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Original Article

Antioxidant Activity of Pink Water Henna Flower Extract (*Impatiens balsamina* L.) with Varying Ethanol Concentrations

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Abstract

Free radicals are highly reactive molecules that can cause damage to cells, contributing to the progression of various degenerative diseases. Plants rich in antioxidants can inhibit free radical reactions. Pink water henna flowers (*Impatiens balsamina* L.) contain secondary metabolites such as flavonoids, alkaloids, saponins, and tannins, which may offer antioxidant benefits. This study aimed to evaluate the characteristics and antioxidant activity of *I. balsamina* flowers extracts using 50%, 70%, and 96% ethanol as solvents, evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The total phenolic and total flavonoid contents were evaluated using folin ciocalteu and $AlCl_3$, respectively. Extraction using different solvents shows variations in antioxidant activity results. The highest antioxidant activity was found in the 96% ethanol extract with an IC_{50} value of 49.872 ppm, classified as very strong. The highest phenolic content was found in 96% ethanol extract with a value of 8.728% and the highest determination of flavonoid levels also found in 96% ethanol extract with a value of 10.917%. The higher the levels of phenols and flavonoids, the greater the ability of antioxidants to donate electrons to neutralize free radicals. This research demonstrates that the 96% ethanol extract of *I. balsamina* flowers possesses very strong antioxidant activity due to its high phenolic and flavonoid content, positioning it as a promising natural source for developing functional ingredients to combat oxidative stress and associated degenerative diseases.

Keywords

Antioxidant, Ethanol, Extract, DPPH, *Impatiens balsamina* L.

1. Introduction

Free radicals can cause cell damage and trigger various degenerative diseases, such as hypertension, stroke, heart disease and cancer. They are produced both from normal cellular metabolism in situ and from external sources such as pollution, smoke, unhealthy lifestyles, and electronic radiation (e.g, phones and computers). When their level exceeds the body's ability to neutralize them, oxidative stress occurs (Fakriah et al., 2019)

The human body has a natural mechanism to fight oxidative stress by producing antioxidants, but the amount is limited, so exogenous antioxidant intake is needed. Exogenous antioxidants can be either synthetic or natural. Synthetic antioxidants have been restricted due to long-term toxicity effects (Salamah & Widyasari, 2015). Therefore, natural antioxidants derived from plants can be an alternative because they are considered safer. They are found in the form of polyphenols, flavonoids, vitamins C and E and are biologically familiar to the human body, often playing roles in the plant's own defense systems. These compounds are generally recognized by consumers as harmless, and they may

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offer additional health benefits beyond simple preservation, such as reducing oxidative stress and inflammation.

Phenolic compounds, especially flavonoids, are important natural antioxidants that are widely found in plants. Water henna flower (*Impatiens balsamina* L.) contains various secondary metabolites, including flavonoids, kaempferol, isoquercertin, quercetin, saponins, and quinones, which can act as antioxidant compounds (Buana et al., 2024). *I. balsamina* are widely found and cultivated in Bali, Indonesia due to their religious and economic value (Lango et al., 2021).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is widely used to measure antioxidant activity due to its practicality, sensitivity, and ease of experimental procedures compared to other methods. The principle is based on a redox reaction, where activity is measured through the IC_{50} value- the lower the IC_{50} value, the higher the antioxidant activity (Purwanti, 2019).

Antioxidant activity is strongly influenced by the type and concentration of solvent used during extraction. Ethanol is a common solvent for polar compounds such as phenols and flavonoids. Of the several extraction solvents, ethanol provides an advantage because it is relatively more suitable for human consumption in terms of safety. Some studies prove that the concentration of ethanol solvent will affect the total flavonoid content obtained (Riwanti et al., 2020).

Therefore, it is important to research the effect of variations in ethanol solvent concentration (50%, 70%, and 96%) on the antioxidant activity of *I. balsamina* flowers. The purpose of this evaluation is to identify the optimal conditions that are most suitable for the extraction of *I. balsamina* flowers and achieve the highest level of antioxidant activity.

2. Methods

2.1. Materials

The materials used for this research include ethanol (food grade), DPPH reagent (Sigma Aldrich), distilled water, methanol (Merck), ethanol (Merck), ascorbic acid, folin ciocalteu, NaOH, gallic acid, quercetin, aluminum chloride, sodium acetate. *I. balsamina* flowers were collected from Badung, Bali.

2.2. Extract preparation

The plant of *I. balsamina* was identified at the Department of Pharmaceutical Biology, Gadjah Mada University. The simplisia was extracted by maceration method using 50%, 70% and 96% ethanol solvents. After the extract was obtained, the yield and organoleptic test were conducted.

2.3. Qualitative and quantitative tests

Phytochemical screening includes alkaloid test with Dragendorff reagent, flavonoid test with concentrated HCl and Mg, steroid test with anhydrous acetic acid and concentrated sulfuric acid, terpenoid test with Bouchardat reagent, saponin test is done by shaking the solution strongly and then dropping hydrochloric acid, tannin test with $FeCl_3$. Testing of total phenol content and total flavonoids refers to the Indonesian Herbal Pharmacopoeia edition II, 2017.

2.4. Antioxidant activity test

From the extract stock solution (1000 ppm), 0.1, 0.2, 0.3, 0.4, and 0.5 mL were pipetted into separate test tubes. Methanol was added to each tube in volumes of 3.9, 3.8, 3.7, 3.6, and 3.5 mL, respectively, to obtain test solutions of 20, 40, 60, 80, and 100 ppm. Then, 1.0 mL of 0.4 mM DPPH was added to each solution. The solutions are stirred until homogeneous and incubated for 30 minutes. The absorbance was then measured at a maximum wavelength of 516 nm with a UV-Vis spectrophotometer. The extract sample test solution and the positive control sample test solution were replicated three times each.

2.5. Statistical analysis

The data in this study were processed with Microsoft Excel software. The analysis included the determination of total phenol content, flavonoids, and IC_{50} value through linear regression equation. The statistical analysis was conducted using One Way ANOVA followed by Duncan post hoc test.

3. Results and Discussion

Table 1 shows that all three types of *I. balsamina* extracts have a similar aroma, which is the typical scent of *I. balsamina* flowers, along with a thick texture and a bitter taste. The 50% ethanol extract is dark brown, the 70% ethanol extract is brown, and the 96% ethanol extract is yellowish-brown. The variation in colour intensity is likely due to the concentration of the ethanol solvent. A higher concentration of a polar solvent like ethanol extracts more polar compounds, such as tannins, leading to a darker filtrate (Fauziyah et al., 2022).

Table 1. Characteristics of ethanol extract of *Impatiens balsamina* L. flowers

Solvent Concentration (%)	Organoleptic				Yield (%)
	Colour	Aroma	Taste	Texture	
50	Dark Brown	Typical *I. balsamina*	Bitter	Thick	47.60
70	Yellowish Brown	Typical *I. balsamina*	Bitter	Thick	46.50
96	Light Brown	Typical *I. balsamina*	Bitter	Thick	24.39

Based on the yield values obtained, it shows that the extract with 50% ethanol solvent gives the highest yield of 47,6% followed by 70% ethanol at 46,5%, and 96% ethanol with the lowest yield of 24,39%. The yield produced in 50% etanol extract is more because increasing water concentration can increase extract yield (Kusuma & Aprileili, 2022). Compounds such as proteins, carbohydrates and soluble polysaccharides dissolve more in polar solvents, so the resulting yield is higher (Padmawati et al., 2020).

Phytochemical tests are carried out to provide an overview of the content of compounds contained in *I. balsamina* flowers so that they can be utilized further (Kumalasari & Andiarna, 2020). Phytochemical screening results showed that ethanol extract of *I. balsamina* flowers contained alkaloid, flavonoid, saponin, tannin, and terpenoid compounds. The presence of alkaloids is indicated by the formation of a brick red precipitate after the addition of Dragendorff reagent (Fajrin & Susila, 2019). Flavonoid compounds are detected through changes in colour to reddish brown due to covalent coordination bonds between magnesium ions and phenolic OH groups of flavonoid compounds (Fadhila et al., 2023).

Table 2. Phytochemical screening of ethanol extract of *Impatiens balsamina* L. flowers

Solvent Concentration (%)	Alkaloids	Flavonoids	Steroids	Terpenoids	Saponins	Tannins
50	+	+	-	+	+	+
70	+	+	-	+	+	+
96	+	+	-	+	+	+

Saponins are identified through the formation of stable foam when the solution is shaken, due to the interaction of hydrophilic and hydrophobic groups in the saponin structure (Elfira et al., 2024). The extract is positive for tannins, as evidenced by the colour change to dark green due to the formation of a coordination covalent bond between iron (III) ions and phenolic OH groups. Qualitative testing of terpenoid compounds in this study was carried out by adding 3 drops of Bouchardat reagent. Positive results of terpenoids are indicated by the formation of orange or reddish brown colour (Fadhila et al., 2023).

Table 3. The TFC, TPC, and IC_{50} values of ethanol extracts of *Impatiens balsamina* L. flowers

Solvent Concentration (%)	TPC (%)	TFC (%)	IC_{50} (ppm)
50	4.995 ± 0.089	1.857 ± 0.267	68.500 ± 1.149
70	7.048 ± 0.081	3.793 ± 0.286	53.998 ± 3.159
70	8.728 ± 0.133	6.076 ± 0.234	49.872 ± 1.493

TPC = Total Phenolic Content, TPC = Total flavonoid content, IC = *Inhibition Concentration*

Determination of total phenol content of *I. balsamina* flower extract using Folin-Ciocalteu reagent and gallic acid as standard. The 96% ethanol extract showed the highest phenolic content (8,728%) compared to 70% (7,048%) and 50% (4,995%) ethanol. This is due to the suitability of 96% ethanol polarity with phenolic compounds of *I. balsamina* flowers, so that these compounds can be extracted maximally and dissolve well in 96% ethanol solvent. The total phenolic content in an extract is strongly influenced by the polarity of the solvent used during the extraction process. This result is in line with the research of (Rahmatullah et al., 2024) on *Labisia pumila* leaves which also showed the highest phenol content in 96% ethanol extract.

Total flavonoid content in *I. balsamina* flower extracts was determined using quercetin as a standard. The 96% ethanol extract had the highest flavonoid content (6,076%), followed by 70% (3,793%) and 50% (1,857%) ethanol. Although 96% ethanol has the lowest polarity, its higher extraction efficiency suggests the dominance of less polar flavonoids in *I. balsamina* flowers which generally have few hydroxyl groups and are not sugar bound, such as isoflavones, flavanones, and flavanols (Hendryani et al., 2015). Flavonoid compounds found in *I. balsamina* flowers include quercetin, kaempferol, myricetin, and isoquercetin (Buana et al., 2024) which belong to the to the less polar flavanone group. These findings align with research by Suharsanti in 2019, who reported the highest flavonoid content in *Cassia angustifolia* leaf extract using 96% ethanol compared to 50% and 70% ethanol solvents (Suharsanti & Ariani, 2019).

This antioxidant activity test was carried out at the maximum wavelength of DPPH 516 nm. Active compounds that act as antioxidants will reduce DPPH free radicals, which are characterized by a change in the colour of the solution from purple to yellow (Listiana et al., 2022). Extraction using different solvents shows variations in antioxidant activity results. The smaller the IC_{50} value, the more effective a compound is as a free radical scavenger. **Table 3** shows that the sample that has the strongest antioxidant activity or the smallest IC_{50} value is 96% ethanol extract (49.69 ppm) with very strong category, 70% ethanol extract (53.998 ppm) has strong antioxidant intensity and 50% ethanol extract (68.50 ppm) has the highest IC_{50} with strong category.

The antioxidant activity is related to the secondary metabolite compounds that are successfully extracted during the extraction process. The difference in the content of secondary metabolites extracted during extraction is due to differences in the polarity of each solvent (Moh, 2024). The highest antioxidant activity was found in 96% ethanol extract, in line with the highest total phenol and flavonoid levels in the extract. The higher the levels of phenols and flavonoids, the greater the ability of antioxidants to donate electrons to neutralize free radicals (Nur et al., 2019).

4. Conclusions

The 96% ethanol extract of *I. balsamina* flowers contained the highest levels of total phenols and flavonoids. Using different solvents for extraction produced varying results in antioxidant activity. The 96% ethanol extract also showed the strongest antioxidant activity, with an IC_{50} value of 49.872 ppm, which is considered very strong. This indicates that antioxidant activity increases along with the total phenol and flavonoid content.

Supplementary Material

No supplementary materials are associated with this manuscript.

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Author Contributions

Ni Nyoman Widhya Sari: conceptualization, data curation resources, and writing – original draft; **Putu Gede Adi Purwahita**: data curation, methodology, formal analysis, investigation, and supervision; **Ni Putu Wintariani:** software, formal analysis, writing – review & editing, and supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

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