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Mineral Composition and Antioxidant Properties of *Plucheaindica* (L.) Leaf Extract

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Abstract—*The mineral composition and antioxidant properties of P. indica (L.) leaves extract were investigated. The mineral composition found in this extract were Potassium (K), Calcium (Ca), Sodium (Na), Phosphorus (P), Magnesium (Mg), Zinc (Zn), Iron (Fe), Manganese (Mn), Selenium (Se), Copper (Cu), and Chromium (Cr). It can be found that the extract has highest amount of Calcium (Ca) compared to others. Meanwhile, the antioxidant properties of the extract were evaluated by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, ABTS radical scavenging ability and Ferric ion reducing antioxidant power (FRAP). The DPPH, ABTS and FRAP of the extract were 17.98 ± 0.01%, 71.47 ± 0.20µg GAE/mL and 3.48 ± 0.03µmol Fe^{2+}/mLrespectively.*

Index Terms—***Mineral composition, DPPH, FRAP, ABTS, Plucheaindica (L.)***

I. INTRODUCTION

In contemporary developed economies, there is a discernible trend towards enhanced dietary practices, catalyzing an escalated consumer interest in "functional foods." These products, which are fortified with essential nutrients such as vitamins, minerals, fiber, and antioxidants, play a pivotal role in the strategic positioning of food companies within industrialized markets[1].

Recently, the incorporation of plant extracts into the food industry has garnered increasing attention, primarily due to their potent antioxidant properties and the subsequent health benefits they confer upon consumers. The efficacy of these extracts is fundamentally linked to their composition, which includes a diverse array of vitamins, minerals, and various phenolic compounds[2]-[3].

Plucheaindica(L.). commonly referred to as *Beluntas*in Malaysia, is a species within the Asteraceae family.*P. indica* (L.) is acclaimed for its diverse medicinal properties and has been utilized to address a

variety of ailments. Traditional applications of *P. indica*(L.) encompass the treatment of inflammation, pain, fever, and gastrointestinal disorders. Moreover, contemporary research has investigated its potential antioxidant, anti-inflammatory, and antimicrobial activities, corroborating its traditional therapeutic uses[4].

Hence, further developmentof leaf extract of *P. indica* (L.) is pivotal in functional food and nutraceutical applications. The analysis of its mineral content and antioxidant properties could offer robust scientific evidence for its potential future applications.

II. METHODOLOGY

A. Sample Extraction

The extraction process of *P. indica* (L.) leaf is according to [5]. The samples were prepared using a 1:20 (w/v) solid-to-solvent ratio, with distilled water serving as the solvent and were stirred until homogeneous by using a magnetic stirrer(IKA C-MAG HS 4 digital, China) [5]. Subsequently, the powdered samples underwent extraction via Ultrasonic-Assisted-Extraction (UAE) using a 25.4-mm-diameter probe on a Branson 450 Digital Sonifier (USA). The parameters used were amplitude (40%), treatment duration (6 minutes), and a constant temperature of 25°C. Meanwhile, the diluted 1:10 of the extract was prepared for analysis of antioxidant properties. The extract was kept at 4°C for further analysis.

B. Minerals

Approximately 0.25 mL of the extract was taken and transferred into a digestion vessel. To this, approximately
10 mL of 70% nitric acid (HNO_3) was slowly added, with gentle swirling to mix. The mixture was allowed to stand for 10 minutes to facilitate the initial digestion process. The vessel was then sealed and placed into the PerkinElmer Titan MPS System (USA).The digestion process was conducted in three distinct steps:

1. The first step involved heating to 160°C with a pressure limit of 30 bar, a ramp time of 5 minutes, a hold time of 5 minutes, and 90% power.
2. The second step raised the temperature to 190°C, maintaining a pressure limit of 30 bar, with a ramp time of 3 minutes, a hold time of 25 minutes, and 100% power.
3. The final step involved cooling to 50°C with a pressure limit of 30 bar, a ramp time of 1 minute, a hold time of 15 minutes, and 0% power.

After digestion, the samples were dissolved in concentrated hydrochloric acid (HCl) and then diluted to a final volume of 100 mL with deionized water. The solution was filtered to remove any particulate matter before mineral analysis.Mineral content was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (PerkinElmer, USA), following the method outlined by Mohd Naeem et al. [6]. All samples were prepared and analyzed in triplicates to ensure the precision and reproducibility of the results.

C. DPPH, FRAP & ABTS

Free Radical Scavenging Activity (DPPH)

The antioxidant properties of *P.indica* (L.) leaf extractswasassessed by evaluating their capacity to scavenge free radicals using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by [7]. In this method, 10 mg of DPPH was dissolved in 100 mL of ethanol. Subsequently, 150 µL of this DPPH ethanolic solution was added to a 96-well microplate, followed by the addition of 50 µL of the extracted sample to each well. Each extracted sample was tested in triplicate. Ethanol acted as the negative control or blank. The mixtures were then incubated for 30 minutes in a dark room before the absorbance was measured at 517 nm using a BioTek microplate reader (ThermoFisher Scientific, USA).The percentage of radical scavenging activity was calculated using a specific equation to determine the inhibition level.

$$\% \text{ Radical Scavenging: } \frac{\text{Abs. control - Abs. sample}}{\text{Abs. control}} \times 100 \qquad (1)$$

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Abs. control: Absorbance of DPPH scavenging + ethanol
Abs. control: Absorbance of DPPH scavenging + extract

ABTS

ABTS radical scavenging activity was determined using a method adopted from [8]-[9] with slight modifications. Initially, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was prepared at a concentration of 7 mM by dissolving it in water. Similarly, potassium persulfate was dissolved in water to achieve a concentration of 2.45 mM. Next, ABTS radical cation (ABTS+) was produced by reacting ABTS+ stock solution with 2.45 mM potassium persulfate to the ratio 1:10 and kept in the dark at room temperature for 16 - 24 hours before use. As for the measurement, the ABTS radical solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. The scavenging activity was determined by mixing 100 ul of diluted extract and 3.9 ml adjusted ABTS radical solution. After incubation for about 10 minutes in thedark at room temperature, the absorbance was determined using a BioTek microplate reader (ThermoFisher Scientific, USA) at 734 nm. The ABTS radical solution with ethanol wasused as a negative control, while gallic acid was used as the positive control. A standard curve was prepared using gallic acid, and the ABTS radical scavenging ability was expressed as µg GAE/mL.

FRAP

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted using the FRAP assay kit (ElabScience, US) [10] to quantify antioxidant activity. The FRAP reagent was prepared by mixing the TPTZ diluent, TPTZ solution, and detection buffer in a volumetric ratio of 10:1:1. This prepared FRAP reagent was maintained on ice and shielded from light to prevent degradation. Subsequently, 5 µL of the sample extract was accurately pipetted into individual wells of a 96-well microplate. To each well, 180 µL of the prepared FRAP reagent was added. The microplate was then incubated at 37°C for 5 minutes to allow the reaction to proceed. Post-incubation, the absorbance of the reaction mixture was measured at a wavelength of 593 nm using a BioTek microplate reader (ThermoFisher Scientific, USA). A standard curve was generated with the equation $y = 0.30304x + 0.001$ and a correlation coefficient, R^2 of 0.99, allowing for the expression of results in terms of micromoles of Fe^{2+} equivalents per millilitre (µmol Fe^{2+}/mL). All measurements were performed in triplicate to ensure accuracy and reproducibility.

D. Statistical Analysis

The commercial Excel Software 2019 (Microsoft, US) was used to determine the mean and standard deviation of mineral composition and antioxidant properties. The mineral composition and antioxidant properties were performed in triplicates. Meanwhile, Minitab software version 21.4.2 was used to perform the Pearson correlation for the mineral composition and antioxidant properties.

III. RESULT AND DISCUSSION

A. Minerals Composition

The mineralogical analysis identified the following elemental constituents: Potassium (K), Calcium (Ca), Sodium (Na), Phosphorus (P), Magnesium (Mg), Zinc (Zn), Iron (Fe), Manganese (Mn), Selenium (Se), Copper (Cu), and Chromium (Cr).

From Table I, it can be seen that Ca has the highest constituent, followed by Na and Se where the concentration was 913.55 ± 9.12, 704.35 ± 2.33 and 104.95 ± 0.64µg/100mL, respectively. Ca is vital for maintaining cell wall integrity and plays a pivotal role in cellular signaling pathways. Na, though generally not essential for most plants, can be beneficial in certain environments for osmotic balance and specific metabolic functions. Se is known for its role in antioxidant systems within plants, helping to mitigate oxidative stress. Thus, several factors related to the plant's physiology and its environment could affect the concentration of these minerals such as soil composition and environmental factors[11]. For instance, if the soil is rich in Ca, Na, or Se, these elements are likely to be absorbed more readily by the *P. indica* (L.) plant.

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Table I: Mineral Composition in *P. indica* (L.) leaf extract

Minerals	**Concentration (µg/100mL)**
Potassium (K)	78.68 ± 0.66
Calcium (Ca)	913.55 ± 9.12
Sodium (Na)	704.35 ± 2.33
Phosphorus (P)	78.68 ± 0.66
Magnesium (Mg)	27.75 ± 0.30
Zinc (Zn)	0.64 ± 0.01
Iron (Fe)	0.01 ± 0.00
Manganese (Mn)	1.23 ± 0.01
Selenium (Se)	104.95 ± 0.64
Copper (Cu)	0.30 ± 0.00
Chromium (Cr)	47.39 ± 2.47

B. Antioxidant Properties

Table II shows the antioxidant properties of *P. indica* (L.) leaf extract. It can be found that the DPPH, ABTS and FRAP assays were 17.98 ± 0.01%, 71.47 ± 0.20 µg GAE/mL and 3.48 ± 0.03 µmol Fe^{2+}/mLrespectively.

Table II: Antioxidant properties of *P. indica* (L.) leaf extract

Antioxidant Properties	**Value**
DPPH (%)	17.98 ± 0.01
ABTS (µg GAE/mL)	71.47 ± 0.20
FRAP (µmol Fe^{2+}/mL)	3.48 ± 0.03

C. Pearson correlation of antioxidant properties (DPPH, ABTS and FRAP)

Pearson correlation is used primarily because it offers a straightforward and widely accepted measure of the linear relationship between two continuous variables [12]. Pearson correlation coefficient quantifies the degree to which two variables are linearly related. It provides a value between -1 and 1, where 1 means a perfect positive linear correlation, -1 means a perfect negative linear correlation, and 0 indicates no linear correlation [12].

Table III shows the Pearson correlation of DPPH, ABTS and FRAP. It can be found that the correlation coefficient of 0.999 indicates an almost perfect positive linear relationship between the ABTS and DPPH assays.This correlation is statistically significant at $p< 0.05$, which means there is a less than 5% probability that this correlation occurred by random chance, confirming the strong association between these two assays.This is attributable to the analogous functionality of DPPH and ABTS radical scavenging assays, which capitalize on their capacity to accept hydrogen ions from hydrogen-atom-donating compounds (antioxidants). These assays are particularly advantageous for evaluating the hydrogen-donating ability of antioxidant compounds [13].

In addition, the correlation coefficient of 0.958between the FRAP and DPPH assays is considered quite high, indicating a strong positive correlation,although there is no significant difference between them. Similarly, the correlation between FRAP and ABTS assays does not have a significant difference, but their correlation coefficient is 0.945, suggesting a strong positive correlation between the them as well.These finding are similar to study reported by[14] which Pearson correlation coefficients indicated strong correlations between DPPH, ABTS, and FRAP assays of black seed and other spices and herbs.

Table III: Pearson correlation of antioxidant properties (DPPH, ABTS and FRAP)

	DPPH	**ABTS**
ABTS	0.999*	
FRAP	0.958	0.945

* means significantly difference at $p < 0.05$

D. Pearson correlation between minerals and antioxidant

Certain minerals serve as vital constituents of antioxidant enzymes, playing a pivotal role in cellular protection against oxidative damage induced by free radicals. The relationship between minerals and antioxidants entails comprehending their functions within the mechanisms of oxidative stress mitigation and antioxidant defense within the body [15] [16]. For example, while iron may act as a pro-oxidant by facilitating the production of free radicals through Fenton reactions, it simultaneously functions as an integral component of catalase. This enzyme is crucial for catalyzing the decomposition of hydrogen peroxide into water and oxygen, thereby alleviating oxidative stress. Apart from that, Selenium also plays a vital role in antioxidant defense. Selenium is an essential component of glutathione peroxidase, an enzyme that safeguards cells from oxidative damage by converting hydrogen peroxide and organic hydroperoxides into water and their corresponding alcohols [17]. Therefore, a correlation between antioxidant properties and minerals arestudied.

Table IV: Pearson correlation of antioxidant properties and minerals of *P. indica* (L.) leaf extract

	DPPH	**ABTS**	**FRAP**	**K**
ABTS	0.999*			
FRAP	0.958	0.945		
K	0.965	0.953	1.000*	
Ca	0.953	0.965	0.827	0.841
Na	0.993	0.997*	0.916	0.926
P	0.976	0.985	0.874	0.886
Mg	0.991	0.996	0.910	0.921
Zn	-0.937	-0.921	-0.998*	-0.996
Fe	0.982	0.989	0.887	0.899
Mn	-0.937	-0.921	-0.998*	-0.996
Se	-0.998*	-0.994	-0.975	-0.980
Cu	-0.933	-0.917	-0.997*	-0.995
Cr	-0.975	-0.965	-0.998*	-0.999*

	Na	**P**	**Mg**	**Zn**
ABTS				
FRAP				
K				
Ca				
Na				
P	0.995			
Mg	1.000*	0.997		
Zn	-0.887	-0.839	-0.880	
Fe	0.998*	1.000*	0.999*	-0.854
Mn	-0.887	-0.839	-0.880	1.000*
Se	-0.983	-0.961	-0.980	0.957
Cu	-0.883	-0.834	-0.876	1.000*
Cr	-0.941	-0.905	-0.936	0.991

	Fe	Mn	Se	Cu
ABTS				
FRAP				
K				
Ca				
Na				
P				
Mg				
Zn				
Fe				
Mn	-0.854			
Se	-0.968	0.957		
Cu	-0.849	1.000*	0.954	
Cr	-0.916	0.991	0.987	0.990

* means significantly difference at $p< 0.05$

Based on Table IV, DPPH shows very high positive correlations with ABTS (0.999), Na (0.993), Mg (0.991), and P (0.976). This indicates that higher DPPH values are associated with higher levels of these minerals. Similarly, ABTS has strong positive correlations with DPPH (0.999), Na (0.997), Mg (0.996), and P (0.985) as well. Meanwhile, FRAP exhibits strong positive correlations with K (1.000), Mg (0.910), and Ca (0.827).

As for minerals, K is highly correlated with other minerals and antioxidants, particularly FRAP (1.000) and Na (0.926). Ca shows strong positive correlations with DPPH, ABTS, and FRAP, and other minerals like Na (0.983) and P (0.996). In addition, Na achieved strong positive correlations with nearly all antioxidants and minerals, especially P (0.996). Meanwhile, P had very high positive correlations with Na (0.996) and other antioxidants and minerals, indicating its central role in mineral balance. Mg showed exceptionally strong positive correlations with ABTS (0.996) and P (1.000). These positive correlations indicate that as the levels of certain antioxidants increase, the levels of certain minerals increase. This suggests that antioxidant has synergistic interactions with minerals.

Furthermore, Zn, Mn, and Cr achieved negative correlations with antioxidants and other minerals. For instance, Zn has high negative correlations with K (-0.996) and P (-0.839), while Se exhibits strong negative correlations with DPPH (-0.998), ABTS (-0.994), and FRAP (-0.975). This implies that a negative correlation indicates that higher levels of some minerals are associated with lower levels of antioxidants, suggesting competitive interactions with inverse proportions of these minerals.

Understanding the correlations between minerals and antioxidants is crucial for optimizing the design of nutritional supplements and dietary plans [18] [19]. For example, higher levels of Zn can diminish the availability of certain antioxidants, highlighting the importance of carefully balancing these nutrients to prevent imbalances. This knowledge provides supplement manufacturers with guidelines to adjust formulations, ensuring that both minerals and antioxidants are included without compromising the efficacy of either [20]. This approach promotes the development of supplements that effectively support overall health.

CONCLUSION

The study of *Plucheaindica* (L.) leaf extract revealed a rich mineral composition, with the highest concentrations of Ca, Na, and Se, alongside notable amounts of K, P, Mg, Zn, Fe, Mn, Cu, and Cr. The extract exhibited significant antioxidant properties, with DPPH radical scavenging activity at 17.98%, ABTS radical scavenging ability at 71.47 µg GAE/mL, and FRAP value at 3.48 µmol Fe^{2+}/mL. Strong positive correlations between antioxidant activities and minerals like Na, Mg, and P were observed, highlighting the importance of these minerals in enhancing the extract's antioxidant efficacy. These findings suggest the extract's potential as a functional food and nutraceutical, with promising applications in various food products such as beverages, gummies and jellies for enhanced health benefits.

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