-1}, and 992.81 cm^{-1}. These spectral differences underscore the utility of the fingerprint region in distinguishing between structurally similar NADES, as each formulation exhibits unique vibrational modes corresponding to its distinct molecular arrangement. The FT-IR spectra of sodium acetate–glycerol-based NADES showed broad and intense absorption in the range of 3,200–3,600 cm^{-1}, corresponding to the O–H stretching vibrations of glycerol and indicative of extensive hydrogen bonding between NADES components. The broad nature of these bands confirms the presence of a strong and complex hydrogen bond network, a defining feature of polyol-based deep eutectic solvents. Additionally, the sharp bands observed within the 1,550–1,650 cm^{-1} range are attributed to the C=O stretching vibrations of the acetate ion, further supporting its involvement in hydrogen bond formation with hydroxyl groups of glycerol. Aliphatic C–H stretching vibrations were also detected between 2,850 and 3,000 cm^{-1}, consistent with the presence of methylene and methyl groups from glycerol.

These spectral features confirm the successful formation of a stable NADES system via non-covalent intermolecular interactions, primarily hydrogen bonding and van der Waals forces. Moreover, comparison of the FT-IR spectra of the NADES before and after maceration revealed significant changes, particularly in three key regions: 1,600–1,650 cm^{-1} (aromatic C=C and carbonyl C=O stretching), 1,000–1,200 cm^{-1} (C–O–C and phenolic C–O stretching), and near 3,300 cm^{-1} (phenolic O–H stretching). These spectral changes suggest the presence of phenolic and flavonoid compounds in the extract, which are characterized by hydroxyl and carbonyl functional groups. These findings are in agreement with previous studies [28,29] emphasized that the physicochemical behavior of NADES—including hydrogen bonding strength and solute selectivity—is strongly influenced by their constituent composition and molar ratios. Therefore, the observed IR spectral shifts in the extracts not only confirm the presence of bioactive compounds but also serve as indirect evidence of the efficiency and selectivity of NADES as green solvents in plant-based extraction processes.

3.1 Changing of Total Phenolic and Flavonoid Content

Phenolic and flavonoid compounds are two primary categories of bioactive molecules commonly found in plants and are recognized for their significant roles in promoting human health. Phenolics, characterized by one or more hydroxyl groups attached to aromatic rings, exhibit strong antioxidant properties that protect cells from oxidative damage. These protective effects are associated with a reduced risk of chronic diseases, including inflammation, cardiovascular disorders, and cancer. Flavonoids, a structurally diverse subclass of polyphenols, demonstrate a wide range of biological activities beyond their antioxidant capacity. These include modulation of enzymatic functions, regulation of signaling pathways, and interactions with various cellular targets. Due to these versatile functions, flavonoids are widely studied in the development of plant-derived pharmaceuticals and nutraceuticals. In phytochemical analysis, total phenolic content (TPC) and total flavonoid content (TFC) are commonly used indicators for assessing antioxidant potential. TPC is typically measured using the Folin–Ciocalteu reagent and expressed as milligrams of gallic acid equivalent (mg GAE) per gram of extract, while TFC is determined via the aluminum chloride colorimetric method and reported as milligrams of quercetin equivalent (mg QE). In this study, standard calibration curves for gallic acid ($y = 0.0137x + 0.063$) and quercetin ($y = 0.0079x + 0.0008$) were employed for quantification, demonstrating reliable linearity and sensitivity (**Fig. 2**).

However, a key limitation of this study is the absence of compound profiling using advanced techniques such as mass spectrometry (MS). While total contents were measured, the specific identities and distribution of individual phenolic and flavonoid compounds remain unknown. Future work should incorporate MS-based characterization, such as LC-MS or GC-MS, to better elucidate the chemical profiles and enhance understanding of NADES selectivity and bioactive compound recovery. This would support more targeted applications in pharmaceutical and functional product development.

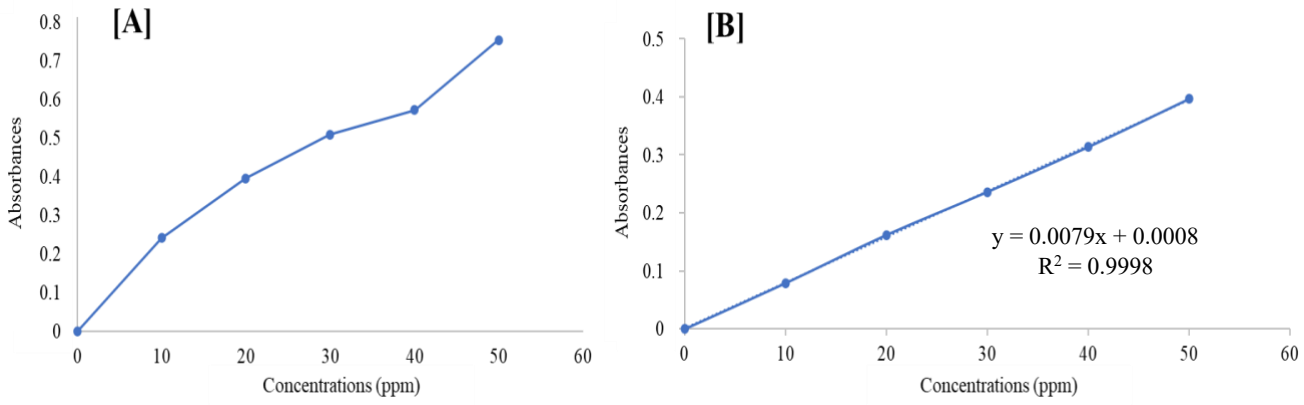

Figure 2 The graph of standar gallic acid 9 [A] and quarcetin [B]

Table 3 Total of phenolic and flavonoids

Samples	Total phenolic ± SD (mgGAE/g)	Total flavonoid ± SD (mgQE/g)
NADES 1	12.81 ± 0.262	5.223 ± 0.252
NADES 2	17.43 ± 0.0947	8.172 ± 0.0682
NADES 3	55.94 ± 0.089	25.418 ± 0.052
NADES 4	58.10 ± 0.082	26.418 ± 0.028

The data reveal notable variations in the total phenolic content (expressed in mg gallic acid equivalent per gram, mg GAE/g) and total flavonoid content (expressed in mg quercetin equivalent per gram, mg QE/g) across the four NADES samples. Among them, NADES 4 exhibited the highest total phenolic content at 58.10 mg GAE/g, followed by NADES 4 with 55.94 mg GAE/g, while NADES 2 showed the lowest value at 17.43 mg GAE/g. In terms of flavonoid content, NADES 3 and NADES 4 both recorded the highest values at 25.418 mg QE/g and 26.418 mg QE/g respectively, whereas NADES 1 and NADES 2 demonstrated lower contents at 5.223 mg QE/g and 8.172 mg QE/g, respectively.

The elevated levels of phenolic and flavonoid compounds observed in NADES 3 suggest a greater antioxidant potential, as these classes of compounds are recognized as major contributors to antioxidant activity. This is consistent with findings by Dai et al. (2013) [17] reported that specific NADES formulations enhance the extraction of phenolic and flavonoid compounds through strong hydrogen bonding interactions with polar functional groups. Accordingly, the superior performance of NADES 3 may be attributed to a more optimal compositional ratio or physicochemical environment that favors the solubilization of bioactive compounds from plant matrices.

These results highlight the critical influence of NADES formulation on extraction efficiency and bioactive compound recovery, reinforcing the importance of tailoring solvent systems based on the chemical nature of target compounds. Further investigations involving compound-specific profiling would provide a deeper understanding of the extraction mechanism and guide the development of high-performance green solvents for functional food and pharmaceutical applications.

3.2 Antioxidant Activity of Extracts against DPPH radical agent

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely recognized, rapid, and reproducible method for assessing the antioxidant capacity of natural compounds and plant-derived extracts. This colorimetric assay operates on the principle of electron or hydrogen atom donation by antioxidants to the stable DPPH radical, resulting in a measurable decrease in absorbance at 517 nm as the purple DPPH solution is reduced to a yellow-colored compound. In this study, the antioxidant activities of *P. canescens* leaf extracts obtained using four different Natural Deep Eutectic Solvent (NADES) formulations—combinations of sodium acetate and glycerol at varying molar ratios—were evaluated using the DPPH assay. The results demonstrated that NADES 3 (sodium acetate:glycerol = 2:1) and NADES 4 (1:3) yielded extracts with the highest antioxidant activities, exhibiting IC_{50} values of 36.30 ppm and 34.20 ppm, respectively. These values are closely comparable to that of the standard antioxidant, ascorbic acid (IC_{50} = 33.41 ppm), indicating the high efficiency of these NADES formulations in extracting and stabilizing antioxidant-active phytochemicals.

Conversely, NADES 1 (1:1) and NADES 2 (1:2) produced extracts with significantly lower antioxidant activity, as reflected in their considerably higher IC_{50} values of 1,023.24 ppm and 634.23 ppm, respectively. This discrepancy may be attributed to the suboptimal polarity and hydrogen bonding capacity of these solvent systems, which could limit their ability to solubilize phenolic and flavonoid compounds or affect the stability of the extracted antioxidants. Importantly, the antioxidant activities observed in this study—particularly for NADES 3 and 4—surpassed those reported in previous studies utilizing conventional organic solvents such as 96% ethanol. This finding underscores the potential of NADES not only as a green and sustainable alternative to traditional solvents but also as a more effective medium for the selective extraction of antioxidant constituents from medicinal plants.

The extraction efficiency of these NADES is closely related to their molar composition, particularly the balance between hydrogen bond

donors (HBD) and acceptors (HBA), which determines polarity, viscosity, and solvation capacity [26,28]. NADES 3, with a higher proportion of acetate, provides a semi-ionic and moderately basic medium favorable for extracting less polar flavonoids, including aglycones. In contrast, NADES 4, rich in glycerol, offers a polar environment saturated with hydroxyl groups capable of forming multiple hydrogen bonds, which facilitates the extraction of highly polar compounds such as phenolic acids [17,28].

Table 4. IC_{50} antioxidant activity of extract

Samples	Concentrations	Abs	% Inhibition	IC_{50} (ppm)
Ascorbic Acid	10	0.636	12.28	33.41
	30	0.486	32.97	
	50	0.102	85.93	
NADES 1	10	0.623	14.07	1,023.24
	30	0.597	17.66	
	50	0.622	14.21	
NADES 2	10	0.628	13.57	634.23
	30	0.612	17.76	
	50	0.598	19.81	
NADES 3	10	0.586	19.17	36.30
	30	0.621	14.34	
	50	0.614	15.31	
NADES 4	10	0.621	14.34	34.20
	30	0.585	19.31	
	50	0.619	14.62	
Ethanol				116 [5]

This mechanistic insight is supported by previous reports showing that hydrogen bonding interactions, ion–dipole attractions, and dipole–dipole interactions significantly enhance the solubilization of antioxidant compounds within eutectic matrices [21,30]. These interactions explain why the composition of NADES significantly influences the extraction profile. NADES 3's semi-ionic nature likely facilitates the solubilization of aromatic and carbonyl-containing structures, whereas NADES 4's high hydrogen bonding density supports the dissolution of phenolic acids and other polar antioxidants. Support for this is found in Fourier Transform Infrared Spectroscopy (FTIR) analysis, which revealed shifts in the –OH and C=O stretching bands in extracts compared to the parent NADES, indicative of strong hydrogen bonding and complexation between solvent and solute molecules. These results are consistent with previous observations in NADES-based extractions of polyphenols and terpenoids [35]. Furthermore, computational modeling studies and molecular dynamics simulations have demonstrated that dipole moment alignment, viscosity reduction, and solvation free energy all vary with HBD:HBA ratio, directly affecting compound accessibility and binding within plant matrices [26,31].

4 Conclusion

The variations in IC_{50} values across different NADES formulations clearly demonstrate the critical role of solvent composition in determining the antioxidant extraction efficiency. NADES 3 (2:1) and NADES 4 (1:3) were particularly effective, likely due to their optimal polarity and interaction profiles, resulting in IC_{50} values comparable to that of ascorbic acid. These findings underscore the potential of tailored NADES formulations as green and sustainable solvents for the extraction of antioxidant-rich natural products. Future studies should focus on profiling individual antioxidant constituents using chromatographic techniques such as LC-MS or HPLC-DAD to confirm the chemical basis of the observed bioactivity.

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