

Optimization of a green ethanol–water extraction system for *Pimenta dioica* leaves: Effects on phytochemical content and antioxidant potential

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ARTICLE INFO	ABSTRACT
<p>Original Research Article Received on January 15, 2026 Revised on January 17, 2026 Accepted on February 14, 2026 Published on February 17, 2026</p> <p>Article Authors Seba Shibu, Lakshmi Priya, Arya S., Santhi W. S., Jisha S., A. Sivaprasad</p> <p>Corresponding Author Email bichudev05@gmail.com</p>	<p>The present study investigated the effect of extraction parameters on the phytochemical composition, total phenolic content, and antioxidant activity of <i>Pimenta dioica</i> (L.) Merrill leaf extracts, emphasizing a green and sustainable approach for the efficient utilization of plant biodiversity. Extraction was performed using an ethanol water solvent system at varying solvent ratios (70:30, 80:20 and 90:10), temperatures (60°C, 70°C, and 80°C), and incubation times (60 and 120 min). Preliminary phytochemical screening revealed the presence of major bioactive constituents such as flavonoids, phenols, tannins, saponins, and glycosides in most extracts, while alkaloids appeared only at specific combinations 80:20 at 80°C and 90:10 at 60°C after 60 min, but were detected in nearly all solvent systems after 120 min. The total phenolic content was highest in extracts obtained at 80:20 solvent ratio, 60°C, and 120 min incubation, indicating the influence of solvent polarity and extraction duration on phenolic recovery. Antioxidant potential evaluated through reducing power and DPPH radical scavenging assays demonstrated that extracts at 60°C exhibited the highest reducing power, whereas the 70:30 solvent ratio showed superior DPPH radical scavenging activity. Overall, ethanol–water extraction proved effective and environmentally benign for recovering phenolic-rich bioactives from <i>P. dioica</i> leaves. The findings highlight optimized extraction as a green strategy to enhance the recovery of natural antioxidants, supporting the sustainable exploitation of plant biodiversity for pharmaceutical and nutraceutical applications. The ethanol–water solvent system represents an environmentally benign alternative to conventional organic solvents, being biodegradable, renewable, and safe. Hence, the extraction approach adopted in this study aligns with green chemistry principles and contributes to sustainable biodiversity utilization.</p>
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Shibu, S., Priya, L., Arya, S., Santhi, W. S., Jisha, S., Sivaprasad, A. (2026) Optimization of a green ethanol–water extraction system for *Pimenta dioica* leaves: Effects on phytochemical content and antioxidant potential, *International Journal of Agricultural Invention*, 11(1): 36-46. DOI: 10.46492/IJAI/2026.11.1.6

Plants serve as invaluable reservoirs of secondary metabolites that play essential ecological and physiological roles, particularly in defense mechanisms, stress tolerance, and interspecies interactions. These compounds comprising phenolics, flavonoids, and tannins are of remarkable biological and economic importance owing to their pharmacological and functional properties. They form the basis of numerous natural therapeutics, nutraceuticals, and eco-friendly preservatives.

The exploration and utilization of such bioactive compounds support biodiversity-based innovation while reducing dependence on synthetic and environmentally hazardous chemicals (Singleton *et al.*, 1999; Panche *et al.*, 2016). Among plant bioresources, *Pimenta dioica* (L.) Merrill, commonly known as allspice or Jamaica pepper, is an aromatic and medicinal species of the family Myrtaceae.

Native to Central America and now cultivated across several tropical regions, *P. dioica* has been traditionally used for managing gastrointestinal, respiratory, and muscular ailments (Nakatani *et al.*, 1986; Cowan, 1999). Its leaves are rich in phenolic constituents such as eugenol, quercetin, and gallic acid derivatives, which contribute to pronounced antioxidant, antimicrobial, and anti-inflammatory activities (Sarathambal *et al.*, 2021; Saddiq and Khayyat, 2010). Thus, the species represents a promising natural resource for developing plant-based products of pharmaceutical and industrial relevance. Extraction is a critical step in recovering bioactive compounds from plant matrices, and its efficiency is highly influenced by factors such as solvent polarity, temperature, extraction time, and solvent-to-sample ratio (Martins *et al.*, 2023).

Conventional extraction methods often employ organic solvents that are toxic, volatile, and environmentally persistent, thereby raising ecological and safety concerns. In contrast, the ethanol–water solvent system offers a green, biodegradable, and renewable alternative that complies with the principles of green chemistry (Awad *et al.*, 2021). Ethanol is recognized as a safe solvent for both food and pharmaceutical applications, and its combination with water improves the extraction of diverse polar and semi-polar phytochemicals while minimizing environmental impact. In this context, the present study focuses on the optimization of a green ethanol–water extraction system for *Pimenta dioica* leaves. The investigation aims to evaluate the influence of key extraction parameters such as solvent ratio, temperature, and extraction time on total phenolic content (TPC) and antioxidant activity. By identifying efficient and environmentally benign extraction conditions, this work contributes to the development of sustainable and eco-friendly extraction strategies that enhance the valorization of plant biodiversity while supporting green innovation in food, pharmaceutical, and bioprocessing sectors.

Materials and Methods

Collection of Plant Material

Fresh leaves of *Pimenta dioica* (L.) Merrill (Allspice) was collected from Alappuzha District, Kerala, India.

The leaves were thoroughly washed several times with tap water, followed by rinsing with distilled and deionized water to remove adhering dust and impurities. The cleaned leaves were air-dried to remove surface moisture and then shade-dried for 4–5 days until completely free of moisture.

Preparation of Plant Material for Extraction

The shade-dried leaves were ground into fine powder using a laboratory grinding machine. The powdered material was weighed and stored in clean, airtight containers under dry conditions until further use for extraction.

Extraction of Plant Material

A green dual-solvent system comprising ethanol and water was used for extraction. Three solvent ratios (70:30, 80:20, and 90:10 v/v ethanol: water) were employed to evaluate the influence of solvent polarity. Extraction was carried out at four different temperatures (Room Temperature (RT), 60 °C, 70 °C, and 80 °C) and for two time intervals (60 and 120 min). For each experiment, 1 g of powdered *P. dioica* leaf material was extracted with 20 mL of the respective ethanol–water mixture in a hot air oven under the specified temperature and duration. The resulting extracts were filtered through Whatman No. 1 filter paper, and the filtrates were stored at 4 °C until further analysis.

Preliminary Phytochemical Analysis

The extracts obtained under different conditions were subjected to qualitative phytochemical screening to detect the presence of major classes of natural compounds such as alkaloids, flavonoids, tannins, phenolics, saponins, and glycosides. The tests were conducted following the standard procedures described by (Sofowora, 1982 and Harborne, 1986).

Estimation of Total Phenolic Content

The total phenolic content (TPC) was determined following the method of (Malik and Singh, 1980) with slight modifications. Briefly, 0.5 g of the plant sample was homogenized with 5 mL of 80% ethanol using a mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 min, and the supernatant was collected.

The residue was re-extracted and centrifuged again under the same conditions. Both supernatants were pooled and evaporated to dryness, and the residue was redissolved in 5 mL of distilled water. An aliquot (0.5 mL) of the extract was made up to 3 mL with distilled water, and 0.5 mL of Folin–Ciocalteu reagent was added. After 3 min, 2 mL of 20% sodium carbonate (Na_2CO_3) solution was added, and the mixture was mixed thoroughly. The tubes were placed in a boiling water bath for 1 min and then cooled to room temperature. Absorbance was measured at 650 nm against a reagent blank. The total phenolic content was expressed as mg gallic acid equivalents (GAE)/g dry weight.

In Vitro Antioxidant Assays **Reducing Power Assay**

The reducing power of the extracts was determined according to the method of (Oyaizu, 1986). Different concentrations of the extracts (250–1000 $\mu\text{g}/\text{mL}$) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution.

The mixtures were incubated in a water bath at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added, and the mixtures were centrifuged at 3000 rpm for 10 min. From the upper layer, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution and allowed to stand for 10 min. The absorbance was recorded at 700 nm. A higher absorbance value indicates greater reducing power. Ascorbic acid was used as a standard reference.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by (Shimada *et al.*, 1992). One mL of the extract (250–1000 $\mu\text{g}/\text{mL}$ in methanol) was mixed with 2 mL of 0.1 mM DPPH solution in methanol. The reaction mixtures were incubated in the dark for 30 min at room temperature, and the absorbance was measured at 517 nm against methanol as blank. Lower absorbance values correspond to higher radical scavenging activity. The percentage inhibition was calculated using the formula:

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Results and Discussion

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of aqueous leaf extracts of *Pimenta dioica* was carried out in. The findings indicated the presence of all the studied phytochemicals in the various extracts.

Phytochemical Analysis of Leaf Extracts at 60 Min. of Incubation

Qualitative phytochemical screening of *Pimenta dioica* leaf extracts at 60 min. revealed (table 1) the presence of major secondary metabolites such as phenols, flavonoids, terpenoids, and saponins, whereas alkaloids were detected only under specific extraction conditions. Extraction performed at 80°C with 80:20 (water: ethanol) solvent ratio exhibited the broadest phytochemical profile, with all tested metabolites showing positive reactions. This suggests that moderate ethanol concentration and elevated temperature enhance solvent polarity balance, facilitating the dissolution

of both polar and semi-polar phytoconstituents (Plášková *et al.*, 2023). At 60°C, higher ethanol proportion (90:10) favored alkaloid and phenolic extraction, while lower ratios were less effective. In contrast, room temperature extractions yielded fewer detectable compounds, highlighting the role of temperature in improving cell wall disruption and metabolite diffusion (Lim *et al.*, 2024). Phenolic and flavonoid compounds were consistently present in most hot extractions, correlating with their heat-stable nature and solvent affinity. Terpenoids and saponins were widely distributed across treatments, indicating their relative abundance and solubility in mixed solvent systems (Waseem *et al.*, 2023). Overall, extraction conditions combining moderate temperature (70–80°C) and balanced solvent polarity (70:30 to 80:20 water:ethanol) significantly improved the recovery of multiple bioactive classes, which is consistent with previous studies emphasizing optimization of temperature and solvent polarity for maximizing phytochemical yield and biological activity (Castro *et al.*, 2024).

Table 1. Phytochemical analysis of *Pimenta dioica* leaf extracts at 60 Min. of incubation

Temp.	Solvent Ratio	Alkaloids	Flavonoids	Phenols	Terpenoids	Saponins
60°C	70:30	-	+	+	+	-
	80:20	-	+	+	+	+
	90:10	+	+	+	+	+
70°C	70:30	-	-	+	+	+
	80:20	-	+	+	+	+
	90:10	-	+	+	+	+
80°C	70:30	-	+	+	+	+
	80:20	+	+	+	+	+
	90:10	-	+	+	+	+
RT	70:30	-	-	-	+	-
	80:20	-	+	+	-	-
	90:10	-	+	+	+	+

Key: + Present, - Absent, RT: Room Temperature

Table 2. Phytochemical analysis of *Pimenta dioica* leaf extracts at 120 Min. of incubation

Temp.	Solvent Ratio	Alkaloids	Flavonoids	Phenols	Terpenoids	Saponins
60°C	70:30	-	+	+	+	-
	80:20	+	+	+	+	+
	90:10	+	+	+	+	+
70°C	70:30	-	+	+	+	+
	80:20	+	+	+	+	+
	90:10	-	+	+	+	+
80°C	70:30	+	+	+	+	+
	80:20	+	+	+	+	+
	90:10	+	+	+	+	+
RT	70:30	-	-	+	+	+
	80:20	+	+	+	+	+
	90:10	-	+	+	+	+

Key: + Present, - Absent, RT: Room Temperature

Phytochemical Analysis of Leaf Extracts at 120 Min. of Incubation

The qualitative phytochemical screening of *Pimenta dioica* leaf extracts (table 2) revealed that extraction temperature and solvent polarity significantly influenced the presence and intensity of bioactive compounds. At 60°C, all solvent ratios showed strong positive reactions for phenols, terpenoids, and saponins, while alkaloids and flavonoids appeared predominantly in the 80:20 and 90:10 (water: ethanol) extracts. This indicates that a moderate ethanol concentration enhances solubility and recovery of both polar and semi-polar compounds (Plášková *et al.*, 2023). At 70°C, phenols, terpenoids, and saponins were consistently detected across all solvent ratios, but alkaloids and

flavonoids were absent at 70:30, suggesting that higher ethanol content aids the extraction of these less polar constituents. Extraction at 80°C produced the most comprehensive phytochemical profile, with all tests showing positive results, confirming that higher temperature promotes better cell wall disruption and diffusion of metabolites into the solvent phase (Lim *et al.*, 2024). Conversely, room temperature extraction resulted in fewer detected compounds, particularly at 70:30, highlighting the role of thermal energy in enhancing phytochemical yield. The overall trend suggests that an 80°C temperature with 80:20 solvent ratio provides optimal extraction conditions for *P. dioica* leaves. Similar temperature solvent interactions enhancing phenolic and flavonoid yields have been reported for other aromatic plants (Waseem *et al.*, 2023).

The TPC of *Pimenta dioica* Leaf Extracts

The total phenolic content (TPC) of *Pimenta dioica* leaf extracts was significantly influenced extraction temperature and solvent composition (fig 1). At 60 minutes of extraction, TPC values ranged from 4.19 to 6.15 mg GAE/g DW, indicating notable variation with the change in temperature and solvent polarity. At room temperature (RT), the TPC values were the lowest across all solvent ratios, varying between 4.19 mg GAE/g (90:10) and 4.66 mg GAE/g (80:20). The reduced extraction efficiency at RT can be attributed to limited solubility and diffusion of phenolic compounds in the solvent system at lower temperatures, which restricts cell wall disruption and phenolic release (Al-Farsi and Lee, 2008). With an increase in temperature to 60°C, there was a noticeable enhancement in TPC, particularly for the 70:30 water–ethanol ratio (5.64 mg GAE/g). However, a further shift to more aqueous (90:10) or ethanol-rich (80:20) systems slightly decreased TPC. This suggests that a moderate solvent polarity (70:30) facilitates optimal solubilization of a wide range of phenolics, as both hydrophilic and moderately lipophilic phenols can be extracted effectively (Do *et al.*, 2014).

At 70°C, the TPC values remained comparable across solvent systems (5.30–5.43 mg GAE/g), showing a narrower variation. This indicates that above 60°C, temperature exerts a stronger effect than solvent ratio, possibly because higher heat enhances solvent penetration and disrupts hydrogen bonding between phenolics and cell wall components (Pinelo *et al.*, 2005). The highest TPC (6.15 mg GAE/g) was obtained at 80°C with a 70:30 water–ethanol ratio, demonstrating that elevated temperature and moderate solvent polarity synergistically improve extraction efficiency. Increased temperature enhances the mass transfer rate and solubility of phenolic compounds but excessive heat can also lead to phenolic degradation (Mohd Zainol *et al.*, 2009). Therefore, 80°C appears to be an optimal condition before thermal degradation becomes significant. Overall, the results highlight that both solvent composition and temperature play crucial roles in determining phenolic yield. A 70:30 water-ethanol mixture at 80°C was found to be the most efficient condition for maximum TPC recovery from *P. dioica* leaves.

At 120 minutes of incubation, the total phenolic content (TPC) of *Pimenta dioica* leaf extracts ranged from 3.64 to 6.70 mg GAE/g DW (fig 2). The maximum TPC (6.70 mg GAE/g) was recorded at 60°C with an 80:20 water–ethanol ratio, while the lowest (3.64 mg GAE/g) occurred at room temperature with a 90:10 solvent ratio. The higher TPC observed at moderate temperatures and mixed solvent systems (70:30–80:20) can be attributed to the synergistic extraction effect of water and ethanol, where water swells plant tissues and ethanol enhances the solubility of less-polar phenolics. This agrees with recent findings that optimal solvent polarity enhances both the diffusion and solubility of phenolic compounds, leading to greater extraction efficiency (Phuyal *et al.*, 2020; Plinelo *et al.*, 2005).

Increasing temperature generally promoted phenolic recovery by improving mass transfer and solvent penetration, but at elevated temperatures ($\geq 80^\circ\text{C}$), a slight reduction or stabilization in TPC was noted, likely due to thermal degradation or oxidation of phenolic constituents (Anokwuru *et al.*, 2022; Mohanty *et al.*, 2023). When compared to the 60-minute extraction, prolonged incubation to 120 minutes enhanced TPC in certain conditions (e.g., at 60°C, 80:20 from 5.24 to 6.70 mg GAE/g), indicating improved diffusion and solvent–solute interaction over time. However, at higher temperatures such as 80°C, a marginal decline in TPC was observed (from 6.15 to 5.59 mg GAE/g), suggesting that extended heating beyond the optimal extraction time may cause partial degradation of heat-labile phenolics (Chen *et al.*, 2021). Thus, a moderate temperature (60–70°C), a balanced solvent system (80:20), and sufficient extraction duration (up to 120 min) appear to be the most favorable conditions for maximizing phenolic yield from *P. dioica* leaves.

In Vitro Anti Oxidant Assays Reducing Power Assay

At 60 min, the reducing power of *Pimenta dioica* leaf extracts varied significantly with both extraction temperature and solvent ratio. The highest reducing power (1.693 ± 0.051) was observed at 60 °C with a 80:20 water–ethanol mixture, indicating optimal extraction of electron-donating antioxidant compounds under moderately polar conditions.

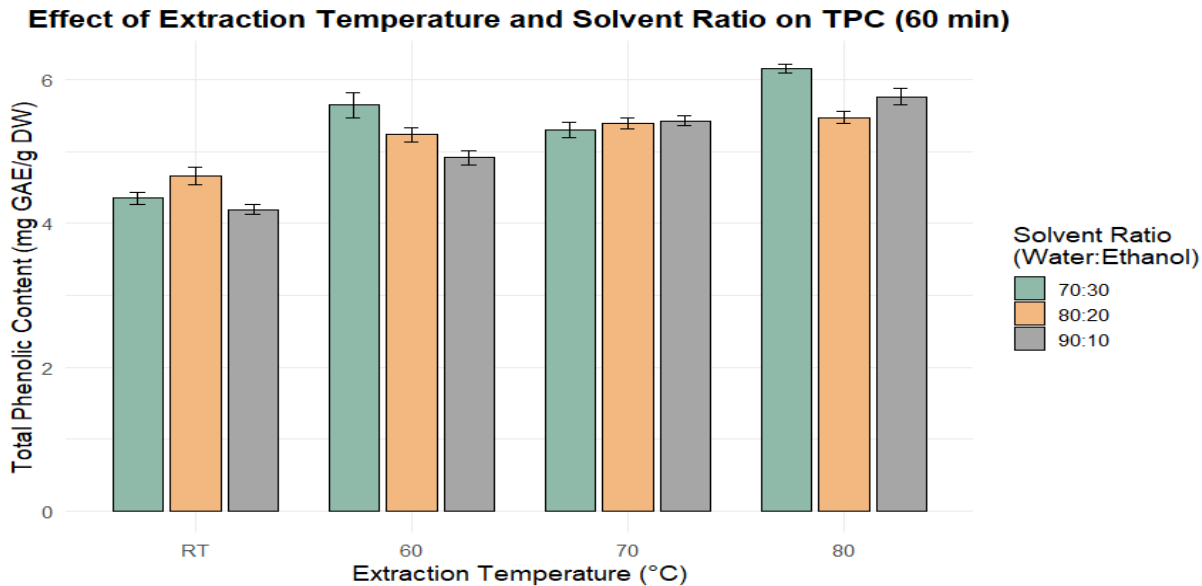


Fig 1. Effect of extraction temperature and solvent ratio on Total Phenol Content (TPC) of the leaf extract at 60 Min.

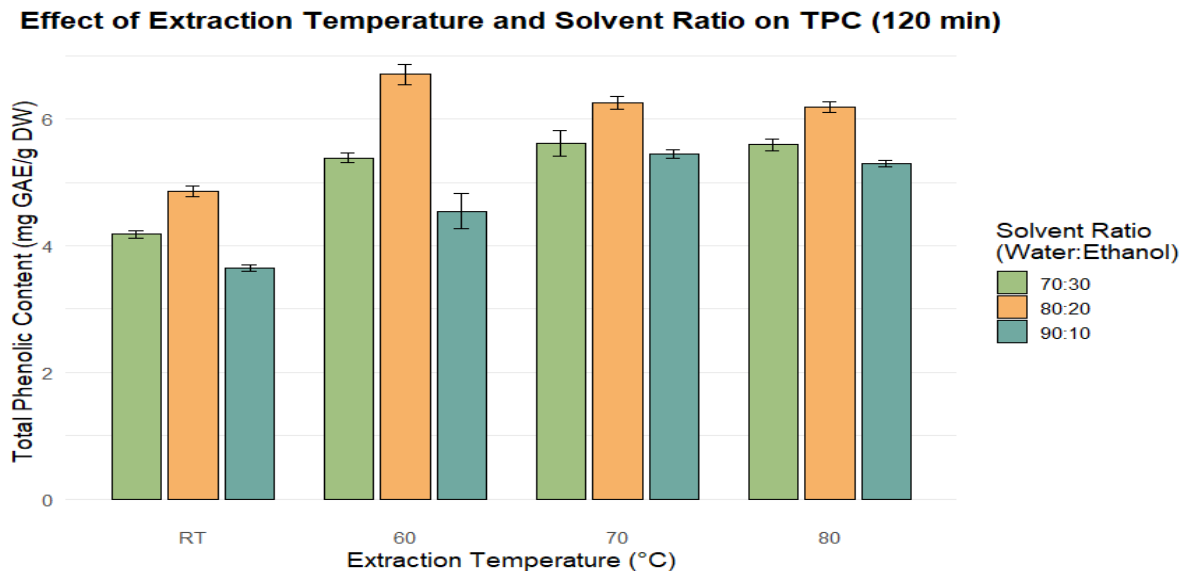


Fig 2. Effect of extraction temperature and solvent ratio on Total Phenol Content (TPC) of the leaf extract at 120 Min.

Further increase in temperature to 70 °C and 80 °C resulted in a decline in reducing capacity, suggesting degradation or transformation of thermolabile phytoconstituents. At room temperature, moderate activity was retained, implying that mild heating facilitates the release of phenolics without extensive oxidation. The superior activity of the 80:20 solvent systems agrees with reports that aqueous alcohol mixtures enhance solubility and diffusion of phenolics and flavonoids compared with either pure water or ethanol alone (Zhang *et al.*, 2023).

Overall, the results demonstrate that extraction conditions critically influence the antioxidant potential of *P. dioica* leaves, with moderate heating (≈ 60 °C) and a balanced solvent polarity yielding extracts with maximum reducing power, consistent with previous findings on temperature-dependent antioxidant recovery from aromatic and medicinal plants (Abd Aziz *et al.*, 2021). At 120 min extraction, the reducing power of *Pimenta dioica* leaf extracts showed clear variations with both temperature and solvent composition.

Table 3. Effect of Extraction Temperature and Solvent ratio on Reducing power assay at 60 min.

Temp. (°C)	Solvent Ratio (Water: Ethanol)	Reducing Power Assay
60	70:30	1.200± 0.050
	80:20	1.693±0.051
	90:10	1.087± 0.042
70	70:30	0.910± 0.046
	80:20	0.223± 0.025
	90:10	0.077± 0.025
80	70:30	0.400± 0.044
	80:20	0.713± 0.041
	90:10	0.299± 0.064
RT	70:30	0.893± 0.051
	80:20	0.708± 0.049
	90:10	0.718± 0.033

The maximum reducing power (2.033 ± 0.038) was recorded at 60 °C with 80:20 water-ethanols, followed by 80 °C (1.403 ± 0.045) under the same solvent system. In contrast, the lowest activity was obtained at 70 °C with 70:30 (0.600 ± 0.044) and 80 °C with 90:10 (0.590 ± 0.036) mixtures. The observed trend indicates that moderate heating and balanced solvent polarity enhance antioxidant extraction, while excessively high or low water content and higher temperatures tend to reduce electron-donating potential. Prolonged extraction time (120 min) improved reducing capacity compared with 60 min values, suggesting increased diffusion of bound antioxidants into the solvent.

The enhanced activity at 60 °C supports earlier findings that moderate thermal treatment promotes cell-wall disruption and solvent penetration, facilitating phenolic release (Zhang *et al.*, 2023). However, further heating (≥70 °C) may cause degradation or polymerization of sensitive phytochemicals, reducing antioxidant potential. The superior performance of the 80:20 solvent ratio aligns with studies indicating that mixtures with intermediate polarity optimize recovery of both hydrophilic and moderately lipophilic phenolics (Barchan *et al.*, 2014). Overall, the results confirm that 60 °C and 80:20 water-ethanol for 120 min represent the most effective extraction conditions for maximizing reducing power in *P. dioica* leaves.

Table 4. Effect of Extraction Temperature and Solvent ratio on Reducing power assay at 120 min.

Temp. (°C)	Solvent Ratio (Water:Ethanol)	Reducing Power Assay
60	70:30	1.093± 0.038
	80:20	2.033± 0.038
	90:10	0.707± 0.051
70	70:30	0.600± 0.044
	80:20	1.167± 0.042
	90:10	0.793± 0.038
80	70:30	0.547± 0.035
	80:20	1.403± 0.045
	90:10	0.590± 0.036
RT	70:30	0.883± 0.035
	80:20	0.693± 0.038
	90:10	0.717± 0.035

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of *Pimenta dioica* leaf extracts varied considerably with extraction temperature and solvent ratio (table 1). At 60 min of incubation, antioxidant activity ranged from 0-78 %, with a marked increase at elevated extraction temperatures and optimal solvent polarity. The highest activity (78 %) was obtained at 60 °C and a 70:30 (water: ethanol) ratio, while the lowest activity (0-5 %) was recorded in extracts prepared at room temperature with the same solvent system. Moderate temperatures (60-80 °C) enhanced radical scavenging efficiency due to improved solubility and diffusion of phenolic and other antioxidant constituents into the solvent phase.

The 70:30 solvent ratio consistently yielded higher DPPH inhibition than either more aqueous (90:10) or ethanol-rich (80:20) mixtures, indicating that intermediate polarity facilitates the extraction of both hydrophilic and lipophilic antioxidants (Phuyal *et al.*, 2020; Sepahpour *et al.*, 2018). An increase in temperature from 60°C to 70°C caused a slight decline in DPPH activity, followed by a mild recovery at 80 °C, suggesting that while moderate heat promotes extraction efficiency, excessive heating may partially degrade thermolabile antioxidant compounds (Mohanty *et al.*, 2023). The consistently low scavenging activity at room temperature indicates limited mass transfer and poor solvent penetration into plant tissues.

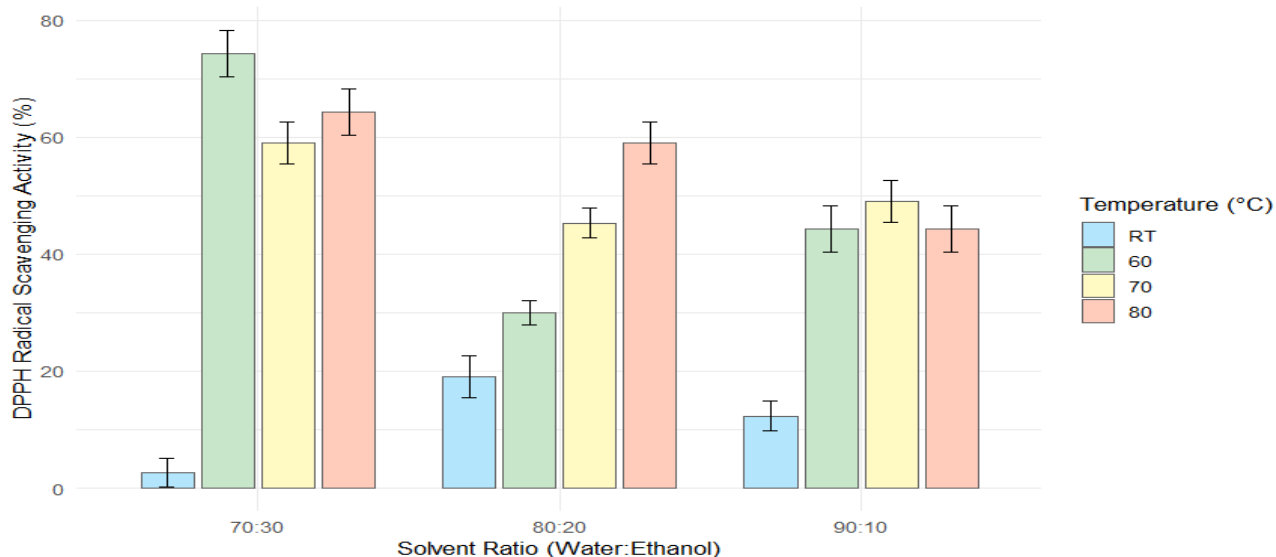
Effect of Temperature and Solvent Ratio on DPPH Radical Scavenging Activity (60 min)

Fig 3. Effect of extraction temperature and solvent ratio on DPPH Radical scavenging assay of the leaf extract at 60 Min.

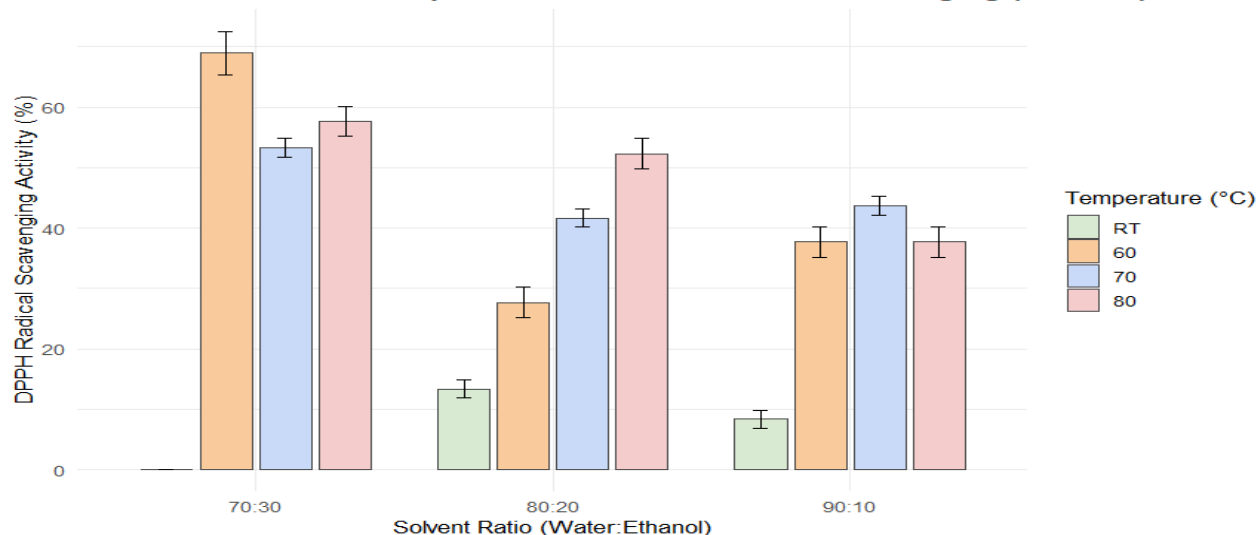
Effect of Solvent Ratio and Temperature on DPPH Radical Scavenging (120 min)

Fig 4. Effect of extraction temperature and solvent ratio on DPPH Radical scavenging assay of the leaf extract at 120 Min.

These findings are in line with reports that elevated extraction temperatures and balanced solvent polarity enhance phenolic recovery and free-radical scavenging capacity (Chen *et al.*, 2021; Antony and Farid, 2022). Overall, the optimal extraction condition for maximum antioxidant potential of *P. dioica* leaves at 60 min was 60 °C using a 70:30 water-ethanol solvent system. At 120 minutes of incubation, the DPPH radical scavenging activity of *Pimenta dioica* leaf extracts ranged from 0 % to 69% (table 1). The highest scavenging activity (69%) was observed at 60°C using a 70:30

(water: ethanol) solvent ratio, while the lowest (0%) was recorded at room temperature with the same solvent system. Similar to the 60 min results, moderate heating and a balanced solvent polarity markedly enhanced antioxidant potential. The 70:30 water–ethanol mixture consistently produced higher scavenging activity than either more aqueous (90:10) or more ethanolic (80:20) mixtures, confirming that intermediate polarity favors the simultaneous extraction of both hydrophilic and lipophilic antioxidant compounds (Phuyal *et al.*, 2020).

Antioxidant activity increased with temperature up to 60 °C and declined slightly thereafter, indicating that moderate heating improved mass transfer and cell wall disruption, whereas prolonged or excessive heating (70-80 °C) may have caused partial oxidation or degradation of phenolic constituents (Mohanty *et al.*, 2023). The very low activity at room temperature reflects limited diffusion and poor solvent penetration into plant tissues. When compared with the 60 min extraction, a marginal decline in DPPH activity was seen at 120 min under most conditions (e.g., 60°C, 70:30 decreased from 74.3% → 69%), suggesting possible degradation of heat-sensitive antioxidants or equilibrium in phenolic diffusion. Prolonged heating thus does not necessarily enhance antioxidant recovery beyond the optimal extraction time, aligning with reports that excessive thermal exposure reduces radical-scavenging efficiency (Chen *et al.*, 2021; Antony and Farid, 2022). Overall, the optimal condition for maximum antioxidant activity of *P. dioica* leaves remained 60 °C with a 70:30 water-ethanol ratio for 60 min extraction.

Conclusion

The present study demonstrated that both temperature and solvent composition play crucial roles in optimizing the green extraction of bioactive compounds from *Pimenta dioica* leaves using an ethanol-water system. The qualitative phytochemical screening confirmed the presence of major secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, and saponins, with their abundance varying according to extraction conditions. Among all treatments, extraction at 80°C with an 80:20 (water: ethanol) ratio yielded the most comprehensive phytochemical profile, indicating that a moderate ethanol proportion and elevated temperature effectively enhance the solubilization of diverse phytochemicals. These findings align with trends observed in the total phenolic content (TPC) and reducing power assay, where the same conditions produced higher antioxidant responses. The results collectively suggest that moderate thermal energy facilitates cell-wall disruption and phenolic diffusion, while balanced solvent polarity ensures efficient extraction of both hydrophilic and semi-polar constituents.

Thus, an 80:20 ethanol-water solvent system at 80°C for 120 min can be considered the optimal green extraction condition for maximizing phytochemical recovery and antioxidant potential in *P. dioica* leaves. This eco-friendly approach offers a sustainable alternative to conventional organic solvent extraction and supports the valorization of *P. dioica* as a natural source of antioxidants for food, nutraceutical, and pharmaceutical applications.

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