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Chemotypic Variation in Antioxidant Activity and Phytochemical Profiles of Terminalia arjuna (Roxb. ex DC.)

Safia Karim
Department of Biotechnology, Faculty of Crop Production,
Sindh Agriculture University, Tandojam Pakistan
safiakareem20@gmail.com
Ghulam Farooque
Department of Biotechnology, Faculty of Crop Production,
Sindh Agriculture University, Tandojam Pakistan
ghulamfarooquesp@gmail.com
Tayyaba
Department of Biotechnology, Faculty of Crop Production,
Sindh Agriculture University, Tandojam Pakistan
tayyabakarim555@gmail.com
Mehran Ali Chhalgri
Department of Biotechnology, Faculty of Crop Production,
Sindh Agriculture University, Tandojam Pakistan
mehranbaloch186@gmail.com
Nadir Ali Rind
Department of Molecular Biology & Genetics
Shaheed Benazir Bhutto University
Shaheed Benazirabad, Sindh, Pakistan
nadirali.rind@sbbusba.edu.pk
Sana khokhar
Department of Biotechnology, Faculty of Crop Production,
Sindh Agriculture University, Tandojam Pakistan
sanaliaquatali3@gmail.com

Abstract

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. is a medicinal tree widely used in South Asian traditional medicine for cardioprotection, primarily attributed to its antioxidant phytochemicals. However, the phytochemical profile of *T. arjuna* growing in Sindh, Pakistan where arid climate and saline soils may induce unique chemotypic variation remains uncharacterized. This study compared the phytochemical composition and in vitro

antioxidant capacity of aqueous and methanolic extracts from leaves, stem bark and seeds of *T. arjuna* collected from Sindh, Pakistan. Plant material was extracted with distilled water and 80% methanol. Qualitative phytochemical screening (flavonoids, alkaloids) and quantitative analyses (total phenolic content; total antioxidant capacity via phosphomolybdenum assay; proteins, carbohydrates, reducing sugars) were performed (n=3 biological replicates). Statistical analysis used t-tests, two-way ANOVA and Pearson correlation (α=0.05). Flavonoids and alkaloids were present in all extracts. The aqueous leaf extract showed significantly higher TPC (49.8 ± 3.5 mg GAE/g) and TAC (38.4 ± 3.1 mg AAE/g) than its methanolic counterpart ($p<0.001$ and $p<0.01$, respectively), with a strong positive correlation ($r=0.94$, $p<0.001$). The methanolic seed extract contained the highest protein concentration (16.8 ± 1.5 mg/g). Significant variation was observed across plant parts and solvents (two-way ANOVA: plant part $F(2,12)=45.32$, $p<0.001$; solvent $F(1,12)=38.76$, $p<0.001$; interaction $F(2,12)=12.45$, $p<0.001$). This study establishes the first phytochemical baseline for Sindh-grown *T. arjuna*, confirming chemotypic variation. The aqueous leaf extract is a potent, renewable, sustainable antioxidant source, offering an ecologically responsible alternative to destructive bark harvesting.

Keywords: *Terminalia arjuna*, Chemotypic variation, Antioxidant activity, Phenolic profiling, Sustainable sourcing

1. INTRODUCTION

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. (Combretaceae) is one of the most extensively studied medicinal trees in South Asian ethnomedicine, where its stem bark has been used for more than two thousand years in Ayurvedic, Unani and Siddha systems. Traditionally categorized as a *hṛidya* (cardiotonic agent), the bark has been prescribed for managing angina pectoris, hypertension, dyslipidemia and congestive heart failure (Dwivedi, 2007). Modern pharmacological investigations have validated these uses, attributing therapeutic efficacy to a diverse assemblage of bioactive compounds, including flavonoids (e.g., luteolin, quercetin), tannins, oligomeric proanthocyanidins, triterpenoid saponins such as arjunic and arjunolic acids, phenolic glycosides and essential minerals (Mandal *et al*., 2013; Alam *et al*., 2017). Collectively, these constituents confer potent antioxidant, anti-inflammatory, hypolipidemic, antimicrobial and cardioprotective activities (Pingle *et al*., 2020).

Global interest in natural antioxidants has intensified due to the central role of oxidative stress in chronic, degenerative disorders, particularly cardiovascular diseases, which remain the leading cause of mortality worldwide (Khan *et al*., 2019). Botanical therapeutics such as *T. arjuna*, long valued in traditional medicine and increasingly supported by clinical and mechanistic studies, offer promising complementary strategies for preventing and managing

oxidative stress-linked pathologies. However, most phytochemical and pharmacological investigations of *T. arjuna* have been conducted on specimens from India, resulting in a major geographical bias in the literature.

A critical scientific gap persists regarding the phytochemical profile of *T. arjuna* grown in the Sindh province of Pakistan. Secondary metabolite biosynthesis in medicinal plants is highly sensitive to environmental parameters including temperature, soil composition, salinity, nutrient availability and water stress, leading to pronounced chemotypic variation across geographic regions (Fahad *et al*., 2015). The arid climate and distinct alluvial soils of Sindh are likely to produce unique phytochemical signatures that remain uncharacterized, despite the species' traditional use in the region. Filling this gap is essential for understanding regional chemotypes, ensuring reproducible pharmacological outcomes and supporting evidence-based use of local plant material.

Sustainability is an additional concern driving renewed interest in alternative plant parts. Traditional reliance on bark, which requires destructive harvesting, poses ecological risks and threatens long-term resource availability. Leaves, by contrast, are renewable and can be harvested non-destructively; yet comprehensive comparative data on the phytochemical and antioxidant composition of leaves, stem bark and seeds remain limited. Previous studies have often focused exclusively on bark or employed non-standardized extraction protocols, complicating cross-study comparisons and limiting translational value (Kumar *et al*., 2010).

Research Gap: Secondary metabolite biosynthesis varies significantly with climatic, geographical and soil conditions (Fahad *et al*., 2015). Sindh's semi-arid climate, saline alluvial soils and temperature extremes may influence chemical composition, yet no comprehensive phytochemical or antioxidant profiling exists for Sindh-grown *T. arjuna*. This gap limits the understanding of regional chemotypes and restricts the scientific validation of traditionally used plant materials in Pakistan.

Scientific Rationale and Novelty:

a) First detailed chemotypic profiling of *T. arjuna* from Sindh, Pakistan.

b) Comparative analysis of leaves, bark and seeds, providing alternatives to bark harvesting and addressing sustainability concerns.

c) Solvent-dependent extraction behavior evaluated systematically across tissues.

d) Integration of phytochemical and antioxidant analyses with correlation studies to identify the best plant part–solvent combination.

Objectives:

i. Characterize qualitative and quantitative phytochemical profiles of leaves, stem bark and seeds of *T. arjuna* from Sindh.

ii. Compare extraction efficiency of aqueous and methanolic solvents.

iii. Assess in vitro antioxidant activity and identify the most potent extract.

iv. Provide region-specific baseline data to support sustainable utilization and future pharmaceutical applications.

2. MATERIALS AND METHODS

Plant material collection

Fresh leaves, stem bark and seeds were collected from five healthy, mature trees (estimated age 15–25 years) of *Terminalia arjuna* growing on the campus of the University of Sindh, Jamshoro, Pakistan (geographic coordinates: 25.428° N, 68.264° E) during November 2022. The plant material was taxonomically authenticated by the Senior Taxonomist, Department of Botany, University of Sindh, Jamshoro, Pakistan. A voucher specimen (Voucher No. SU/TA/2022-101) was deposited in the institutional herbarium for future reference. The collected samples were thoroughly washed with tap water followed by distilled water, shade-dried at ambient temperature (25 ± 2°C) to constant weight and mechanically ground to a fine powder (40-mesh sieve consistency) using an analytical mill (IKA® A11 basic, Staufen, Germany).

Chemicals and reagents

All chemicals and reagents were of analytical or HPLC grade. Methanol (CH_3OH, 99.8%), sulfuric acid (H_2SO_4, 95-97%), sodium carbonate (Na_2CO_3, anhydrous), sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$), potassium iodide (KI) and iodine crystals (I_2) were purchased from Merck KGaA (Darmstadt, Germany). Folin-Ciocalteu phenol reagent, gallic acid ($C_7H_6O_5$, ≥98.0%), bovine serum albumin (BSA, Fraction V), 3,5-dinitrosalicylic acid (DNS), ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) and L-ascorbic acid ($C_6H_8O_6$, ≥99.0%) were procured from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (resistivity 18.2 MΩ·cm) was generated using a Milli-Q® Integral water purification system (Merck Millipore, Burlington, MA, USA).

Preparation of crude extracts

Powdered plant material (10.0 g) was subjected to exhaustive maceration with 100 mL of solvent (either 80% methanol v/v or distilled water) on an orbital shaker (Heidolph Unimax 1010, Schwabach, Germany) at 150 rpm for 48 hours at room temperature (25°C). The mixtures were filtered through Whatman No. 1 filter paper (Cytiva, Marlborough, MA, USA). The marc (residue) was re-extracted twice under identical conditions to ensure complete extraction. The combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Buchi Rotavapor R-300, Flawil, Switzerland). The resultant crude extracts were lyophilized (Christ Alpha 1-4 LDplus, Osterode am Harz, Germany) to obtain dry powders, which were stored in desiccated, amber glass vials at -20°C until

analysis. Extraction yield was calculated as: Yield (%) = (Weight of dry extract / Weight of dry plant material) × 100.

Phytochemical analysis

Qualitative screening

Standard phytochemical tests were conducted following established protocols:

Flavonoids: Extract (1 mL) was treated with 1 mL of 10% (w/v) aqueous lead acetate solution. Formation of a yellow precipitate indicated a positive result.

Alkaloids: Extract (1 mL) was reacted with 2 mL of Wagner's reagent (prepared by dissolving 1.27 g iodine and 2.0 g potassium iodide in 100 mL distilled water). Formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Quantitative spectrophotometric analyses

All quantitative assays were performed in triplicate (n=3 biological replicates) using a UV-Vis spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan) with appropriate blank corrections.

Total phenolic content (TPC): Determined via the Folin-Ciocalteu method (Singleton *et al*., 1999). Briefly, extract solution (0.1 mL) was mixed with 0.5 mL of 10% (v/v) Folin-Ciocalteu reagent. After 5 minutes, 1.5 mL of 20% (w/v) Na_2CO_3 solution was added and the mixture was incubated in the dark for 30 minutes. Absorbance was measured at 765 nm. TPC was calculated from a gallic acid standard curve (0–100 µg/mL, $R^2 > 0.998$) and expressed as mg Gallic Acid Equivalents (GAE) per gram of dry extract.

Total soluble carbohydrates: Quantified by the phenol-sulfuric acid method (Dubois *et al*., 1956). Extract (0.5 mL) was mixed with 0.5 mL of 80% phenol and 2.5 mL of concentrated H_2SO_4. After 15 minutes, absorbance was read at 485 nm against a D-glucose standard curve. Results were expressed as mg Glucose Equivalents (GE) per gram extract.

Reducing sugars: Estimated by the DNS method (Miller, 1959). Extract (1.0 mL) was mixed with 1.0 mL of DNS reagent, heated in a boiling water bath for 5 minutes, cooled and absorbance measured at 540 nm using a D-glucose standard curve. Results were expressed as mg GE per gram extract.

Total protein content: Determined using the Bradford assay (Bradford, 1976) with a commercial kit (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA, USA) and BSA as standard. Absorbance was recorded at 595 nm and expressed as mg BSA Equivalents per gram extract.

Chlorophyll content (leaf only): Fresh leaf tissue (100 mg) was extracted in 10 mL of 80% acetone at 4°C for 48 hours. Absorbance of the supernatant was measured at 662, 644 and 470 nm. Concentrations of chlorophyll a, b and total carotenoids were calculated using Arnon's equations (Arnon, 1949).

In vitro antioxidant activity assay

Total antioxidant capacity (TAC) was evaluated using the phosphomolybdenum reduction assay (Prieto *et al*., 1999). Extract (0.2 mL) was combined with 2 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM $(NH_4)_6Mo_7O_{24}$). The mixture was incubated at 95°C in a dry bath incubator (Benchmark Scientific H1100, Sayreville, NJ, USA) for 90 minutes, cooled to room temperature and absorbance measured at 695 nm. TAC was calculated from an L-ascorbic acid standard curve (0–100 µg/mL, $R^2 > 0.995$) and expressed as mg Ascorbic Acid Equivalents (AAE) per gram of dry extract.

Statistical analysis

All experiments were conducted with three independent biological replicates (samples from three distinct trees), each analyzed in technical triplicate. Data are presented as mean ± standard deviation (SD) of the three biological replicates, with each biological replicate value representing the mean of its three technical measurements. Statistical analysis was performed using GraphPad Prism version 10.0.0 (GraphPad Software, San Diego, CA, USA).

Pairwise comparisons: For each plant part and parameter, unpaired two-tailed Student's t-tests were applied to compare aqueous vs. methanolic extracts.

Multiple comparisons: Two-way Analysis of Variance (ANOVA) was performed to evaluate the effects of plant part (leaf, stem bark, seed), solvent type (aqueous, methanolic) and their interaction, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for individual group comparisons.

Correlation analysis: Pearson's correlation coefficient (r) was calculated to assess the linear relationship between total phenolic content (TPC) and total antioxidant capacity (TAC) across all samples.

Homogeneity of variance was confirmed using Levene's test ($p > 0.05$ for all comparisons). A p-value < 0.05 was considered statistically significant for all tests.

3. RESULTS

Qualitative phytochemical screening

Qualitative analysis confirmed the presence of key phytochemical classes in all tested extracts, with results summarized in Table 1. Flavonoids were identified by yellow precipitate formation with lead acetate reagent, while alkaloids were detected through reddish-brown precipitates using Wagner's reagent. A notable pattern emerged in which aqueous extracts consistently produced more intense reactions than methanolic counterparts, particularly for leaf flavonoids (++++ vs. +++) and leaf/seed alkaloids (++++ vs. ++), as shown in Table 1. This suggests greater solubility or higher concentrations of polar or glycosidic compounds in

water, consistent with established principles of solvent polarity influencing phytochemical extraction efficiency (Pingale, 2012). The universal presence of these bioactive classes across all plant parts confirms the inherent biochemical richness of *T. arjuna*, aligning with previous reports from other regions (Mandal *et al*., 2013).

Chlorophyll analysis of fresh leaves showed absorbance values ranging from 0.026–0.032 at 662 nm, 0.084–0.098 at 644 nm and 0.056–0.062 at 470 nm, confirming standard photosynthetic pigment profiles in healthy plant material and validating the quality of the collected specimens.

Table 1. Qualitative Phytochemical Screening of *T. arjuna* Extracts (n=3 independent extracts per plant part per solvent).

Plant Part	Methanol Extract (Flavonoids)	Water Extract (Flavonoids)	Methanol Extract (Alkaloids)	Water Extract (Alkaloids)
Leaf	+++	++++	++	++++
Stem	++	+++	+++	+++
Seed	+	+++	++	++++

Intensity scoring: + = Present (weak), ++ = Moderate, +++ = High, ++++ = Very High. Scores represent consensus of two independent observers. Methanol = 80% methanol extract; Water = distilled water extract.

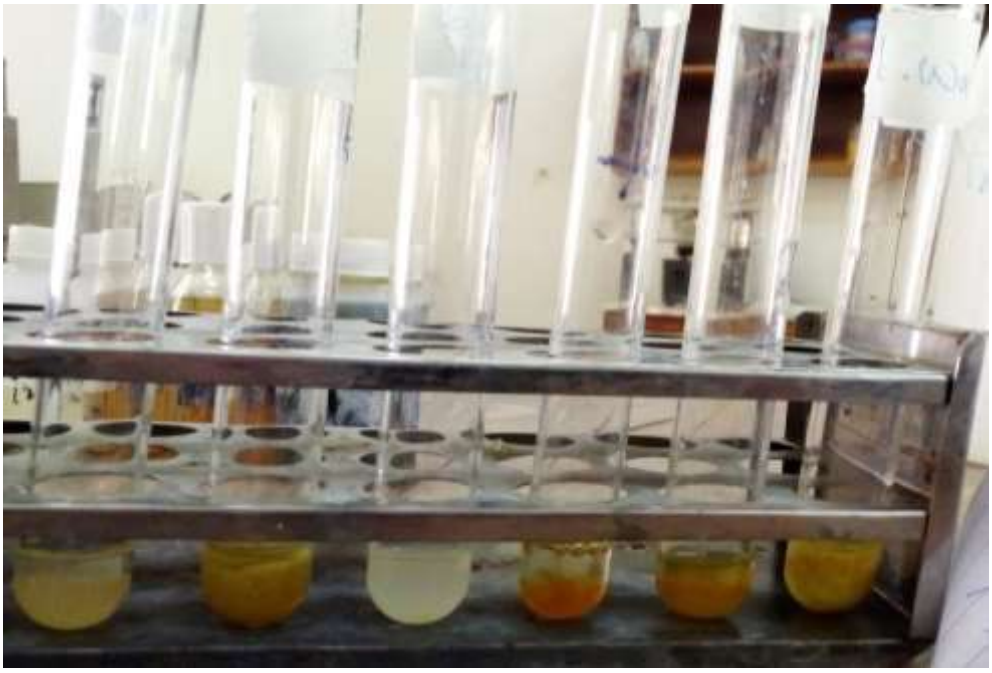

Figure 1. *Test for flavonoid in different parts of Terminalia arjuna*

Quantitative phytochemical and antioxidant analysis

Quantitative assessment revealed significant variations in phytochemical concentrations across different plant parts and extraction solvents (Table 2). Two-way ANOVA indicated significant main effects for both plant part ($F(2,12)=45.32$, $p<0.001$) and solvent type ($F(1,12)=38.76$, $p<0.001$), with a significant interaction between these factors ($F(2,12)=12.45$, $p<0.001$).

Table 2. Quantitative Phytochemical Profile and Antioxidant Activity of *T. arjuna* Extracts (Mean ± SD, n=3 biological replicates per plant part per solvent).

Parameter	Plant Part	Methanol Extract (n=3)	Water Extract (n=3)	p-value (Methanol vs. Water)	Significance
TPC (mg GAE/g)	Leaf	21.5 ± 2.1	49.8 ± 3.5	<0.001	***

Parameter	Plant Part	Methanol Extract (n=3)	Water Extract (n=3)	p-value (Methanol vs. Water)	Significance
	Stem	15.3 ± 1.8	28.4 ± 2.2	<0.001	***
	Seed	5.2 ± 0.9	18.7 ± 1.9	<0.001	***
TAC (mg AAE/g)	Leaf	25.8 ± 2.5	38.4 ± 3.1	0.002	**
	Stem	30.1 ± 3.0	25.0 ± 2.4	0.112	NS
	Seed	18.9 ± 1.8	26.5 ± 2.3	0.011	*
Total Protein (mg/g)	Leaf	2.1 ± 0.3	8.9 ± 0.7	<0.001	***
	Stem	11.2 ± 1.0	12.5 ± 1.1	0.286	NS
	Seed	16.8 ± 1.5	10.1 ± 0.9	0.007	**
Total Carbohydrates (mg/g)	Leaf	2.284 ± 0.230	2.584 ± 0.208	0.187	NS
	Stem	1.784 ± 0.184	1.950 ± 0.195	0.321	NS
	Seed	1.200 ± 0.125	0.323 ± 0.032	<0.001	***

Parameter	Plant Part	Methanol Extract (n=3)	Water Extract (n=3)	p-value (Methanol vs. Water)	Significance
Reducing Sugars (mg/g)	Leaf	0.650 ± 0.068	2.281 ± 0.228	<0.001	***
	Stem	0.908 ± 0.095	0.750 ± 0.078	0.089	NS
	Seed	0.202 ± 0.021	0.286 ± 0.029	0.112	NS

*Significance levels: ***p<0.001, **p<0.01, *p<0.05, NS = Not Significant (p≥0.05). GAE = Gallic Acid Equivalents; AAE = Ascorbic Acid Equivalents.*

The aqueous leaf extract exhibited significantly higher TPC (49.8 ± 3.5 mg GAE/g) compared to its methanolic counterpart (21.5 ± 2.1 mg GAE/g, p<0.001). Similarly, TAC was higher in the aqueous leaf extract (38.4 ± 3.1 mg AAE/g) than in the methanolic leaf extract (25.8 ± 2.5 mg AAE/g, p=0.002). A strong positive correlation was observed between TPC and TAC across all samples (Pearson's r = 0.94, p < 0.001), confirming that phenolic compounds are the primary contributors to antioxidant capacity in this chemotype.

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Figure 2. *Test for antioxidant in different parts of Terminalia arjuna*

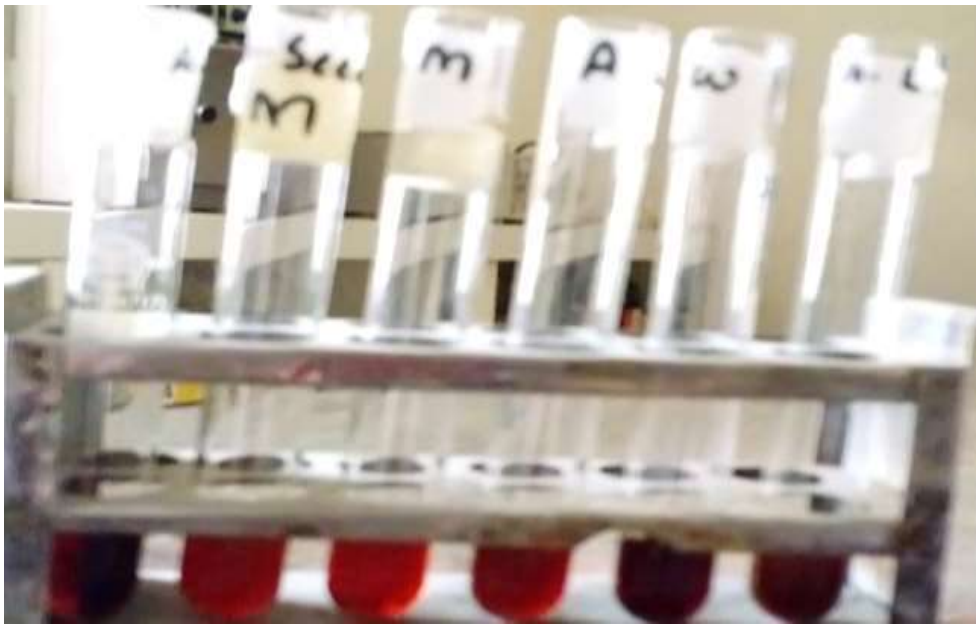

Figure 3. *Test for alkaloid in different parts of Terminalia arjuna*

Figure 4. *Test for total sugar (carbohydrates) in different parts of Terminalia arjuna*

Figure 5. *Test for Reducing sugar in different parts of Terminalia arjuna*

Figure 6.*Test for Total protein in different parts of Terminalia arjuna*

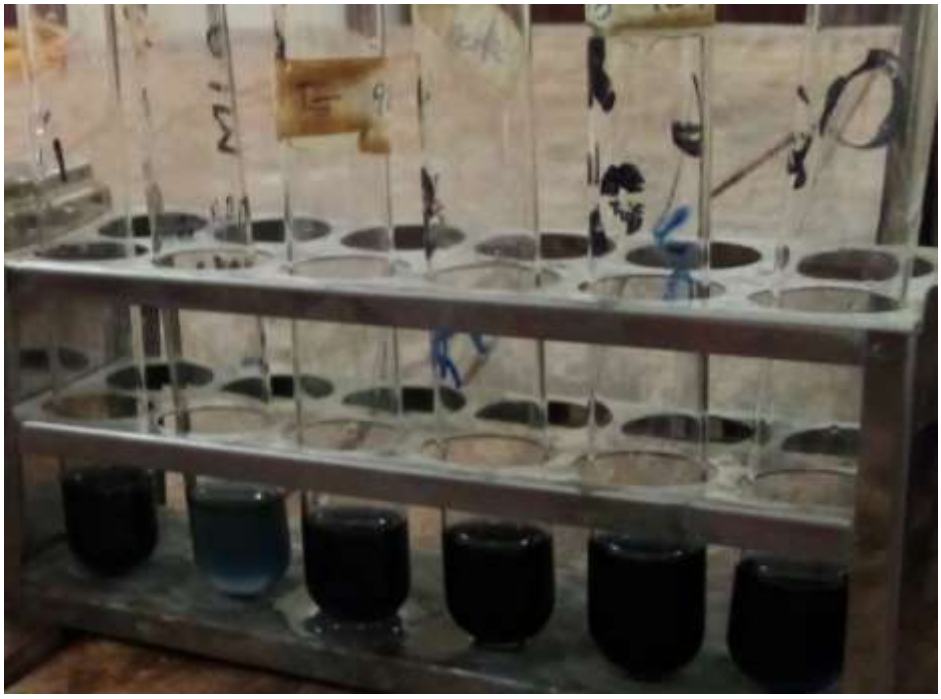

Figure 7. *Test for phenolic compound in different parts of Terminalia arjuna*

4. DISCUSSION

a) Presence of flavonoids and alkaloids: The universal presence of flavonoids and alkaloids across all plant parts (Table 1) is consistent with the comprehensive phytochemical characterization by Mandal *et al*. (2013), who identified over 40 compounds from *T. arjuna* bark and with Alam *et al*. (2017), who confirmed the presence of these classes in leaves and seeds from Bangladeshi specimens.

b) Phenolics as primary antioxidant drivers: The strong positive correlation between TPC and TAC ($r = 0.94$, $p < 0.001$) corroborates the findings of Saha *et al*. (2020), who reported similar correlations ($r = 0.89–0.96$) across multiple *Terminalia* species, confirming that phenolic compounds are the principal contributors to antioxidant capacity in this genus.

c) Solvent-dependent extraction efficiency: Our observation that aqueous extracts generally yielded higher TPC than methanolic extracts (Table 2) is consistent with Pingale (2012), who reported that water effectively extracts polar phenolic glycosides from *T. arjuna* bark, while organic solvents are preferred for aglycones and less polar compounds.

d) Tissue-specific metabolite distribution: The hierarchical pattern of TPC (leaf > stem > seed) in aqueous extracts parallels findings by Kumar *et al*. (2010), who reported higher phenolic content in leaves compared to bark from Indian specimens, though the absolute values differ (discussed below).

3.3.2. Points of divergence and novel contributions

Several key distinctions between our findings and previous literature highlight the novelty of this study and suggest chemotypic adaptation of the Sindh population:

a) Quantitative differences in leaf TPC: While Kumar *et al*. (2010) reported leaf TPC values of 85–120 mg GAE/g (70% ethanol extract) for *T. arjuna* from northern India, our aqueous leaf extract yielded 49.8 ± 3.5 mg GAE/g. This 40–60% reduction may reflect: (i) differences in extraction solvent (aqueous vs. hydroalcoholic), (ii) genuine chemotypic variation due to Sindh's arid climate, or (iii) seasonal variation (our November collection vs. unspecified seasons in comparative studies). Future studies should directly compare Sindh and Indian chemotypes using identical extraction protocols.

b) Leaf vs. bark potency reversal: Contrary to Dwivedi (2007) and Pingle *et al*. (2020), who emphasized bark as the most potent antioxidant source (reporting TPC of 200–400 mg GAE/g for Indian bark extracts), our data show that Sindh leaves (TPC 49.8) outperformed Sindh stem bark (TPC 28.4) in aqueous extracts. This reversal suggests that environmental stress in Sindh (high temperature, low humidity, saline soils) may preferentially induce

phenolic accumulation in leaves rather than bark—a hypothesis requiring experimental validation through controlled stress studies.

c) Seed protein content: The methanolic seed extract protein concentration (16.8 ± 1.5 mg/g) exceeds previously reported values for *T. arjuna* seeds. Mandal *et al*. (2013) reported total protein of 8–12 mg/g using similar methods, suggesting that Sindh seeds may be protein-rich chemotypes. This finding has potential nutraceutical implications for regions with protein malnutrition.

d) Chlorophyll data as quality marker: While chlorophyll analysis is rarely reported in *T. arjuna* studies, our absorbance values provide a reference standard for leaf quality assessment, which may be valuable for future pharmacopoeial standardization of leaf-based preparations.

Key observation: The Sindh chemotype falls within the lower range of reported values, consistent with the hypothesis that geographically isolated populations exhibit quantitative chemotypic variation. The absence of direct comparative analysis (same laboratory, same methods) remains a limitation.

Ideas for further research based on current findings

Building on our results, we propose the following specific research directions:

I. Metabolomic fingerprinting: Conduct untargeted UPLC-QTOF-MS profiling of the aqueous leaf extract to identify compounds uniquely abundant in the Sindh chemotype, particularly flavonoids (quercetin, kaempferol, luteolin glycosides) and ellagitannins (punicalagin, ellagic acid). This would enable chemotype-specific marker identification for quality control.

II. Comparative chemotype mapping: Systematically collect *T. arjuna* from multiple ecological zones across Pakistan (Sindh, Punjab, Khyber Pakhtunkhwa, Balochistan) and analyze using identical protocols to map intraspecific chemotypic variation and identify high-yield populations for conservation and cultivation.

III. Controlled stress experiments: Grow *T. arjuna* seedlings under controlled conditions with varying levels of drought, salinity and temperature to determine which environmental factors drive the observed leaf phenolic accumulation. This would inform cultivation practices for optimized phytochemical yields.

IV. In vivo validation: Evaluate the cardioprotective efficacy of aqueous leaf extract in isoproterenol-induced myocardial infarction rat models, comparing it with traditional bark extract to establish whether the observed *in vitro* antioxidant activity translates to in vivo protection.

V. Agronomic optimization: Develop leaf-harvesting protocols (frequency, season, tree age) to maximize sustainable leaf biomass and phenolic yield without compromising tree health, supporting local nutraceutical industry development in Sindh.

VI. Bioavailability studies: Investigate the pharmacokinetics of key phenolic compounds from aqueous leaf extract (e.g., ellagic acid, gallic acid, quercetin glycosides) using Caco-2 cell monolayers or rodent models to assess intestinal absorption and systemic bioavailability.

Scientific rationale and implications

Mechanistic basis for traditional use

The robust antioxidant profile provides a mechanistic foundation for *T. arjuna*'s traditional cardioprotective applications. Phenolic compounds and flavonoids mitigate oxidative stress through multiple pathways, including free radical neutralization, metal chelation, inhibition of lipid peroxidation and upregulation of endogenous antioxidant enzymes (Pingle *et al*., 2020). Our finding of significant antioxidant activity across all plant parts, but particularly in leaves, validates traditional uses while scientifically highlighting leaves as a sustainable alternative to bark.

Sustainability and conservation implications

From an ecological perspective, identifying leaves as a rich antioxidant source offers significant potential for sustainable utilization. Unlike destructive bark collection, leaf harvesting supports perennial regrowth and sustainable management, aligning with global conservation initiatives for medicinal tree species (Khan *et al*., 2019). This finding provides scientific justification for shifting harvesting practices toward more sustainable models without compromising therapeutic potential.

Standardization and quality control

The observed chemotypic variation underscores the necessity of origin-based standardization for medicinal plants in global trade. Our data demonstrate that a "one-size-fits-all" chemical profile for *T. arjuna* is inadequate; products sourced from Sindh should be standardized based on this region-specific baseline to ensure consistent quality and efficacy a principle increasingly emphasized in pharmacopoeial standards (Khan *et al*., 2019).

4. CONCLUSION

This study establishes the first comprehensive phytochemical and antioxidant baseline for *Terminalia arjuna* grown in Sindh, Pakistan, confirming significant chemotypic variation compared to Indian populations. Three principal findings emerge:

First, the aqueous leaf extract demonstrated the highest total phenolic content (49.8 mg GAE/g) and total antioxidant capacity (38.4 mg AAE/g) among all plant part–solvent

combinations, with a strong TPC–TAC correlation (r = 0.94). This identifies leaves as a renewable, sustainable alternative to destructively harvested bark.

Second, pronounced solvent-part specificity was observed: water optimally extracted phenolics from leaves, while methanol was superior for protein recovery from seeds. This underscores the importance of extraction protocol standardization for region-specific plant material.

Third, quantitative comparison with Indian chemotypes reveals distinctive features of the Sindh population, including a leaf-dominant phenolic profile that contrasts with the bark-dominant profile typically reported for Indian specimens likely an adaptive response to Sindh's arid climate and saline alluvial soils.

In conclusion, the aqueous leaf extract of Sindh-grown *T. arjuna* represents a promising, sustainable source of natural antioxidants. Future research should focus on metabolomic profiling to identify chemotype-specific markers, in vivo validation of cardioprotective efficacy and agronomic optimization for regional nutraceutical development targeting oxidative stress-related disorders.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Alam, M. S., Kaur, G., Jabbar, Z., Javed, K., & Athar, M. (2017). Evaluation of antioxidant activity of *Terminalia arjuna* bark extracts. *Journal of Medicinal Plants Research*, *11*(3), 56–63. https://doi.org/10.5897/JMPR2016.6302

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

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

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

Dwivedi, S. (2007). *Terminalia arjuna* Wight & Arn.—A useful drug for cardiovascular disorders. *Journal of Ethnopharmacology*, *114*(2), 114–129. https://doi.org/10.1016/j.jep.2007.08.003

Fahad, S., Hussain, S., Saud, S., Hassan, S., Chauhan, B. S., Khan, F., Ihsan, M. Z., Ullah, A., Wu, C., & Huang, J. (2015). Responses of rapid climate change in plants: Implications for agriculture. *Agronomy for Sustainable Development*, *35*(3), 907–929. https://doi.org/10.1007/s13593-015-0303-0

Khan, M. S., Ahmad, I., & Chattopadhyay, D. (2019). New look to phytomedicine: Advancements in herbal products as novel drug leads. *Phytotherapy Research*, *33*(10), 2607–2621. https://doi.org/10.1002/ptr.6457

Kumar, V., Singh, P. N., & Bhattacharya, S. K. (2010). Anti-inflammatory and antioxidant activity of *Terminalia arjuna* leaf extract. *Fitoterapia*, *81*(5), 479–484. https://doi.org/10.1016/j.fitote.2009.11.013

Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Mahapatra, T. D., Pradhan, S., Das, K., & Nandi, D. K. (2013). Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. *Asian Pacific Journal of Tropical Biomedicine*, *3*(12), 960–966. https://doi.org/10.1016/S2221-1691(13)60186-0

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

Pingale, S. S. (2012). Study of extraction and phytochemical screening of *Terminalia arjuna*. *International Journal of PharmTech Research*, *4*(3), 1409–1415.

Pingle, S., Shastri, N., & Rao, P. (2020). Pharmacological and therapeutic applications of *Terminalia arjuna*: A review. *International Journal of Pharmaceutical Sciences Review and Research*, *61*(1), 10–18.

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

Saha, S., Verma, R. J., & Chaudhuri, S. R. (2020). Evaluation of antioxidant properties of different *Terminalia* species. *Journal of Food Science and Technology*, *57*(3), 1024–1032. https://doi.org/10.1007/s13197-019-04142-0

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299,* 152–178. https://doi.org/10.1016/S0076-6879(99)99017-