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Effect of Natural Deep Eutectic Solvents and Conventional Solvents on Extraction Yield, Antioxidant Activity, and Toxicity of *Peperomia pellucida* (L.) Kunth

Aniza Saini[1a], Mohammad Amil Zulhilmi Benjamin[2b], Nor Azizun Rusdi[3c], Ahmad Hazim Abdul Aziz[4a] and Mohd Azrie Awang[5ad]*

Abstract: *Peperomia pellucida* (L.) Kunth, commonly known as 'Sirih Cina,' is a botanical plant recognised for its traditional application in various therapeutic contexts due to its bioactive compounds. Despite its potential benefits, its properties are sometimes underappreciated. The choice of solvent extraction significantly influences its biological properties. This study investigates the impact of different solvents on the extraction yield, antioxidant activity, and toxicity of *P. pellucida* leaf extracts. The selected solvents include natural deep eutectic solvents (NADES), distilled water, methanol, ethanol, and ethyl acetate. The extraction of *P. pellucida* leaves was conducted using an ultrasonic water bath apparatus. The aluminium chloride colorimetric assay was employed to determine the total flavonoid content (TFC), while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess antioxidant activity. Moreover, the toxicological assessment of *P. pellucida* leaf extracts was performed using the brine shrimp lethality assay (BSLA) to determine LC_{50} (lethal concentration 50) values. NADES emerged as the most efficient solvent for extraction, yielding the highest extraction yield (17.39 ± 0.03%) and DPPH scavenging activity (83.31 ± 0.03%), while demonstrating non-toxicity in the BSLA (LC_{50} = 1597.62 µg/mL). Although NADES ranked third in terms of TFC, a moderate correlation between TFC and DPPH suggests that factors beyond TFC influence antioxidant activity. Overall, NADES exhibited antioxidant activity and showed non-toxicity towards brine shrimp. Therefore, NADES is a suitable solvent for exploring the medicinal potential of *P. pellucida* leaves as a source for therapeutic applications.

Keywords: *Peperomia pellucida, extraction yield, flavonoids, antioxidant, toxicity.*

1. Introduction

In this era, the extraction of natural compounds from medicinal plants has garnered significant attention due to the abundant presence of bioactive molecules, including phenolic and flavonoid compounds. Extraction solvents are commonly selected based on their polarity. Solvents such as methanol, ethanol, and ethyl acetate, which have high polarity, are often used for extracting polyphenols, while non-polar compounds are better extracted using solvents like hexane (Ng et al., 2020). Achieving effective extraction and purification of antioxidant and phytochemical compounds from plant materials depends on various factors, including duration, temperature, solvent concentration, and polarity. Given the differing polarities of various phytochemicals, no single solvent can effectively extract all compounds. This highlights the importance of carefully selecting suitable solvents to ensure thorough extraction (Nawaz et al., 2020).

Natural deep eutectic solvents (NADES) represent a solvent system comprising natural elements such as organic acids and amines that form a specialised mixture. This innovation is gaining attention as an eco-friendlier substitute for traditional organic solvents in extraction processes. It offers benefits such as being biodegradable, highly soluble, stable, and easy to produce, making it a promising green choice for extracting natural substances (Liu et al., 2018). Despite being less explored for plant extractions, there is increasing evidence supporting NADES as a potential alternative to conventional solvents. For instance, Oomen et al. (2020) found that, despite the high hydrophilicity of NADES, glycosides with greater water affinity were extracted less than their aglycones. This highlights NADES as a potential medium for extracting *Scutellaria baicalensis* compounds with diverse hydrophilic properties. Additionally, NADES proved to be as effective as traditional eco-friendly solvents in extracting polyphenols, offering the added advantage of operating at milder temperatures. This method avoids flammable solvents and utilises sustainable, natural compounds in the extraction of polyphenols from ground coffee (García-Roldán et al., 2023). Their environmentally friendly nature and compatibility with the environment make them a viable green option for natural product extraction (Popovic et al., 2022).

Authors information:
[a]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA. E-mail: anizasaini96@gmail.com[1]; hazim.aziz@ums.edu.my[4]
[b]Borneo Research on Algesia, Inflammation and Neurodegeneration (BRAIN) Group, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA. E-mail: mohammad_amil_zulhilmi_dm22@iluv.ums.edu.my[2]
[c]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA. E-mail: azizun@ums.edu.my[3]
[d]Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA. E-mail: ma.awang@ums.edu.my[5]
*Corresponding Author: ma.awang@ums.edu.my

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Peperomia pellucida (L.) Kunth, also referred to as 'Sirih Cina,' is a plant that thrives in moist surroundings. Belonging to the Piperaceae family, it is predominantly distributed across regions such as Central and South America, Africa, Australia, and Southeast Asia (Alves et al., 2019). Its distinct characteristics include heart-shaped leaves, a smooth surface, juicy stems, and shallow roots, contributing to its significance as both a culinary and medicinal asset that benefits human well-being (Alves et al., 2019; Ho et al., 2022). In Southeast Asian countries, *P. pellucida* is frequently employed to manage particular skin issues in Iloilo, Philippines (Tantiado, 2012). Additionally, in Singapore, people use a concoction of the entire plant to alleviate joint discomfort (Siew et al., 2014). Meanwhile, in Malaysia, *P. pellucida* has a longstanding tradition of being used as a plant decoction for managing rheumatism (Ibrahim & Hamzah, 1999). Phytochemical analysis of crude extracts reveals the presence of flavonoids, alkaloids, carbohydrates, carotenoids, depsides, phenols, quinones, sterols, tannins, saponins, azulenes, reducing sugars, and triterpenoids (Alves et al., 2019). Results from a range of in vivo, in vitro, and clinical investigations suggest that extracts of *P. pellucida* exhibit promising pharmacological properties. These extracts have demonstrated potential in various areas, including antioxidant, analgesic, antibacterial, antifungal, anti-inflammatory, antidiabetic, anti-hypercholesterolemia, toxicological, and cytotoxic effects (Alves et al., 2019; Ho et al., 2022). While the extract showed mild toxicity in animal models, it was non-toxic to normal cell lines (HEK-293) compared to HeLa and HepG2 cancer cell lines, as well as to brine shrimps and rodents (Ho et al., 2022).

Despite its potential for various biological activities, the utilisation of *P. pellucida* leaf extract remains limited. Further exploration is necessary to thoroughly examine the choice of suitable solvents for extraction, aiming to uncover environmentally conscious methods and compare them with alternative solvent options. Hence, this study aimed to assess the extraction yield and antioxidant activity of *P. pellucida* leaf extracts using different solvents, including NADES, distilled water, methanol, ethanol, and ethyl acetate. Moreover, the study also sought to evaluate the toxicity levels associated with these extracted compounds.

2. Experimental Methods

Chemicals and Solvents

NADES consisting of choline chloride and lactic acid, along with methanol, ethanol, and ethyl acetate acquired from Merck (Darmstadt, Germany), were employed as solvents for the extraction process. Sigma-Aldrich (Burlington, MA, USA) provided ascorbic acid, aluminium chloride, potassium dichromate, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

Plant Materials

P. pellucida leaves were gathered near the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Sabah, Malaysia. The fresh samples were washed with tap water to remove surface impurities and subsequently dried using an oven dryer (ED 23, Binder, Neckarsulm, Germany) at 60 °C for 4 h until fully dried (Awang et al., 2021a). Following drying, the samples were finely ground into a consistent powder and stored at 4 °C for future analyses.

NADES Preparation

NADES were prepared using a modified heating and stirring technique based on the method by Rosarina et al. (2022). Choline chloride (a hydrogen bond acceptor, HBA) and lactic acid (a hydrogen bond donor, HBD) were combined in a molar ratio of 1:2. The water content of NADES was adjusted by adding deionised water. These constituents were mixed in a glass beaker at 70 °C under continuous stirring until a clear and homogeneous liquid was obtained. The resulting NADES were then stored at room temperature for subsequent use.

Sample Extraction

This study was conducted using the ultrasonic extraction technique implemented through an ultrasonic water bath. Five types of solvents were utilised: ethanol, methanol, ethyl acetate, distilled water, and NADES. Approximately 5 g of powdered *P. pellucida* leaves were added to 150 mL of an extraction solvent using an ultrasonic water bath (CPX8800H, Branson, Brookfield, CT, USA). The ultrasonic extraction was carried out for 60 min, starting at an initial temperature of 50 °C. The resulting extract supernatant was collected and filtered, while the residue was discarded. The filtered extract underwent further separation under reduced pressure at 45 °C using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany) and was stored in aluminium-wrapped tubes. Subsequently, these tubes were placed in an oven dryer and dried for 24 h at 50 °C to ensure complete solvent evaporation from the samples. The percentage of sample obtained from different solvents was calculated using Eq. (1):

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried sample (g)}} \times 100 \quad (1)$$

Antioxidant Analysis

Total Flavonoid Content

The total flavonoid content (TFC) of the sample was determined based on the formation of the flavonoid-aluminium complex, as described by Awang et al. (2021b). Approximately 1 mg of the extract was combined with 1 mL of a 2% methanolic-aluminium chloride solution. The complex was allowed to form during a 15-min incubation period and was subsequently measured at a wavelength of 430 nm using a UV-Vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA).

DPPH Assay

The DPPH assay was conducted following the protocol outlined by Stephenus et al. (2023), with minor adjustments. Approximately 1 mL of the extract or ascorbic acid (positive control) was combined with 1 mL of methanolic-DPPH solution.

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The mixture was vigorously agitated and subsequently incubated in the absence of light at room temperature for 20 min. The decrease in absorbance was measured at 517 nm against a blank without DPPH using a UV-Vis spectrophotometer. Afterward, the percentage of DPPH radical scavenging activity was determined using Eq. (2):

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \qquad (2)$$

where A_c and A_s represent the absorbance values of the control and sample, respectively.

Brine Shrimp Lethality Assay

The brine shrimp lethality assay (BSLA) protocol was adapted from the method outlined by Benjamin et al. (2022), with slight adjustments. Brine shrimp were hatched from brine shrimp eggs in an aerated aquarium filled with seawater over a 48-h period. Once hatched, the nauplii were collected from a well-lit area, ensuring they were free from eggshells, and subsequently used for the assay. One hundred nauplii were meticulously transferred using a micropipette and glass capillary into a petri dish containing 20 mL of seawater.

In each petri dish, 1 mL of the sample was added to 20 mL of the brine shrimp solution and left at room temperature for 24 h in the presence of light. After that, the remaining larvae were enumerated using a magnifying glass. The experiment included a positive control (potassium dichromate) and various concentrations of the extract solution (1000, 500, 300, and 100 µg/mL), with each set containing three tubes. Mortality (%) was calculated using Eq. (3):

$$\text{Mortality (\%)} = \frac{\text{Number of deaths}}{\text{Total number of individuals}} \times 100 \qquad (3)$$

The chronic LC_{50} (lethal concentration 50), representing 50% mortality within 24 h, was used to assess extract toxicity through probit analysis, utilising correlated concentrations and fatality percentages on a probit scale.

Statistical Analysis

The data was gathered in triplicate and provided as the mean ± standard deviation. Statistical analysis was conducted using IBM SPSS Statistics (Version 28). A one-way analysis of variance (ANOVA) was used to assess the data, followed by Tukey's Honestly Significant Difference (HSD) post hoc test to identify significant differences across the samples, with the level of significance set at 95% ($p < 0.05$). For correlation analysis, the Pearson correlation coefficient (r) was used.

3. Results and Discussion

Extraction Yield

An ultrasonic water bath is a device used in extraction processes to enhance the efficiency of extracting compounds from plant materials. It operates by subjecting the mixture of solvent and plant material to high-frequency sound waves, creating microscopic bubbles that implode upon collapsing. This phenomenon, known as cavitation, generates intense local pressure and temperature changes that aid in breaking down the cell walls of the plant and promoting better solvent penetration (Santos & Capelo, 2007; Kallioinen & Mänttäri, 2011). Factors such as frequency, amplitude, solvent choice, temperature, and extraction time influence its effectiveness. The benefits include efficient mass transfer, reduced extraction time, selectivity, and sustainability due to lower energy requirements (Vilkhu et al., 2008; Chemat et al., 2017). Hence, this method optimises extraction yields while maintaining the quality of extracted compounds.

Table 1 presents a comparison of the results obtained from five different solvent types used in *P. pellucida* leaf extracts, showing significant differences. Considering how different solvents interact with plant compounds, NADES stands out due to its strong extraction ability, yielding 17.39 ± 0.04%. This efficacy can be attributed to the unique characteristics of NADES, which allow it to establish robust interactions with the compounds due to its distinct polarity. In contrast, ethyl acetate, characterised by lower polarity, yields the least efficient extraction at 2.87 ± 0.15%, possibly due to its comparatively weaker solvent-target interactions. Distilled water, methanol, and ethanol, which fall within the middle range of polarities, show extraction yields of 8.24 ± 0.05%, 7.46 ± 0.00%, and 6.70 ± 0.02%, respectively, indicating their moderate ability to interact with the compounds of interest.

Table 1. Extraction yield of *P. pellucida* leaf extracts based on different solvents

Solvent Extraction	Extraction Yield (%)
NADES	17.39 ± 0.04[a]
Distilled water	8.24 ± 0.05[b]
Methanol	7.46 ± 0.00[c]
Ethanol	6.70 ± 0.02[d]
Ethyl acetate	2.87 ± 0.15[e]

The presented data represent means ± standard deviations from triplicates. Distinct letters (within a column) indicate significant differences established through one-way ANOVA and Tukey's HSD test ($p < 0.05$).

The choice of solvent significantly influences the extraction yield. Solvents with different polarities interact differently with the target compounds in plant material (Rezaie et al., 2015). The higher extraction yield obtained with NADES can be attributed to its distinct polarity, which allows it to effectively interact with the

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compounds and facilitate their release (Hikmawanti et al., 2021). This study also highlighted that diluting NADES with distilled water and increasing the temperature aim to reduce its high viscosity, thereby facilitating the extraction process (Shishov et al., 2020). Furthermore, surface tension and viscosity are crucial factors in ultrasonic extraction. Reduced surface tension and viscosity facilitate the formation of explosive cavities. Liquids with lower viscosity tend to generate stable foam fractions, while those with high viscosity produce larger cavities (Kadoi & Nakae, 2011; Kallioinen & Mänttäri, 2011). Hence, this enhances the diffusion of NADES into plant samples.

On the other hand, solvents with lower polarity, such as ethyl acetate, demonstrated lower extraction yields due to their weaker interactions with the target compounds. This aligns with the findings of Koch et al. (2020), who reported that ethyl acetate has limited efficacy as an extraction solvent in different methods. The lower extraction yield of ethyl acetate, coupled with its lower polarity, could also be influenced by its viscosity. The reduced viscosity of ethyl acetate might hinder its ability to effectively penetrate plant material and establish strong interactions, contributing to the observed lower extraction efficiency (Rezaie et al., 2015). Recent studies highlight that solvent polarity significantly influences the extraction process, affecting the amount and quality of extracted compounds, secondary metabolites, and biological activity (Rafińska et al., 2019). These findings emphasise the importance of selecting the appropriate solvent to achieve better extraction results, particularly in obtaining greater amounts of desired compounds.

Antioxidant Activity

Table 2 presents the outcomes of solvent extraction on *P. pellucida* leaf extracts, including TFC and DPPH scavenging activity. Among the solvents used, ethanol proved to be the most efficient, yielding the highest TFC value of 18.74 ± 0.10 mg RE/g, demonstrating its strong capacity for flavonoid extraction. Furthermore, distilled water yielded a TFC value of 17.89 ± 0.05 mg RE/g, followed by NADES (17.24 ± 0.17 mg RE/g) and methanol (16.43 ± 0.02 mg RE/g). Conversely, ethyl acetate yielded the lowest TFC value at 4.92 ± 0.02 mg RE/g, indicating its relatively limited capacity for flavonoid extraction. These significant variations in TFC values highlight pronounced differences in extraction efficiency among the solvents.

Table 2. Antioxidant activity of *P. pellucida* leaf extracts based on different solvents

Solvent Extraction	TFC (mg RE/g)	DPPH (%)
NADES	17.24 ± 0.17^{c}	83.31 ± 0.03^{b}
Distilled water	17.89 ± 0.05^{b}	70.83 ± 0.44^{d}
Methanol	16.43 ± 0.02^{d}	79.12 ± 0.02^{c}
Ethanol	18.74 ± 0.10^{a}	79.48 ± 0.03^{c}
Ethyl acetate	4.92 ± 0.02^{e}	68.22 ± 0.07^{e}
Ascorbic acid	NA	95.01 ± 0.06^{a}

The presented data represent means ± standard deviations from triplicates. Distinct letters (within a column) indicate significant differences established through one-way ANOVA and Tukey's HSD test ($p < 0.05$).

NA: Not Applicable.

Regarding DPPH scavenging activity (Table 2), the solvents demonstrated the following percentages: NADES exhibited the highest activity at 83.31 ± 0.03%, highlighting its strong antioxidant potential. Meanwhile, ethanol and methanol showed comparable DPPH scavenging activities at 79.48 ± 0.03% and 79.12 ± 0.02%, respectively, indicating their effective free radical neutralisation capabilities. Distilled water recorded a DPPH scavenging activity of 70.83 ± 0.44%, reflecting variations in antioxidant effectiveness. However, ethyl acetate exhibited the lowest DPPH scavenging activity at 68.22 ± 0.07%, suggesting its relatively limited capacity to counteract free radicals.

Polarity is highly sensitive to changes in water content due to hydrogen bonding disruptions, leading to alterations in the polarity index. Therefore, polarity is a crucial aspect of NADES and is closely related to its solubilising ability. The increased antioxidant efficacy of NADES might be attributed to the release of hydrophilic phenolic compounds present in *P. pellucida* leaf extracts (Mohammad Salamatullah et al., 2022). The elevated antioxidant activity in NADES can also be attributed to its unique blend of choline chloride and lactic acid, forming deep eutectic solvents. This combination strengthens hydrogen bond interactions and polarity, facilitating efficient electron donation to counteract DPPH radicals (Doldolova et al., 2021). The notable polarity of NADES further assists in dissolving and extracting hydrophilic antioxidants from the sample, resulting in better scavenging activity when compared to solvents such as ethyl acetate. The distinct composition and polarity of NADES enhance its ability to engage with DPPH radicals, making it a promising solvent choice for antioxidant studies.

Distilled water exhibits slightly lower DPPH activity than NADES due to its lack of solute compounds. In contrast, NADES contains choline chloride and lactic acid, which enhance hydrogen bond interactions and polarity (Ling et al., 2020). These components contribute to electron donation against DPPH radicals, leading to increased antioxidant activity (Jurić et al., 2021). On the other hand, the higher polarity of distilled water and the absence of these solutes hinder its ability to interact with and neutralise DPPH radicals as effectively as NADES. Meanwhile, methanol and ethanol exhibit similar DPPH radical scavenging activities due to their moderate polarity, allowing them to interact moderately with DPPH radicals and demonstrate noticeable scavenging activity (Liu et al., 2018). However, their DPPH activity is weaker compared to NADES, possibly due to differences in specific polarities and interactions with DPPH radicals.

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As shown in Table 3, the correlation analysis between TFC and DPPH revealed a moderate positive correlation, with an r value of 0.67. This finding indicates a significant relationship between flavonoids (TFC) and antioxidant activity (DPPH) in *P. pellucida* leaf extracts from different solvents.

Table 3. Correlation analysis between TFC and DPPH of *P. pellucida* leaf extracts.

	DPPH	
	r	***p*-Value**
TFC	0.67*	0.00

* Correlation is significant at the 0.01 level (2-tailed).

The moderate positive correlation indicates that as TFC increases, DPPH scavenging activity tends to increase as well. This aligns with the general understanding that flavonoids are often associated with antioxidant properties, and higher flavonoids may contribute to enhanced free radical scavenging abilities (Aryal et al., 2019). Notably, although NADES ranked third in TFC among the solvents, it exhibited the highest DPPH scavenging activity. While the correlation is moderate, additional factors beyond flavonoids could also influence antioxidant activity, including the presence of other bioactive compounds such as phenolics (Aryal et al., 2019).

Toxicity

As noted by Benjamin et al. (2022), in relation to the BSLA, the toxicity categorisation for plant extracts is as follows: extracts with LC_{50} values exceeding 1000 µg/mL are deemed non-toxic; those ranging from 500 µg/mL to 1000 µg/mL exhibit mild toxicity; and extracts with LC_{50} values below 500 µg/mL are classified as toxic. This assay is commonly used to determine whether an extract ranges from toxic to non-toxic, serving as a preliminary screening for cytotoxicity or safety for consumption (Adelegan et al., 2023; Kharisma et al., 2023). Hence, Table 4 presents the toxicity of *P. pellucida* leaf extracts with various solvents using BSLA. Based on the findings, both NADES and distilled water displayed LC_{50} values > 1000 µg/mL, implying that at concentrations above this threshold, these solvents exerted a non-toxic effect on the test organisms. Conversely, methanol and ethanol had significantly lower LC_{50} values of 403.66 µg/mL and 198.45 µg/mL, respectively, indicating mild toxicity. Ethyl acetate exhibited the lowest LC_{50} at 129.90 µg/mL, indicating toxicity compared to the other solvents. These results highlight the diverse toxic effects of different solvents on the test organisms.

Table 4. BSLA of *P. pellucida* leaf extracts based on different solvents

Solvent Extraction	Concentration (µg/mL)	Mortality (%)	LC_{50} (µg/mL)
NADES	1000	9	1597.62
	500	6	
	300	2	
	100	0	
Distilled water	1000	10	1561.44
	500	5	
	300	3	
	100	0	
Methanol	1000	70	403.66
	500	50	
	300	40	
	100	30	
Ethanol	1000	80	198.45
	500	70	
	300	50	
	100	40	
Ethyl acetate	1000	100	129.90
	500	100	
	300	70	
	100	40	
Potassium dichromate	1000	100	300.66
	500	40	
	300	30	
	100	20	

The high toxicity observed in methanol and ethanol, with LC_{50} values below 500 µg/mL, can be attributed to their properties as common organic solvents. These solvents interact with living systems, resulting in toxicity (Popovici et al., 2021). Similarly, ethyl acetate, which is less polar, exhibited higher toxicity, with an LC_{50} value of 129.90 µg/mL due to its distinct properties and interactions with organisms. Its lower polarity might influence how it interacts with organisms, potentially harming cells and metabolic processes (Al-Saeedi et al., 2017). The increased toxicity of ethyl acetate underscores its unsuitability for applications requiring low toxicity. In contrast, NADES and distilled water exhibited no toxic effects, even at concentrations > 1000 µg/mL, highlighting their potential suitability for various applications without posing substantial harm to organisms. This can be attributed to their unique compositions and low toxicity profiles, making them safe choices for use in environments where minimising harm to living organisms is crucial (Benjamin et al., 2022; Usmani et al., 2023).

The aim of the BSLA was to assess the functional properties of *P. pellucida* leaf extracts. However, a notable gap in global knowledge exists regarding the impact of solvent extracts on the toxicity of these extracts. This study employs the BSLA as a reliable method for the initial assessment of extract toxicity (Ntungwe N et al., 2020). Additionally, the BSLA results for *P. pellucida* leaf extracts are compared using different solvents to explore their

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potential medicinal value for future development as functional food ingredients. The ultimate goal is to formulate products with pharmaceutical and nutraceutical properties. Notably, NADES and distilled water exhibit no harmful effects, even at concentrations > 1000 µg/mL, indicating their potential suitability for various applications without causing significant harm to organisms. Further investigations could explore their in vivo effects using animal models.

4. Conclusion

Overall, NADES stands out as the superior solvent, delivering the highest extraction yield, remarkable antioxidant potential, and non-toxic attributes for *P. pellucida* leaf extracts. NADES emerges as the optimal solvent for *P. pellucida* leaf extraction, yielding both the highest crude extract and moderate TFC. This solvent also exhibits the most potent antioxidant activity, attributed to its ability to control DPPH reactivity kinetics. Notably, NADES demonstrates exceptional non-toxicity, as indicated by an LC_{50} of 1597.62 µg/mL in the BSLA. Therefore, these results highlight the promising role of NADES in advancing therapeutics by harnessing the medicinal value of the plant.

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6. References

Adelegan, A. A., Dokunmu, T. M., & Iweala, E. E. J. (2023). In-vitro antioxidant activity and cytotoxic effect of ethanol leaf extract and fractions of *Olax subscorpioidea* Oliv. (Olacaceae). *Tropical Journal of Natural Product Research*, *7*(8), 3806–3812.

Al-Saeedi, A. H., Al-Ghafri, M. T. H., & Hossain, M. A. (2017). Brine shrimp toxicity of various polarities leaves and fruits crude fractions of *Ziziphus jujuba* native to Oman and their antimicrobial potency. *Sustainable Chemistry and Pharmacy*, *5*, 122–126.

Alves, N. S. F., Setzer, W. N., & da Silva, J. K. R. (2019). The chemistry and biological activities of *Peperomia pellucida* (Piperaceae): A critical review. *Journal of Ethnopharmacology*, *232*, 90–102.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants*, *8*(4), 96.

Awang, M. A., Chua, L. S., Abdullah, L. C., & Pin, K. Y. (2021a). Drying kinetics and optimization of quercetrin extraction from *Melastoma malabathricum* leaves. *Chemical Engineering and Technology*, *44*(7), 1214–1220.

Awang, M. A., Daud, N. N. N. N. M., Ismail, N. I. M., Cheng, P. G., Ismail, M. F., & Ramaiya, S. D. (2021b). Antioxidant and cytotoxicity activity of *Cordyceps militaris* extracts against human colorectal cancer cell line. *Journal of Applied Pharmaceutical Science*, *11*(7), 105–109.

Benjamin, M. A. Z., Ng, S. Y., Saikim, F. H., & Rusdi, N. A. (2022). The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. *Molecules*, *27*(19), 6458.

Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, *34*, 540–560.

Doldolova, K., Bener, M., Lalikoğlu, M., Aşçı, Y. S., Arat, R., & Apak, R. (2021). Optimization and modeling of microwave-assisted extraction of curcumin and antioxidant compounds from turmeric by using natural deep eutectic solvents. *Food Chemistry*, *353*, 129337.

García-Roldán, A., Piriou, L., & Jauregi, P. (2023). Natural deep eutectic solvents as a green extraction of polyphenols from spent coffee ground with enhanced bioactivities. *Frontiers in Plant Science*, *13*, 1072592.

Hikmawanti, N. P. E., Ramadon, D., Jantan, I., & Mun'im, A. (2021). Natural deep eutectic solvents (NADES): Phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development. *Plants*, *10*(10), 2091.

Ho, K. L., Yong, P. H., Wang, C. W., Kuppusamy, U. R., Ngo, C. T., Massawe, F., & Ng, Z. X. (2022). *Peperomia pellucida* (L.) Kunth and eye diseases: A review on phytochemistry, pharmacology and toxicology. *Journal of Integrative Medicine*, *20*(4), 292–304.

Ibrahim, F. H., & Hamzah, N. (1999). The use of medicinal plant species by the Temuan tribe of Ayer Hitam Forest, Selangor, Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *22*(2), 85–94.

Jurić, T., Mićić, N., Potkonjak, A., Milanov, D., Dodić, J., Trivunović, Z., & Popović, B. M. (2021). The evaluation of phenolic content, in vitro antioxidant and antibacterial activity of *Mentha piperita* extracts obtained by natural deep eutectic solvents. *Food Chemistry*, *362*, 130226.

Kadoi, K., & Nakae, H. (2011). Relationship between foam stabilization and physical properties of particles on aluminum foam production. *Materials Transactions*, *52*(10), 1912–1919.

DOI:https//doi.org/10.22452/mjs.vol44no1.5
Malaysian Journal of Science 44(1): 34-41 (March 2025)

Kallioinen, M., & Mänttäri, M. (2011). Influence of ultrasonic treatment on various membrane materials: A review. *Separation Science and Technology*, *46*(9), 1388–1395.

Kharisma, A. D., Nisa, U. C., & Yasman. (2023). Evaluation of antioxidant activity and toxicity of *Cinnamomum burmannii* B. from different provinces of Indonesia. *Journal of Hunan University Natural Sciences*, *50*(4), 177–188.

Koch, W., Kukuła-Koch, W., Czop, M., Helon, P., & Gumbarewicz, E. (2020). The role of extracting solvents in the recovery of polyphenols from green tea and its antiradical activity supported by principal component analysis. *Molecules*, *25*(9), 2173.

Ling, J. K. U., Chan, Y. S., Nandong, J., Chin, S. F., & Ho, B. K. (2020). Formulation of choline chloride/ascorbic acid natural deep eutectic solvent: Characterization, solubilization capacity and antioxidant property. *LWT*, *133*, 110096.

Liu, Y., Friesen, J. B., McAlpine, J. B., Lankin, D. C., Chen, S.-N., & Pauli, G. F. (2018). Natural deep eutectic solvents: Properties, applications, and perspectives. *Journal of Natural Products*, *81*(3), 679–690.

Mohammad Salamatullah, A., Hayat, K., Mabood Husain, F., Asif Ahmed, M., Arzoo, S., Musaad Althbiti, M., … Bourhia, M. (2022). Effects of different solvents extractions on total polyphenol content, HPLC analysis, antioxidant capacity, and antimicrobial properties of peppers (red, yellow, and green (*Capsicum annum* L.)). *Evidence-Based Complementary and Alternative Medicine*, *2022*, 7372101.

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*, e17129.

Ng, Z. X., Samsuri, S. N., & Yong, P. H. (2020). The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. *Journal of Food Processing and Preservation*, *44*(9), e14680.

Ntungwe N, E., Domínguez-Martín, E. M., Roberto, A., Tavares, J., Isca, V. M. S., Pereira, P., … Rijo, P. (2020). *Artemia* species: An important tool to screen general toxicity samples. *Current Pharmaceutical Design*, *26*(24), 2892–2908.

Oomen, W. W., Begines, P., Mustafa, N. R., Wilson, E. G., Verpoorte, R., & Choi, Y. H. (2020). Natural deep eutectic solvent extraction of flavonoids of *Scutellaria baicalensis* as a replacement for conventional organic solvents. *Molecules*, *25*(3), 617.

Popovic, B. M., Micic, N., Potkonjak, A., Blagojevic, B., Pavlovic, K., Milanov, D., & Juric, T. (2022). Novel extraction of polyphenols from sour cherry pomace using natural deep eutectic solvents – Ultrafast microwave-assisted NADES preparation and extraction. *Food Chemistry*, *366*, 130562.

Popovici, V., Bucur, L., Popescu, A., Schröder, V., Costache, T., Rambu, D., … Badea, V. (2021). Antioxidant and cytotoxic activities of *Usnea barbata* (L.) F.H. Wigg. dry extracts in different solvents. *Plants*, *10*(5), 909.

Rafińska, K., Pomastowski, P., Rudnicka, J., Krakowska, A., Maruśka, A., Narkute, M., & Buszewski, B. (2019). Effect of solvent and extraction technique on composition and biological activity of *Lepidium sativum* extracts. *Food Chemistry*, *289*, 16–25.

Rezaie, M., Farhoosh, R., Iranshahi, M., Sharif, A., & Golmohamadzadeh, S. (2015). Ultrasonic-assisted extraction of antioxidative compounds from Bene (*Pistacia atlantica* subsp. *mutica*) hull using various solvents of different physicochemical properties. *Food Chemistry*, *173*, 577–583.

Rosarina, D., Narawangsa, D. R., Shaffa, N., Chandra, R., Sari, E., & Hermansyah, H. (2022). Optimization of ultrasonic—assisted extraction (UAE) method using natural deep eutectic solvent (NADES) to increase curcuminoid yield from *Curcuma longa* L., *Curcuma xanthorrhiza*, and *Curcuma mangga* Val. *Molecules*, *27*(18), 6080.

Santos, H. M., & Capelo, J. L. (2007). Trends in ultrasonic-based equipment for analytical sample treatment. *Talanta*, *73*(5), 795–802.

Shishov, A., Pochivalov, A., Nugbienyo, L., Andruch, V., & Bulatov, A. (2020). Deep eutectic solvents are not only effective extractants. *Trends in Analytical Chemistry*, *129*, 115956.

Siew, Y.-Y., Zareisedehizadeh, S., Seetoh, W.-G., Neo, S.-Y., Tan, C.-H., & Koh, H.-L. (2014). Ethnobotanical survey of usage of fresh medicinal plants in Singapore. *Journal of Ethnopharmacology*, *155*(3), 1450–1466.

Stephenus, F. N., Benjamin, M. A. Z., Anuar, A., & Awang, M. A. (2023). Effect of temperatures on drying kinetics, extraction yield, phenolics, flavonoids, and antioxidant activity of *Phaleria macrocarpa* (Scheff.) Boerl. (mahkota dewa) fruits. *Foods*, *12*(15), 2859.

Tantiado, R. G. (2012). Survey on ethnopharmacology of medicinal plants in Iloilo, Philippines. *International Journal of Bio-Science and Bio-Technology*, *4*(4), 11–26.

DOI:https//doi.org/10.22452/mjs.vol44no1.5
Malaysian Journal of Science 44(1): 34-41 (March 2025)

Usmani, Z., Sharma, M., Tripathi, M., Lukk, T., Karpichev, Y., Gathergood, N., … Gupta, V. K. (2023). Biobased natural deep eutectic system as versatile solvents: Structure, interaction and advanced applications. *Science of The Total Environment*, *881*, 163002.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry — A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161–169.

DOI:https//doi.org/10.22452/mjs.vol44no1.5
Malaysian Journal of Science 44(1): 34-41 (March 2025)