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Effect of extraction solvents on yield, chemical composition and antioxidant activity of *Peperomia pellucida* (L.) Kunth seed extract

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ABSTRACT

Peperomia pellucida (L.) Kunth is a valuable herbal plant with numerous medicinal properties, offering potential for the development of herbal products with antioxidant benefits. Extraction is a critical step in the recovery and analysis of bioactive compounds in botanical materials. The judicious selection of extraction methodologies and solvents is important in ensuring the recovery and protection of the biological efficacy of these compounds, thereby facilitating the standardization of herbal products. This study investigates the influence of solvent on the yield, chemical composition, and antioxidative activity of P. Pellucida seed extract. The results underscore a propensity towards maximal yield with ethanol employment. Furthermore, all extracts manifest a moderate antioxidative capacity, with the ethyl acetate extract particularly demonstrating heightened activity. Dill apiol is identified as the predominant constituent in all extracts, constituting up to 83% of the composition. The study's findings suggest the promising potential of P. Pellucida seed extract as a valuable source of antioxidants, paving the way for the creation of beneficial herbal products.

1. INTRODUCTION

Peperomia pellucida (L.) Kunth is an annual, shallow-rooted herb, native to Central and South America, China, Nigeria, Angola, and Zambia. The plant is identified by its succulent stems, glossy heart-shaped fleshy leaves, and diminutive dot-like seeds attached to fruiting spikes. It is commonly found in humid and damp areas, existing both in the wild and cultivated, typically reaching a height of 6-18 inches. *P. pellucida* is widely acknowledged for its utilization in folk medicines to address a variety of ailments, including diabetes, hypertension, skin sores, gastrointestinal disorders, rheumatic pain, headache, fatigue, malaria, fever, and reduction of cholesterol levels. Its medicinal properties have been substantiated by numerous studies (Alves et al., 2019). Extracts from the plant's aerial parts exhibit anti-inflammatory activity and dose-dependent analgesic properties, with these effects linked to interference with prostaglandin synthesis (Aziba et al., 2001; Arrigoni-Blank et al., 2002, 2004). A study by Florence and co-authors has highlighted the fracture-healing effect of the aqueous and ethanol extracts of *P. pellucida*. Both aqueous and ethanol extracts of *P. pellucida* have demonstrated efficacy in promoting fracture healing by enhancing mineral deposition and improving the microarchitecture of regenerating bone (Florence et al., 2017). Furthermore, the ethanolic extracts from the leaves of *P. pellucida* have demonstrated broad-

spectrum bactericidal activity, as documented by Alves et al. (2019). Additionally, Wei et al. (2011) reported the presence of phytol, decahydro-2-naphthalenol, and methyl palmitate in the methanol leaf extract of P. pellucida, attributing them to its antimicrobial properties.

The bioactivity of *P. pellucida* is attributed to its secondary metabolites, particularly alkaloids, flavonoids, glycosides, terpenoids, and steroids. These bioactive compounds are typically found in low concentrations in plants, which makes the choice of extraction technique important to obtain a high yield while minimizing the impact on their functional properties (Quispe-Condori et al., 2008; Hernández et al., 2009). Consequently, it is essential to choose an appropriate extraction method and solvent, taking into consideration the characteristics of the sample matrix, the chemical properties of the analytes, the interactions between the matrix and the analytes, as well as the overall efficiency and desired attributes of the extracted compounds (Ishida et al., 2001; Hayouni et al., 2007).

Maceration is an extraction technique with roots dating back to ancient times, commonly employed for solid-liquid extraction processes. This method involves the careful selection of a solvent with suitable polarity, aiming to enhance the diffusion of target compounds from plant material into the solvent. In comparison to other traditional extraction techniques such as Soxhlet extraction and hydrodistillation, maceration is preferred for its simplicity, convenience, and cost-effectiveness. A notable advantage of this technique is that it does not necessitate any specialized equipment. Furthermore, this method can be tailored to extract a variety of compounds by utilizing different solvents, temperatures, and agitation techniques, which facilitates a more selective and efficient transfer of valuable chemicals from biomass. (Mathews et al., 2024).

Despite the current proven biological activities of *Peperomia pellucida*, most studies have primarily focused on the plant's leaves and roots, with limited data available on the pharmacological activity of its seeds. Therefore, this investigation aims to assess the influence of various solvents on the quantity and chemical composition as well as antioxidant activity of extracts derived from *P. pellucida* seeds by the maceration technique.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of Peperomia pellucida (L.) Kunth were collected from a farm in Binh Phuoc Province, Vietnam. The plant's identification and authentication were confirmed by the Department of Biology at Can Tho University and verified through http://www.theplantlist.org. Peperomia pellucida (L.) Kunth is recognized as an accepted scientific name according to the data from the WFO plant list.

DPPH and L- Ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hexane, ethanol 96^o, and ethyl acetate was provided by Acros, Thermal Fisher Scientific (MA, USA); All other chemicals and reagents were of the highest grade commercially available.

2.2. Preparation of seed extract

The plant seed extract was prepared by soaking the seed in solvents of varying polarities, such as ethanol, ethyl acetate, and hexane (Do et al., 2014). The extraction took place at ambient temperature using a solid-to-liquid ratio of 1:10 (w/v). The mixture was stirred periodically and macerated for 72 hours to enhance the extraction process. This entire procedure was repeated three times with a new solvent each time for a complete extraction. Following extraction, the solid and liquid components were separated using filtration. The crude extract was obtained after removing the solvent under reduced pressure. All plant seed extracts were then stored at 4°C for further analysis. The extraction yield was determined based on the mass of the crude extract obtained and was calculated as follows:

$$\%\, Yield = \frac{dried\ mass\ of\ extract}{dried\ mass\ of\ seed} \times 100 \quad (1)$$

2.3. GS- MS analysis of P. *pellucida* seed extracts

Chemical composition of P. *pellucida* seed extracts was determined by gas chromatography/mass spectrometry (GC-MS). A fused silica column DB-5ms (5%-phenyl-methylpolysiloxane) (30 m x 0.25 mm i.d. x 1 µm film thickness, Agilent Technologies) was employed for the analysis. Injector and ion source temperature were set at 280^{o}C, and 200^{o}C, respectively. The initial column temperature was 80^{o}C, raised to 120 ^{o}C at 8^{o}C/min, increased to 180^{o}C at 5^{o}C/min; 10°C/min to 280°C, and held for 3^{o}C. Helium was used as a carrier gas at a flow rate of 2 mL/min. The identification of

compounds was performed by comparison of mass spectrum and retention index (RI) with data present in the libraries NIST17.

2.4. DPPH radical scavenging assay

Antioxidant activity of the plant seed extracts was measured by the DPPH radical scavenging assay. Typically, 100 µL of each sample of various concentrations (50- 400 µg/mL) was mixed with 100 µL of DPPH solution in ethanol in a 96 well plate. The mixtures were incubated in dark for 30 min at 25-30 °C. After the reaction; the absorbance was measured at 517 nm with vitamin C as standard. The percentage of DPPH radical scavenging and antioxidant activity index (AAI) were calculated based on the equations (2) and (3):

Percentage of DPPH radical scavenging (%) = $[(A_0-A_i)/A_0] \times 100$ (2)

Where A_o was the absorbance of control, and A_i was the absorbance of sample i

$$\text{AAI} = \frac{\text{final concentration of DPPH}^{\cdot}\ (\mu g.ml^{-1})}{IC_{50}\ (\mu g.ml^{-1})} \quad (3)$$

3. RESULTS AND DISCUSSIONS

3.1. Effect of solvent on the yield and chemical composition of *P. pellucida* seed extract

The extraction yield and chemical composition of *P. pellucida* seed extracts are presented in Table 1 and Table 2, respectively. Hexane, ethyl acetate, and ethanol were chosen as nonpolar, moderately polar, and polar solvents for extracting *P. Pellucida* seeds. Among the selected solvents, ethanol yielded the highest extraction compared to ethyl acetate and hexane ($P \leq 0.5$). The extraction process is known to be influenced by several critical factors, including the plant matrix, solvent, temperature, pressure, and time (Hernández et al., 2009). Of particular significance is the choice of solvent, as the efficiency of the extract is predominantly contingent upon this selection. The polarity of the target compounds serves as the decisive factor in solvent selection. Additionally, factors such as the molecular affinity between the solvent and solute, mass transfer, the use of co-solvents, environmental safety, human toxicity, and financial feasibility are essential considerations when selecting a solvent for extracting bioactive compounds. In this study, the higher extraction yield observed with ethanol is due to its ability to dissolve both polar and non-polar molecules (Mujtaba et al., 2016). Therefore selection of proper solvents is important and depends upon the type and polarity of the plant components (Do et al., 2014; Vieito et al., 2018).

Dill apiol was detected as the predominant component in all extracts, comprising up to 83 wt.%, followed by α-ylangene**.** Others were found in minor quantities (Table 2). The results obtained are consistent with other studies (Table 3). Dill apiol is an organic compound commonly found in dill weed and various plants, contributing to the antioxidant activity, antibacterial properties, and flavor of the plant's seeds. Furthermore, dill apiol, along with apiol and myristicin, has been reported as a specific inhibitor of aflatoxin G1 production (Razzaghi-Abyaneh et al., 2007). Upon comparison with the plant extract, it is observed that the seed extract of *P. pellucida* contains similar components but demonstrates heightened antioxidant activity. This elevated activity can be attributed to the higher concentration of dill apiol.

Table 1. Extraction yield and antioxidant activity of *P. Pellucida* seed extracts

Extraction Solvent	Extraction yield (wt.%)	Antioxidant activity IC_{50} (µg/ml)	AAI index
Ethanol	3.41 ± 0.13	190.77 ± 6.2	0.52
Ethyl acetate	2.47 ± 0.14	141.51 ± 5.1	0.71
Hexane	2.00 ± 0.21	231.54 ± 6.8	0.43
P. pellucida plant extract		369.01 ± 7.7	0.27
Vitamin C		11.52 ± 0.7	8.68

Table 2. Chemical composition of *P. Pellucida* seed 'extracts using different solvents

Chemical composition	Hexane extract	Ethyl acetate extract	Ethanol extract
Cetane	---	4.06	7.01
α-Ylangene	**8.93**	**3.78**	**6.28**
Dill apiol	**83.08**	**83.26**	**76.97**
Mono(2-ethylhexyl) phthalate	---	---	9.74
Bis(2-ethylhexyl) phthalate	---	8.90	---
β-Elemene	0.36	---	---
β-Longipinene	0.33	---	---
Nonadecane	4.17	---	---
(±)-β-Acoradiene	1.10	---	---
Myristicin	0.95	---	---
1,2-Dimethoxy-4-(2 methoxyethenyl)benzene	0.50	---	---
β-Acorenol	0.31	---	---
Behenyl acrylate	0.27	---	---

Table 3. Essential oil composition of *P. Pellucida* from different studies

Plant part extract	Compounds	References
Seed	Dill apiols (76.97 -83.26%); α-Ylangene (3.78 – 8.93%)	**This study**
Leaf	Germacrene D (10.3%), myristicin (11.3%) and dillapiole (37.8%)	(François et al., 2013)
Whole plant	Carotol (26.6–32.0%), dillapiole (25.1–30.2%), pygmaein (5.5–10.5%), and β caryophyllene (5.6–8.3%)	(Verma et al., 2014)
Leaf	Carotol (32.1%), dillapiole (20.7%), and β-caryophyllene (7.6%)	(Verma et al., 2014)
Root	Dillapiole (63.9%), apiole (9.2%), and β-caryophyllene (4.4%)	(Verma et al., 2014)
Leaf	Dillapiole (39.7%), β-caryophyllene (10.7%), and bicyclogermacrene (4.9%)	(DaSilva et al., 1999)
Leaf	Dillapiole (36.9%), carotol (13.4%) and 5-hydroxy-3,4-methylenedioxy allylbenzenea (10.6%)	(Moreira et al., 1999)
Whole plant	Dillapiole (55.3%), β-caryophyllene (14.3%) and carotol (8.1%)	(De Lira et al., 2009)

3.2. Antioxidant activity screening of total extract and different fractions of *P. pellucida* seed extracts

DPPH is a well-established method for measuring the antioxidant potential of a compound, an extract, or other biological sources. Antioxidants react with DPPH and convert it to DPPH-H, which causes a color change from dark purple to light yellow or colorless (Baliyan et al., 2022; Carvalho et al., 2010; Dröge, 2002; Halliwell, 2012). The degree of discoloration is proportional to the scavenging potential of an antioxidant. The DPPH free radical scavenging activity of extracts from different solvents is presented in Table 1. Experimental results indicated that the ethyl acetate extract possesses the highest DPPH radical scavenging activity (IC50=141.51 ± 5.11 mg/mL), followed by ethanol and hexane extracts. Based on a study conducted by Scherer & Godoy (2009), the antioxidant activity of plant extracts can be evaluated using the Antioxidant Activity Index (AAI). The AAI values of the *P. pellucida* seed extracts from various solvents closely correspond to their IC values (Table 1). Furthermore, based on the AAI value, it is evident that *P. pellucida* seed demonstrate superior antioxidant properties compared to the plant extract. This heightened antioxidant activity in the seed extracts is attributed to their higher dill apiol content, known for its antioxidant and anti-inflammatory (Aneesa et al., 2019). Additionally, antioxidant properties of plant extracts can also be attributed to the presence of high polyphenols and flavonoids - the secondary metabolites of plants- which function in various biological processes and aid in the neutralization of free radicals, preventing undesired health problems caused by oxidative stress (Middleton Jr. et al., 2000; Nichols & Katiyar, 2010). The study's findings suggest that the extract of P. pellucida seed could serve as a valuable source of antioxidants, offering potential benefits in treating conditions such as diabetes and hypertension that are linked to oxidative stress. Further research on the flavonoids

and polyphenols in the seed extract is needed to gain a deeper understanding of its biological activities.

4. CONCLUSION

This study is a significant contribution as it is the first report on the chemical composition and antioxidant activity of P. pellucida seed extract. Among the solvents tested, ethanol achieved the highest extraction yield at 3.41%, while ethyl acetate exhibited the highest antioxidant activity. The GC/MS analysis of the volatile compounds in the extract identified dill apiol as the major component, comprising up to 83% by weight. These results suggest that P. pellucida seed is a rich source of bioactive compounds and antioxidants, highlighting its potential as a valuable resource for the development of nutritional supplements.

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