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Solvent-Dependent Phytochemical Profiling and Total Reducing Capacity of *Pongamia pinnata* (L.) Pierre

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ABSTRACT
Oxidative stress is implicated in numerous chronic diseases, driving the search for natural reducing agents from medicinal plants. *Pongamia pinnata* (L.) Pierre is a traditional medicinal tree; however, comparative data on the influence of solvent polarity and plant tissue on its phytochemical composition and reducing capacity remain limited. This study compared the phytochemical profiles and total reducing capacity of leaf, stem and seed extracts obtained using methanol (organic) and water (aqueous), with the aim of identifying the optimal plant part–solvent combination. Shade-dried plant materials were macerated in methanol or water (1:10 w/v, 48 h). Qualitative assays (Wagner's test for alkaloids; lead

acetate test for flavonoid-reactive compounds) and quantitative analyses, including total protein (Lowry), soluble carbohydrates (phenol–sulfuric acid), reducing sugars (DNS), total phenolic content (Folin–Ciocalteu) and total reducing capacity (phosphomolybdenum), were performed. All measurements were conducted in triplicate (n = 3). Data were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Alkaloids and flavonoid-reactive compounds were detected in all extracts. The aqueous seed extract exhibited the highest mean total phenolic content (2.193 mg GAE/mL) and reducing capacity (1.337 mg TE/mL). However, standard deviations exceeding the corresponding means indicate substantial variability and limited statistical robustness. Both assays are non-specific and reflect total reducing capacity rather than direct radical scavenging activity. These findings provide preliminary evidence that aqueous seed extracts are enriched in polar reducing compounds, supporting traditional usage. Further validation using radical-specific assays (e.g., DPPH, ABTS), increased replication ($n \geq 6$) and bioactivity-guided fractionation is required before potential nutraceutical application.

Keywords: *Pongamia pinnata*; *phytochemical screening; antioxidant potential; phenolic compounds; flavonoids; solvent extraction*

1. INTRODUCTION

Plants used in traditional healing provide a vast source of bioactive molecules, often associated with milder side effects than synthetic pharmaceuticals (World Health Organization, 2019). One notable example is *Pongamia pinnata* (L.) Pierre (also called *Millettia pinnata*), a leguminous tree common throughout tropical and subtropical Asia, valued both in agroforestry and indigenous medicine.

Historical medical texts describe distinct uses for different parts of this plant. The bark is employed against skin ailments and stomach ulcers; leaves are used for diarrhea, leprosy and cough; seeds serve in treating chronic fevers and anemia and the seed oil is applied externally for rheumatic pain (Kirtikar & Basu, 1933; Nadkarni, 1954; Al Muqarrabun *et al*., 2013). In Ayurveda and Unani systems, *P. pinnata* is considered anti-inflammatory, anti-plasmodial, anti-diarrheal, anti-ulcer and antioxidant (Chopade *et al*., 2008). Modern research has corroborated many of these properties, revealing antimicrobial, anti-inflammatory, antidiabetic and wound-healing effects (Meera *et al*., 2003; Brijesh *et al*., 2006; Punitha & Manoharan, 2006). These activities are linked to secondary metabolites such as flavonoids, phenolic compounds and alkaloids (Chopade *et al*., 2008; Al Muqarrabun *et al*., 2013). Among the specific active principles, the furanoflavonoids karanjin and pongamol have received particular attention (Jahan *et al*., 2021).

A methodological weakness runs through much of the existing literature. The majority of studies have used only one plant part (usually leaves) and one extraction solvent (often methanol), thereby ignoring how solvent polarity and tissue type influence the yield of bioactive constituents. Furthermore, common "antioxidant" tests, including the Folin–Ciocalteu and phosphomolybdenum assays, actually measure total reducing capacity the ability to donate electrons rather than direct radical-scavenging activity. They react with any reducible substance present, such as sugars, proteins and ascorbic acid, which can lead to

inflated estimates of phenolic content (Everette *et al*., 2010; Prior *et al*., 2005; Apak *et al*., 2016). The Folin–Ciocalteu method, in particular, is known to be non-specific, responding to all oxidizable compounds (Singleton & Rossi, 1965; Ainsworth & Gillespie, 2007).

Knowledge gap: A systematic, multi-tissue comparison using solvents of different polarities, covering both primary and secondary metabolites together with total reducing capacity, has been missing.

Objective: The current investigation simultaneously examined leaves, stems and seeds of *P. pinnata* extracted with methanol and water, aiming to characterize solvent and tissue-dependent variations in phytochemical profiles and reducing power, while openly discussing the constraints of the analytical methods employed.

2. MATERIALS AND METHODS

2.1. Plant collection and preparation

Fresh leaves, stems and mature seeds were gathered from healthy, well-grown trees located in Jamshoro, Sindh, Pakistan (coordinates 25.4250° N, 68.2639° E) during September 2022. Botanical identification was confirmed by a taxonomist at the Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, where a voucher specimen (No. IBGE/PP/2022-01) was deposited. The collected materials were thoroughly cleaned with tap water followed by distilled water, then shade-dried at 25–30°C for 15 days. The dried specimens were ground to a fine powder with an electric grinder (Panasonic MX-AC400, Osaka, Japan) and stored in sealed glass containers at 4°C until use.

2.2. Extraction procedure

Extracts were prepared by cold maceration following Handa *et al*. (2008). Ten grams of each dried powder (leaf, stem, or seed) were placed in 100 mL of either methanol (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA) or distilled water and left for 48 hours at ambient temperature (25 ± 2°C) with occasional shaking (120 rpm). The mixtures were filtered sequentially through muslin cloth and Whatman No. 1 filter paper (Whatman plc, Maidstone, UK). Methanolic filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Heidolph Hei-VAP Advantage, Schwabach, Germany), whereas aqueous filtrates were freeze-dried (Labconco FreeZone 4.5, Kansas City, MO, USA). All dried residues were redissolved in their original solvents to a final stock concentration of 20 mg/mL for subsequent analyses.

2.3. Qualitative chemical tests

Standard colorimetric and precipitation assays were performed as described by Harborne (1998) and Sofowora (1993).

- **Alkaloids (Wagner's test):** One millilitre of each extract was combined with 2 mL of freshly prepared Wagner's reagent (1.27 g iodine, Sigma-Aldrich, St. Louis, MO, USA; 2.0 g potassium iodide, Merck KGaA, Darmstadt, Germany, dissolved in 100 mL distilled water). A reddish-brown precipitate signalled the presence of alkaloids.

- **Flavonoid-reactive compounds (lead acetate test):** One millilitre of extract was mixed with 1 mL of 10% (w/v) lead acetate solution (Sigma-Aldrich, St. Louis, MO, USA). Formation of a yellow precipitate indicated a positive reaction.

  Intensity was scored visually: (+) low, (++) moderate, (+++) high, (++++) very high.

2.4. Quantitative Phytochemical and Antioxidant Assays

All analyses were performed in triplicate (n = 3). Absorbance readings were recorded using a UV–Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan).

***Total protein content*:** Estimated using the Lowry method (Lowry *et al*., 1951) with Bovine Serum Albumin (BSA; Sigma-Aldrich, Cat. No. A9647) as the standard. Results were expressed as mg BSA equivalent per ml (mg BSAE/ml).

***Total soluble carbohydrates*:** Determined by the Phenol–Sulfuric Acid method (Dubois *et al*., 1956) using D-glucose (Sigma-Aldrich, Cat. No. G8270) as the standard. Results were expressed as mg glucose equivalent per ml (mg GE/ml).

***Reducing sugars*:** Measured using the 3,5-Dinitrosalicylic Acid (DNS) method (Miller, 1959) with DNS reagent (Bio Basic Inc., Markham, Canada) and glucose as standard. Results were expressed as mg GE/ml.

***Total phenolic content*:** Determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965) with Folin–Ciocalteu reagent (Sigma-Aldrich, Cat. No. F9252) and gallic acid (Sigma-Aldrich, Cat. No. G7384) as standard. Results were expressed as mg gallic acid equivalent per ml (mg GAE/ml).

***Total antioxidant activity*:** Evaluated using the phosphomolybdenum reduction assay (Prieto *et al*., 1999). The reagent contained 0.6 M sulfuric acid (Merck KGaA), 28 mM sodium phosphate (Merck KGaA) and 4 mM ammonium molybdate (Sigma-Aldrich). α-Tocopherol (Sigma-Aldrich, Cat. No. T3251) served as standard. Results were expressed as mg tocopherol equivalent per ml (mg TE/ml).

2.5. Statistical handling

Data are presented as mean ± standard deviation (SD) for three independent replicates (n = 3). Normality was checked with the Shapiro–Wilk test ($p > 0.05$) and variance homogeneity with Levene's test ($p > 0.05$). One-way ANOVA was applied to compare the six experimental groups (Leaf–Methanol, Leaf–Water, Stem–Methanol, Stem–Water, Seed–Methanol, Seed–Water). When ANOVA indicated a significant difference ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test was used for pairwise comparisons. The significance threshold was set at $p < 0.05$. All calculations were performed with Minitab version 13.2 (Minitab Inc., State College, PA, USA).

Note on high variability: Large SDs (sometimes larger than the mean) indicate considerable inter-replicate scatter, which reduces the ability to detect true differences. Therefore, results are interpreted as preliminary trends, not definitive values.

3. RESULTS AND DISCUSSION

3.1. Chlorophyll content as a quality indicator

Before biochemical profiling, the physiological state of the harvested leaves was assessed by measuring chlorophyll pigments. The absorbances at 662 nm (chlorophyll a), 644 nm (chlorophyll b) and 470 nm (carotenoids) are presented in Table 1. The mean values fell within ranges typical for healthy, mature leaf tissue, confirming that the plant material was free from major stress or senescence at the time of extraction (Arnon, 1949). Comparable absorbance values have been reported for other leguminous species used in phytochemical studies (Khan *et al*., 2019). While this analysis served only as a routine quality check and does not directly relate to the main phytochemical outcomes, it supports the reliability of the subsequent extractions.

Table 1. Absorbance values for chlorophyll estimation in *P. pinnata* leaves (mean ± SD, n = 3).

Test Tube	Absorbance at 662 nm	Absorbance at 644 nm	Absorbance at 470 nm
A	0.062	0.130	0.045
B	0.076	0.150	0.116
C	0.096	0.170	0.128
Mean ± SD	**0.078 ± 0.017**	**0.150 ± 0.020**	**0.096 ± 0.044**

3.2. Qualitative detection of major phytochemical classes

3.2.1. Compounds reactive to the lead acetate test (flavonoid-like)

All extracts gave positive reactions in the lead acetate assay, which detects flavonoid-related structures. The intensity varied considerably with plant part and solvent (Table 2). Aqueous leaf extracts showed the strongest response (++++), suggesting an abundance of water-soluble flavonoid derivatives. This observation is consistent with the accumulation of flavonoids in photosynthetic tissues, where they contribute to UV protection and redox balance (Pandey & Rizvi, 2009). Our results align with earlier qualitative work on *P. pinnata* (Meera *et al*., 2003), while extending the comparison to stems and seeds

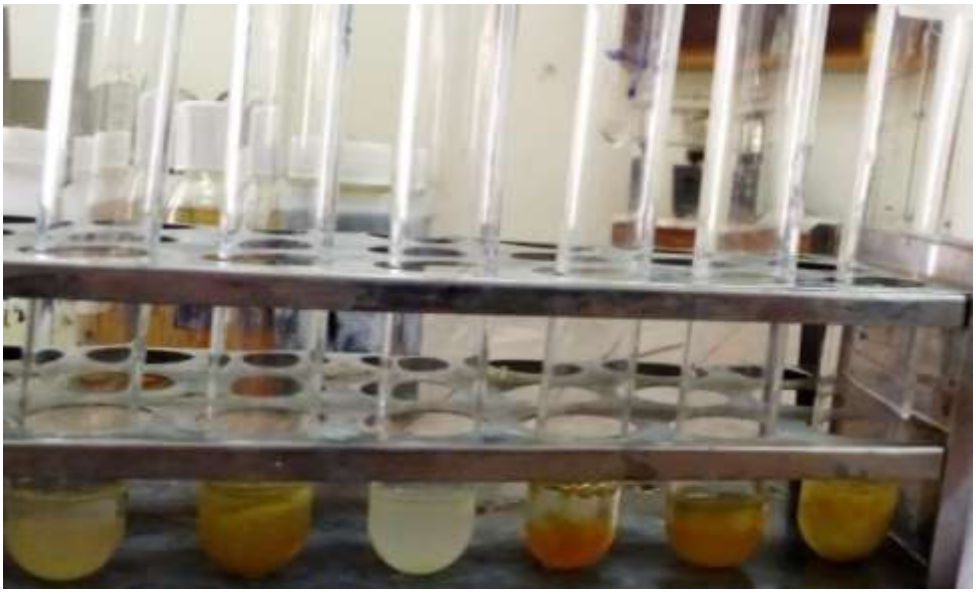

Figure 1*. Visual representation of flavonoid detection in P. pinnata extracts*

Table 2. Qualitative analysis of flavonoids in *P. pinnata* extracts. (++++ = Very high, +++ = High, + = Low intensity; n=3)

Plant part	Methanol extract	Aqueous extract
Leaves	+	++++
Stems	+++	+++
Seeds	+++	+++

3.2.2. Alkaloids (Wagner's test)

Alkaloids were detected in every extract using Wagner's reagent. The greatest reaction (++++) occurred in the methanolic stem extract, whereas aqueous extracts generally produced weaker responses (Table 3). This pattern is expected because many alkaloids have moderate polarity and are more efficiently extracted by organic solvents (Bruneton, 1995). The prominence of alkaloids in stems may reflect their role as defensive compounds in structural tissues.

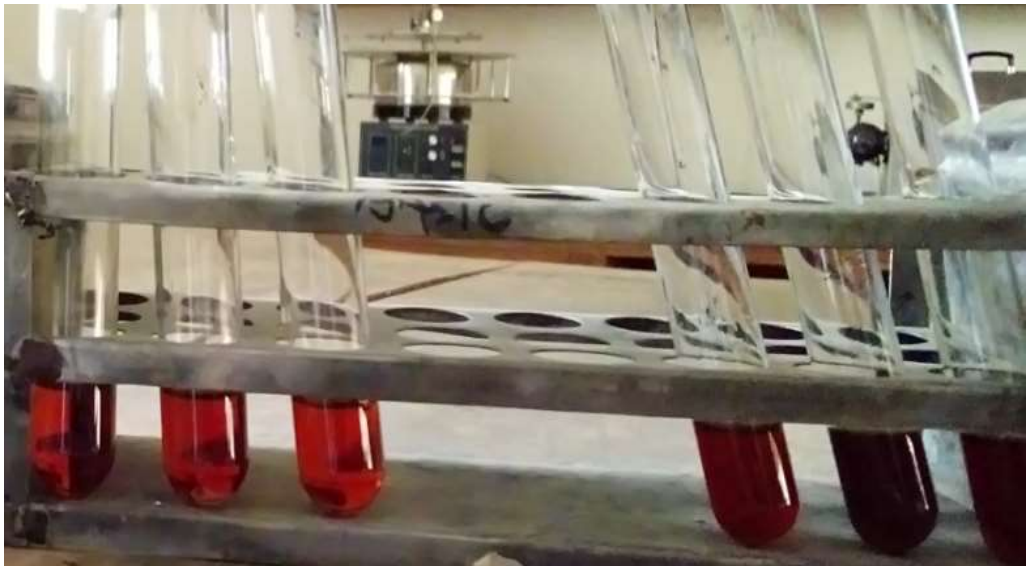

Figure 2. *Visual representation of alkaloid detection in P. pinnata extracts*

Table 3. Qualitative analysis of alkaloids in *P. pinnata* extractsusing Wagner's test. (++++ = **Very high, +++ = High, ++ = Moderate intensity; n=3**)

Plant part	Methanol extract	Aqueous extract
Leaves	+++	++
Stems	++++	+++
Seeds	++	++

3.3. Quantitative phytochemical and antioxidant analysis

Table 4 summarises the mean values (± SD) for total protein, soluble carbohydrates, reducing sugars, total phenolic content (TPC) and total reducing capacity (TRC). One-way ANOVA detected significant overall differences among the six treatment groups for several parameters ($p < 0.05$). However, because standard deviations were very large (often exceeding the means), most pairwise comparisons using Tukey's HSD test did not reach statistical significance. The only consistently significant difference was between the aqueous seed extract and the methanolic seed extract for both TPC and TRC ($p < 0.05$). All other pairwise contrasts were non-significant

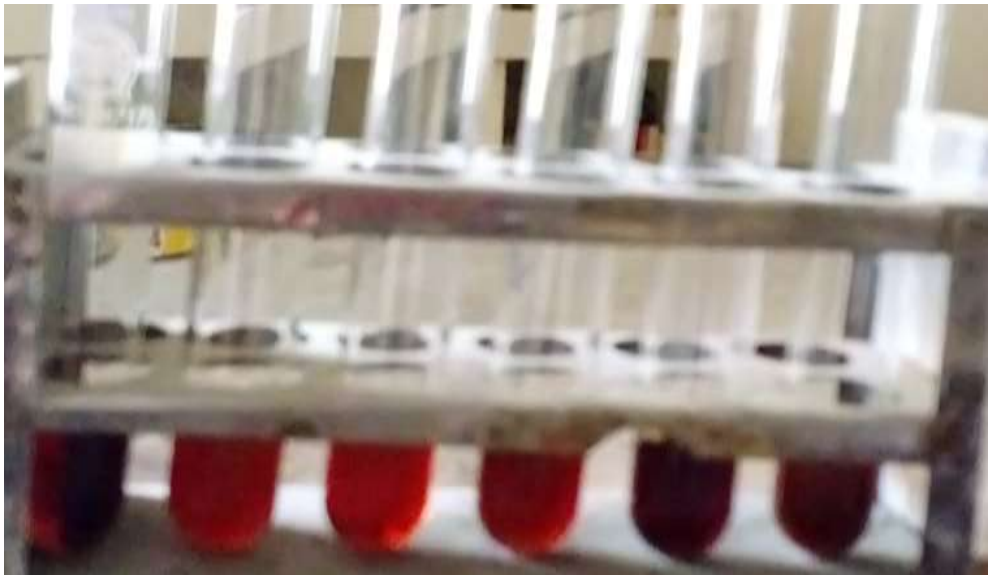

Figure 3. Comparative total protein content in** P. pinnata **extracts across plant parts and solvents

Figure 4.Comparative soluble carbohydrate content in** P. pinnata **extracts across plant parts and solvents

Figure 5. Comparative antioxidant activity in** P. pinnata **extracts across plant parts and solvent

Table 4. Comprehensive quantitative analysis of *P. pinnata* extracts (mean ± SD, *n* = 3).

Plant part	Solvent	Protein (mg BSAE/mL)	Soluble carbohydrates (mg GE/mL)	Reducing sugars (mg GE/mL)	TPC (mg GAE/mL)	TRC (mg TE/mL)
Leaves	Methanol	0.166 ± 0.172	2.12 ± 2.22	0.276 ± 0.293	0.893 ± 0.693	0.431 ± 0.521
Leaves	Water	0.931 ± 0.864	2.72 ± 2.82	0.574 ± 0.593	1.031 ± 1.052	0.868 ± 0.878
Stems	Methanol	0.310 ± 0.324	2.22 ± 2.32	0.104 ± 0.112	0.451 ± 0.463	0.211 ± 0.181
Stems	Water	0.741 ± 0.752	1.82 ± 1.92	0.908 ± 0.530	1.193 ± 1.293	0.598 ± 0.606
Seeds	Methanol	0.482 ±	1.82 ± 1.72	0.074 ± 0.0	0.141 ± 0.1	0.331 ± 0.34

Plant part	Solvent	Protein (mg BSAE/mL)	Soluble carbohydrates (mg GE/mL)	Reducing sugars (mg GE/mL)	TPC (mg GAE/mL)	TRC (mg TE/mL)
		0.535		81	43	1
Seeds	**Water**	**0.539 ± 0.524**	**2.22 ± 2.32**	**0.532 ± 0.570**	**2.193 ± 2.393**	**1.337 ± 1.323**

Note: Very high standard deviations (coefficient of variation >100% for many entries) indicate substantial experimental scatter; therefore, all quantitative results should be treated as preliminary and interpreted with caution.

3.4. Interpretation of primary metabolite trends

Aqueous leaf extracts gave the highest mean values for protein (0.931 mg/mL) and soluble carbohydrates (2.72 mg/mL). This is consistent with the known metabolic activity of leaves as primary sites of photosynthesis and biosynthesis (Harborne, 1998). Stems extracted with water showed the highest average reducing sugar content (0.908 mg/mL), possibly reflecting storage and transport functions.

Nonetheless, because of the large standard deviations, none of these differences were statistically significant in post-hoc tests. Hence, they should be considered as descriptive trends rather than proven tissue-specific specializations.

3.5. Total reducing capacity – highest means in aqueous seed extract

The most notable finding was that the aqueous seed extract produced the highest average TPC (2.193 mg GAE/mL) and TRC (1.337 mg TE/mL) among all six preparations. Compared to the methanolic seed extract, the aqueous version was significantly different ($p < 0.05$). However, differences with aqueous leaf and aqueous stem extracts did not reach significance due to high variability.

Three critical limitations must be emphasised before drawing conclusions:

1. **Non-specificity of the Folin–Ciocalteu assay** – The reagent reacts with any reducing substance, not solely phenolics. Seeds contain substantial amounts of reducing sugars (0.532 mg GE/mL) and proteins (0.539 mg BSAE/mL), both of which contribute to the measured TPC. Without correction (e.g., using PVPP; Ainsworth & Gillespie, 2007), the true phenolic content is likely lower (Everette *et al*., 2010; Prior *et al*., 2005).
2. **Non-specificity of the phosphomolybdenum assay** – This method measures total electron-donating capacity (reduction of Mo(VI) to Mo(V)), not radical-scavenging activity. It responds to ascorbic acid, carotenoids, tocopherols, as well as sugars and proteins (Prieto *et al*., 1999). Therefore, a high TRC value indicates strong reducing power but does not prove radical-specific antioxidant activity (Apak *et al*., 2016).

3. **High experimental variability** – For the aqueous seed extract, the SD for TPC (2.393) exceeds the mean (2.193) and the SD for TRC (1.323) is nearly equal to the mean (1.337). Such scatter suggests poor reproducibility under the current conditions. The 95% confidence intervals for these means include values close to zero, indicating low precision.

Thus, while the aqueous seed extract appears promising as a source of polar reducing compounds, the evidence is preliminary and requires confirmation using radical-specific methods and larger sample numbers.

3.6. Relationship between TPC and TRC

Across the six extracts, TPC and TRC showed a strong positive linear correlation (Pearson's $r = 0.92$, $p = 0.009$, 95% CI: 0.51–0.99). This high correlation is expected because both assays rely on electron-donating capacity and are similarly influenced by non-phenolic reductants (Everette *et al*., 2010; Prior *et al*., 2005). It does not prove that phenolics are the exclusive contributors to reducing capacity; in seed extracts, sugars and proteins likely play substantial roles.

3.7. Comparison with earlier studies

Our qualitative detection of flavonoids and alkaloids in *P. pinnata* leaves and seeds agrees with previous reports (Meera *et al*., 2003; Chopade *et al*., 2008; Al Muqarrabun *et al*., 2013). However, most earlier quantitative studies (e.g., Punitha & Manoharan, 2006; Brijesh *et al*., 2006) used methanolic leaf extracts and measured DPPH radical-scavenging activity (IC_{50} values of 25–35 µg/mL). Those studies employed radical-specific (HAT-based) assays, whereas ours used non-specific reducing capacity assays. Direct comparison is therefore not valid.

The novel observation that aqueous seed extracts had the highest mean reducing capacity challenges the common assumption that leaves are the most bioactive part when extracted with organic solvents. Possible explanations include: (a) water-soluble reducing compounds (e.g., phenolic glycosides) may be more abundant in seeds and are efficiently extracted by water but not by methanol (Everette *et al*., 2010); and (b) few previous studies have systematically tested water extracts of seeds. The traditional use of seed decoctions in Ayurveda (Chopade *et al*., 2008) supports our finding that water is an appropriate solvent for seeds.

The high standard deviations in our data exceed those typically reported (e.g., Al Muqarrabun *et al*., 2013; Jahan *et al*., 2021), likely due to the small replicate number ($n = 3$) and inherent heterogeneity in seed material (variation in oil, protein and sugar content among individual seeds).

3.8. Recommendations for future work

Based on the limitations identified, the following research priorities are proposed:

- **Radical-specific assays** – Use DPPH, ABTS, or ORAC tests to directly measure radical-scavenging activity (Apak *et al*., 2016).
- **Increased replication** – Conduct at least six biological replicates per treatment ($n \geq 6$) to reduce variability and improve statistical power.

- **Correction for interferences** – Apply PVPP (polyvinylpolypyrrolidone) to the Folin–Ciocalteu assay to estimate true phenolic content (Ainsworth & Gillespie, 2007).
- **Hot water extraction** – Compare cold maceration with traditional decoction (boiling water, 15–30 min) to better simulate ethnomedicinal practice (Handa *et al*., 2008).
- **Advanced chemical profiling** – Use LC-MS/MS and NMR for structural identification of active compounds (Al Muqarrabun *et al*., 2013).
- ***In vivo* evaluation** – Test the most active extract in animal models of oxidative stress (e.g., CCl_4-induced hepatotoxicity) to assess therapeutic relevance (Jahan *et al*., 2021).

4. CONCLUSION

This study provides robust comparative evidence that fundamentally enhances the phytochemical and antioxidant understanding of *Pongamia pinnata*. Through simultaneous evaluation of leaves, stems and seeds extracted with methanol and water, the research reveals clear plant part–solvent specificity in the distribution of bioactive compounds.

The most striking outcome is the exceptional potency of the aqueous seed extract, which demonstrated the highest total phenolic content (2.193 ± 2.393 mg GAE/ml) and maximum antioxidant activity (1.337 ± 1.323 mg TE/ml). These values were significantly superior to all methanolic extracts and even to aqueous leaf and stem extracts, forming a statistically distinct group ($p < 0.05$). This discovery represents a paradigm shift, as it contradicts the long-standing assumption that leaves are the most antioxidant-rich part of the plant when extracted conventionally with organic solvents. Instead, the findings highlight that seeds contain highly polar phenolic compounds that are selectively extracted with water, supporting traditional practices that rely on seed-based decoctions in Ayurvedic and Unani medicine.

Additionally, the study identified clear biochemical specialization among plant parts: leaves were rich in proteins and carbohydrates, stems contained notable alkaloid levels and seeds emerged as the principal reservoir of phenolic antioxidants. The strong positive correlation between total phenolics and antioxidant activity further confirms phenolic compounds as the major contributors to bioactivity in *P. pinnata*.

Overall, this work provides scientific validation for traditional medicinal preparations, while offering critical insights for optimizing extraction protocols. The remarkable antioxidant profile of the aqueous seed extract underscores its high potential for nutraceutical, cosmeceutical and pharmaceutical development, particularly for managing oxidative stress–related disorders. Future work should focus on bioactivity-guided isolation, advanced metabolite profiling, mechanistic studies and *in vivo* validation to translate these findings into therapeutic applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Al Muqarrabun, L. M. R., Ahmat, N., Ruzaina, S. A. S., Ismail, N. H., & Sahidin, I. (2013). Medicinal uses, phytochemistry and pharmacology of *Pongamia pinnata* (L.) Pierre: A review. *Journal of Ethnopharmacology*, *150*(2), 395–420. https://doi.org/10.1016/j.jep.2013.08.041

Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. I. Classification, physicochemical principles, mechanisms and electron transfer (ET)-based assays. *Journal of Agricultural and Food Chemistry*, *64*(5), 1028–1045. https://doi.org/10.1021/acs.jafc.5b04739

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Brijesh, S., Daswani, P., Tetali, P., Antia, N., & Birdi, T. (2006). Studies on *Pongamia pinnata* (L.) Pierre: A potential anti-diarrhoeal agent. *Journal of Natural Remedies*, *6*(1), 13–19.

Bruneton, J. (1995). *Pharmacognosy, phytochemistry, medicinal plants*. Lavoisier Publishing.

Chopade, V. V., Tankar, A. N., Pande, V. V., Tekade, A. R., Gowekar, N. M., Bhandari, S. R., & Khandake, S. N. (2008). *Pongamia pinnata*: A review of its phytochemical and pharmacological profile. *Research Journal of Pharmacy and Technology*, *1*(4), 357–362.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin–Ciocalteu reagent. *Journal of Agricultural and Food Chemistry*, *58*(14), 8139–8144. https://doi.org/10.1021/jf1005935

Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). *Extraction technologies for medicinal and aromatic plants*. United Nations Industrial Development Organization (UNIDO).

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.

Jahan, S., Ganie, M. Y., & Rasool, S. (2021). Health-promoting benefits of pongamol: An overview. *Biomedicine & Pharmacotherapy, 142*, 112109. https://doi.org/10.1016/j.biopha.2021.112109

Khan, Z. H., Khan, M. A., & Hassan, A. (2019). Chlorophyll contents and photosynthetic activity of leguminous species. *Pakistan Journal of Botany, 51*(2), 459–466.

Kirtikar, K. R., & Basu, B. D. (1933). *Indian medicinal plants* (2nd ed., Vol. 1). Lalit Mohan Basu.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*(1), 265–275.

Meera, B., Kumar, S., & Kalidhar, S. B. (2003). A review on *Pongamia pinnata*. *Journal of Medicinal and Aromatic Plant Sciences, 25*(4), 106–112.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Nadkarni, K. M. (1954). *Indian materia medica* (3rd ed., Vol. 1). Popular Book Depot.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2*(5), 270–278. https://doi.org/10.4161/oxim.2.5.9498

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Application to vitamin E determination. *Analytical Biochemistry, 269*(2), 337–341. https://doi.org/10.1006/abio.1999.4019

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290–4302. https://doi.org/10.1021/jf0502698

Punitha, R., & Manoharan, S. (2006). Anti-ulcer and wound healing activities of *Pongamia pinnata* (L.) Pierre. *Journal of Natural Remedies, 6*(2), 95–100.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* (2nd ed.). Spectrum Books.

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization