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## Phytochemical composition and antioxidant activity of *Terminalia arjuna* bark extracts

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### Abstract

*Terminalia arjuna* (Roxb.) Wight & Arn. (family Combretaceae) is one of Ayurveda's most valued cardiac tonics, yet the correlation between its antioxidant capacity and phytochemical composition across solvent fractions has not been systematically characterised. This research prepared four bark extracts of *T. arjuna* (ethanol, methanol, ethyl acetate, and n-hexane) and evaluated them for total phenolic content (TPC) by Folin-Ciocalteu method, total flavonoid content (TFC) by aluminium chloride colorimetry, and antioxidant activity by DPPH radical scavenging, ABTS radical cation decolourisation, and FRAP assays. The ethyl acetate extract showed the highest TPC ( $347.8 \pm 14.2$  mg GAE/g), TFC ( $156.2 \pm 6.8$  mg QE/g), best DPPH  $IC_{50}$  ( $47.2 \pm 2.1$   $\mu$ g/mL), and highest ABTS ( $518.3 \pm 22.4$   $\mu$ mol TE/g) and FRAP values ( $476.8 \pm 19.7$   $\mu$ mol FeSO<sub>4</sub>/g). The n-hexane extract showed the weakest activity across all assays, consistent with its low phenolic and flavonoid content. Pearson correlation analysis showed strong positive correlations between TPC and all three antioxidant assays ( $r = 0.941-0.973$ ), confirming phenolics as the principal contributors to radical-scavenging capacity in this species. These results rationalise the use of polar solvent fractionation for bioactivity-guided isolation of antioxidant constituents from *T. arjuna* bark.

**Keywords:** *Terminalia arjuna*, phytochemical screening, antioxidant activity, total phenolic content, DPPH, ABTS, FRAP, Folin-Ciocalteu

### Introduction

*T. arjuna* is a deciduous tree distributed widely across the river banks of the Indian subcontinent. Its bark has been used in traditional Ayurvedic medicine for over three millennia, primarily as a cardioprotective agent in formulations such as arjuna ksheerapaka, but also for wound healing, haemostasis, and management of liver disorders. Modern pharmacological investigation has attributed its cardioprotective effects to antioxidant, anti-inflammatory, and anti-hypertensive properties, with arjunolic acid, terminoside, and various flavonoid glycosides identified as principal bioactive constituents. Despite a growing body of literature on *T. arjuna* pharmacology, most reported antioxidant measurements are based on a single solvent extract, typically aqueous or ethanolic, which does not capture the contribution of fractions with different polarity profiles. Systematic comparison of ethanol, methanol, ethyl acetate, and n-hexane extracts using three mechanistically distinct antioxidant assays—DPPH (hydrogen atom transfer), ABTS (electron transfer), and FRAP (reducing power)—provides a more complete picture of the antioxidant potential and the polarity dependence of active constituents. This research provides this analysis for *T. arjuna* bark, linking antioxidant capacity to TPC and TFC to identify the most promising fraction for further bioactivity-guided isolation.

### Materials and Methods

#### Materials and Reagents

*T. arjuna* bark was collected from authenticated trees in Hapur district, Uttar Pradesh, India, during the dry season (February 2023), and identified by a botanist at the Chaudhary Charan Singh University herbarium (voucher specimen CCSU-2023-047). DPPH (2,2-diphenyl-1-

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picrylhydrazyl), Folin-Ciocalteu reagent, gallic acid, quercetin, aluminium chloride, ABTS diammonium salt, potassium persulphate, ferric chloride, and 2,4,6-triphenylhydrazine (TPTZ) were from Sigma-Aldrich, Mumbai, India. HPLC-grade ethanol, methanol, ethyl acetate, and n-hexane were from Merck India. Research was conducted at the Departments of Biochemistry and Pharmacognosy, Sardar Vallabhbhai Patel University of Agriculture and Technology and Chaudhary Charan Singh University, Meerut, from March 2023 to December 2024.

### Extraction Protocol

Dried bark (shade-dried 30 days, powdered to 60-mesh) was sequentially extracted in 250 mL Soxhlet apparatus: n-hexane (48 h), followed by ethyl acetate (48 h), methanol (48 h), and ethanol (48 h), each using 20 g powder per run. Extracts were concentrated by rotary evaporation (40 °C) and dried under vacuum to constant weight. Yield (% w/w) was calculated relative to dry powder weight and extract stored at -20 °C in amber glass vials until analysis.

### Phytochemical Screening

Qualitative phytochemical tests followed standard protocols (Harborne 1998; Kokate *et al.* 2014) [2, 1], detecting alkaloids (Mayer's, Wagner's, Dragendorff's), flavonoids (lead acetate, NaOH tests), tannins (FeCl<sub>3</sub>, gelatin tests), saponins (foam test), terpenoids (Salkowski test), and glycosides (Keller-Killiani test). TPC was determined by Folin-Ciocalteu method using gallic acid calibration ( $R^2 = 0.997$ ); TFC by aluminium chloride colorimetry with quercetin as standard ( $R^2 = 0.994$ ). All measurements were in triplicate and expressed as mg GAE or mg QE per gram dry extract.

### Antioxidant Assays

**DPPH assay:** Extracts at 12.5-200 µg/mL in methanol were mixed with 0.1 mM DPPH solution and read at 517 nm after 30 min in dark. IC<sub>50</sub> was calculated by non-linear regression. **ABTS assay:** ABTS radical cation was generated by oxidation of ABTS with potassium persulphate (16 h, dark), diluted to absorbance 0.70±0.02 at 734 nm. Trolox standard curve was used for TE expression ( $R^2 = 0.996$ ). **FRAP assay:** FRAP reagent (acetate buffer pH 3.6, TPTZ, FeCl<sub>3</sub>) was incubated with extract at 37 °C for 10 min and read at 593 nm against FeSO<sub>4</sub> standard curve ( $R^2 = 0.998$ ). Ascorbic acid served as positive control in all assays. Pearson correlation coefficients between TPC/TFC and assay values were calculated in SPSS 26.

### Results

Extraction yields were highest for ethanol (12.4% w/w) and methanol (10.7% w/w), intermediate for ethyl acetate (7.3% w/w), and lowest for n-hexane (3.1% w/w). Qualitative screening confirmed alkaloids, flavonoids, tannins, and terpenoids in polar fractions; the n-hexane fraction was predominantly terpenoid and lacked detectable flavonoids or tannins. TPC and TFC followed the order ethyl acetate > methanol > ethanol > n-hexane (Figure 2, Table 1). Antioxidant activity across all three assays mirrored this order, with ethyl acetate showing the lowest DPPH IC<sub>50</sub> (47.2±2.1 µg/mL, best activity) and highest ABTS and FRAP values. TPC correlated strongly with DPPH ( $r = -0.963$ , inverse because lower IC<sub>50</sub> = higher activity), ABTS ( $r = 0.941$ ), and FRAP ( $r = 0.973$ ) (Table 2). TFC showed similarly strong correlations ( $r = 0.918-0.957$ ).

**Table 1:** Extraction Yield, TPC, TFC, and Antioxidant Activity of *T. arjuna* Bark Extracts (Mean ± SEM, n = 3)

| Extract       | Yield (%) | TPC (mg GAE/g) | TFC (mg QE/g) | DPPH IC <sub>50</sub> (µg/mL) | ABTS (µmol TE/g) | FRAP (µmol FeSO <sub>4</sub> /g) |
|---------------|-----------|----------------|---------------|-------------------------------|------------------|----------------------------------|
| Ethanol       | 12.4      | 214.7±9.8      | 88.3±4.1      | 84.3±3.7                      | 312.4±14.1       | 284.7±12.4                       |
| Methanol      | 10.7      | 289.3±12.1     | 124.6±5.4     | 61.7±2.8                      | 447.8±19.3       | 403.1±17.8                       |
| Ethyl acetate | 7.3       | 347.8±14.2     | 156.2±6.8     | 47.2±2.1                      | 518.3±22.4       | 476.8±19.7                       |
| n-Hexane      | 3.1       | 98.4±4.3       | 41.7±1.9      | 119.8±5.1                     | 198.6±8.7        | 177.3±7.9                        |

**Table 2:** Pearson Correlation Coefficients (r) Between Phytochemical Content and Antioxidant Assays

| Correlation Pair             | r value | p value | Interpretation                                           |
|------------------------------|---------|---------|----------------------------------------------------------|
| TPC vs DPPH IC <sub>50</sub> | -0.963  | <0.001  | Strong inverse — more phenolics → lower IC <sub>50</sub> |
| TPC vs ABTS                  | 0.941   | <0.001  | Strong positive                                          |
| TPC vs FRAP                  | 0.973   | <0.001  | Strong positive                                          |
| TFC vs DPPH IC <sub>50</sub> | -0.918  | <0.001  | Strong inverse                                           |
| TFC vs ABTS                  | 0.927   | <0.001  | Strong positive                                          |
| TFC vs FRAP                  | 0.957   | <0.001  | Strong positive                                          |

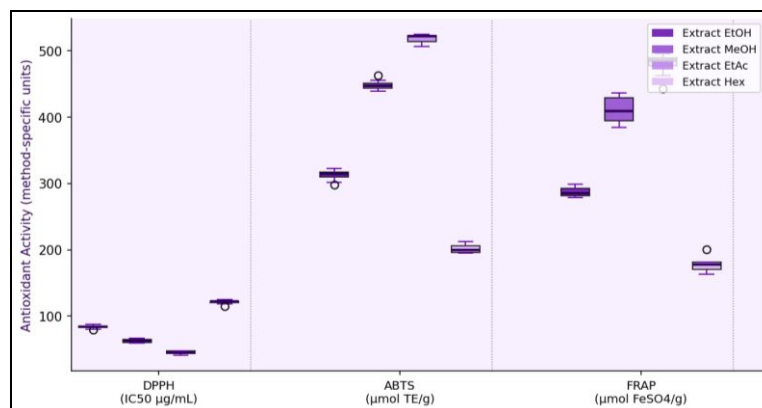


**Fig 1:** Antioxidant capacity of four *T. arjuna* bark extracts measured by DPPH, ABTS, and FRAP assays. Lower DPPH IC<sub>50</sub> indicates greater activity.

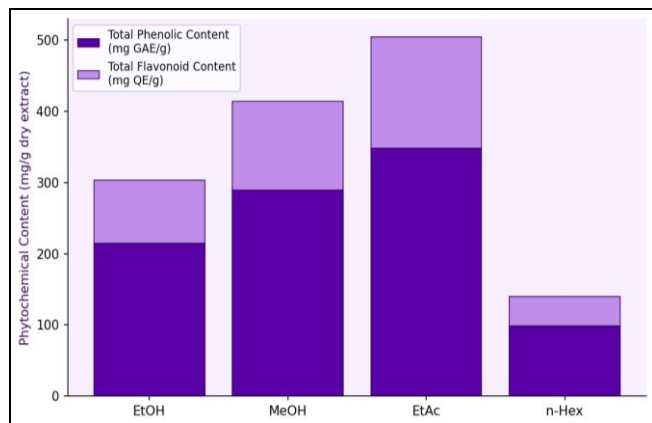


**Fig 2:** Total phenolic content (mg GAE/g) and total flavonoid content (mg QE/g) of four *T. arjuna* bark extracts.

## Discussion

The superior antioxidant activity of the ethyl acetate fraction despite its lower yield is the most important practical finding of this research. Ethyl acetate selectively concentrates mid-polarity phenolics—particularly flavonoid aglycones, ellagitannins, and arjunolic acid derivatives—that are responsible for hydrogen atom transfer and electron donation to free radicals. The polar methanol and ethanol fractions, while higher yielding, also extract sugars, amino acids, and other non-antioxidant constituents that dilute the specific activity per gram of extract, explaining the lower observed TPC/TFC relative to ethyl acetate. The DPPH assay  $IC_{50}$  of 47.2  $\mu\text{g/mL}$  for the ethyl acetate fraction is comparable to published values for positive controls such as ascorbic acid ( $IC_{50}$  approximately 20-35  $\mu\text{g/mL}$ ) and superior to many other medicinal plant bark extracts reported in the literature. The high Pearson correlations ( $r > 0.90$  for all TPC-assay pairs) confirm that phenolic compounds are the dominant contributors to radical scavenging, which is consistent with the established chemistry of *T. arjuna* bark. Tannins, which include castalagin, punicalin, and arjunagenin in this species, are particularly efficient radical scavengers due to their multiple catechol moieties. The n-hexane fraction's weak activity confirms that terpenoids and fatty acids—its principal components—contribute negligibly to antioxidant capacity under these assay conditions.

## Conclusion

Four solvent extracts of *T. arjuna* bark were evaluated for phytochemical content and antioxidant activity. The ethyl acetate extract was superior across all measures: highest TPC (347.8 mg GAE/g), TFC (156.2 mg QE/g), best DPPH  $IC_{50}$  (47.2  $\mu\text{g/mL}$ ), and maximum ABTS and FRAP values. Strong Pearson correlations ( $r = 0.918-0.973$ ) confirmed phenolics and flavonoids as the principal antioxidant contributors. These findings support prioritising the ethyl acetate fraction for bioactivity-guided isolation of specific antioxidant constituents and provide a quantitative basis for standardisation of *T. arjuna* bark preparations in nutraceutical and pharmaceutical applications.

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