The Journal of Phytopharmacology 2026; 15(1):20-25
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2026; 15(1): 20-25
January- February
Received: 13-11-2025
Accepted: 11-03-2026
Published: 28-03-2026

doi: 10.31254/phyto.2026.15103

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Analytical elucidation of phytoconstituents in *Terminalia arjuna* bark through HPTLC and GC-MS approaches

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ABSTRACT

Background: *Terminalia arjuna (Roxb.) Wight and Arn*., a well-documented medicinal plant, is traditionally acclaimed for its cardioprotective efficacy along with several other properties. **Objective**: The present study aimed to evaluate the phytochemical composition and characterize the bioactive constituents of the ethanolic extract of *T. arjuna* bark. **Materials and Methods**: The bark was authenticated, shade dried, powdered and extracted with ethanol using the cold maceration method. Qualitative phytochemical analysis was performed to reveal the presence of bioactive phytoconstituents. Quantification of the phytochemicals was carried out by HPTLC, along with the estimation of total phenols and total alkaloid content. **Results:** The percentage yield of the extract was found to be 17.15%. The qualitative phytochemical analysis of the *T. arjuna* bark extract tested positive for alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phytosterols, saponins, tannins and terpenoids. HPTLC analysis confirmed the presence of quercetin in the extract with a mean concentration of 96.07 µg/100 mg of the extract. GC-MS analysis identified 15 major components, providing phytochemical evidence supporting the plant's antioxidant and cardioprotective potential. **Conclusion**: The study provides scientific validation for therapeutic significance of *T. arjuna* bark and establishes a foundation for future phytopharmacological and formulation-based research.

Keywords: *Terminalia arjuna*, Phytochemical profile, Quercetin, HPTLC, GC-MS.

INTRODUCTION

India is renowned for its rich biodiversity harbouring around 8000 plant species recognized for its therapeutic properties [1]. Traditional healing systems such as siddha, Ayurveda continue to attract global attention owing to their multiconstituent nature, synergistic effect and minimal adverse effects compared to conventional medicine. Among the vast variety of medicinal flora, the family combretaceae comprises of nearly 200 species among the world. One among them is the species *Terminalia arjuna* (Roxb.) Wight and Arn., commonly known as 'Arjuna' native to India, Burma, Sri Lanka and Mauritius. It is predominantly found along the river banks and sub-Himalayan tracts [2]. Several ancient medicinal texts such as Charaka Samita, Sushruta Samhita and Astanga Hridayam have described the plant as an ayurvedic remedy. Several parts of the plant, *viz*., bark, leaves, fruits, roots have been extensively used for cardiopathy, fracture, ulcers, wound, headache, diabetes, anemia, cirrhosis, hematuria, skin diseases. The therapeutic use of stem bark in cardiac disorders was first advocated by the ancient physician, Vagabhatta, for which it is popularly entitled as 'guardian of heart' [3]. The bark of this plant has have demonstrated numerous pharmacological benefits, including inotropic, anti-ischemic, antioxidant, antihypertensive, antiplatelet, hypolipidemic, antiatherogenic, antihypertrophic, anti-inflammatory, anticancer and antimicrobial effects [2]. These pharmacological properties have been attributed to its rich phytochemical profile including triterpenoids, flavonoids, glycosides, tannins, saponins, phenolic constituents, sterols, minerals such as calcium, aluminium, zinc and aminoacids such as tryptophan, tyrosine, histidine and cysteine [2]. Recent research on herbal medicine primarily focuses on the characterization and standardization of previously established medicinal plants. Thus, the present study focuses on the phytochemical analysis and characterization of *T. arjuna* bark, aiming to correlate its chemical composition with its therapeutic potential, supporting its use in phytopharmacology.

MATERIAL AND METHODS

The Collection and authentication of bark

T. arjuna bark was procured from local traders in Namakkal and it was identified, duly authenticated by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai. The collected bark was shade dried, powdered and stored for further preparation of ethanolic extract.

Preparation of *Terminalia arjuna* bark extract

T. arjuna bark ethanolic extract was prepared as per the procedure followed by Dube *et al.* with slight modifications [4]. 50 g of powdered *T. arjuna* bark was mixed with 500 mL of ethanol and subjected to continuous agitation on a rotary shaker for 72 hours. The mixture was then filtered through Whatman filter paper No.1 and the filtrate was incubated at 45 °C for 24 h to facilitate solvent evaporation. The collected extract was stored in an airtight container for further analysis.

Estimation of yield percentage

The extraction yield of *Terminalia arjuna* bark ethanolic extract was calculated by the formula given below

$$\text{Extraction yield in percentage} = \frac{\text{Weight of the crude extract (g)}}{\text{Weight of the powdered bark (g)}} \times 100$$

Qualitative phytochemical analysis

Qualitative phytochemical analysis of *T. arjuna* bark extract was carried out to detect the presence of the active ingredients [5-7].

High performance thin layer chromatography analysis

The quercetin in *T. arjuna* bark extract was quantified using high performance thin layer chromatography based on the method of Patel *et al.* with slight modifications [8]. Standards and samples were prepared by dissolving them in methanol to known concentrations. Different concentrations of standards and sample were spotted on pre-coated silica gel 60F 254 HPTLC plates using a CAMAG Linomat V sample applicator. A 20 x 10 twin trough glass chamber was used, which had been saturated for 20 minutes at room temperature with the mobile phase (Toluene: Ethyl acetate: Formic acid in the ratio 10.9:8.7:0.4). After development, plates were sprayed with anisaldehyde sulphuric acid reagent and visualized under 254 nm and 366 nm wavelengths using visualizer. Scanning was done using a TLC scanner IV at a speed of 20 mm/s, with a slit dimension of 6 x 0.45 mm. Quercetin in *T. arjuna* bark extract was quantified using calibration curve based on retardation factor (Rf) values.

Estimation of total phenol content

Total phenol content was estimated by Folin-Ciocalteau reagent method [9]. *T. arjuna* bark extract was taken at the rate of 0.1 mg/mL and was mixed with 5 mL of Folin-Ciocalteau reagent (1:10 dilution with distilled water) and 4 mL of 7.5% sodium carbonate solution. The reaction mixture was incubated for 30 min at room temperature and the absorbance was measured at 765 nm using UV-Vis spectrophotometer. Total phenol content was calculated from standard curve generated using gallic acid at concentration of 10 - 60 µg/mL and expressed as mg of gallic acid equivalent (GAE) per g of extract.

Estimation of total alkaloid content

Alkaloid content was estimated according to Harborne *et al.* [10]. To 1.0 g of *T. arjuna* bark extract, 40 mL of 10 % acetic acid in ethanol was added and allowed to stand for 4 h. The mixture was filtered and concentrated to one fourth of its original volume by placing on water bath. Concentrated ammonium hydroxide was added drop by drop to the filtrate until the precipitation was complete. The solution was filtered and the precipitate was washed with diluted ammonium hydroxide, filtered again and dried. The residue was weighed and the alkaloid content was expressed in per cent.

Gas chromatography-mass spectroscopic (GC-MS) analysis

GC-MS analysis of *T. arjuna* bark extract was performed using Perkin Elmer Clarus 500 GC-MS. The apparatus consisted of an Elite-5 non-polar capillary column with dimensions of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness. Helium (purity of 99.9%) was used as a carrier gas at a flow rate of 1 mL/minute. The injector temperature was kept at 280 °C and the oven temperature was programmed as follows: initially set as 60°C, then increased gradually to 150 °C at a rate of 6 °C/min for 2 min, followed by 10 min hold, and finally increased by 4 °C/minute to 280 °C. Mass spectra fragments collected at 70 eV were scanned with a range of 40 to 450 Da. The obtained components were identified based on the National Institute of Standards and Technology library database.

RESULTS

Percentage yield

The present study was undertaken to screen, characterize and quantify the phytochemical constitution of *T. arjuna* bark ethanolic extract. The extract obtained was brown in colour with crystalline nature. The percentage yield of the ethanolic extract was found to be 17.15 % (w/w) with respect to dried bark powder.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis of *T. arjuna* bark ethanolic extract revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phytosterols, saponins, tannins and terpenoids, whereas protein was not detected (Figure 1). The results are presented in Table 1.

High performance thin layer chromatography analysis

After optimization of mobile phase, HPTLC fingerprinting of *T. arjuna* bark ethanolic extract was performed to identify and quantify the marker compound quercetin. The chromatogram visualized under UV light at 366 nm exhibited a distinct band at an Rf value of 0.53, which corresponded precisely to that of the standard quercetin (Rf = 0.53), confirming its presence in the extract. Quantitative estimation was carried out using the calibration curve of the standard compound and the concentration of the quercetin in the extract was calculated (Figure 2). The mean quercetin content in *T. arjuna* bark ethanolic extract was determined to be 96.07 µg per 100 mg of extract.

Estimation of total phenol content

The mean concentration of total phenols in the *T. arjuna* bark ethanolic extract was 45.13 ± 0.37 mg of GAE/g of the extract (Figure 3). This value was estimated with the standard curve obtained using gallic acid as the standard. The calibration curve exhibited good linearity with the regression equation $y = 0.0118x + 0.0244$ and a correlation coefficient of $R^2 = 0.977$, indicating its accuracy and prediction.

Estimation of total alkaloid content

The mean concentration of alkaloids calculated by precipitation method in ethanolic extract of *T. arjuna* bark extract was found to be 81.20 ± 2.08 mg/g of extract.

Table 1: Qualitative Phytochemical screening of *T. arjuna* bark ethanolic extract

S. No.	Phytochemical	Indication
1	Alkaloids	Present
2	Carbohydrates	Present
3	Cardiac glycosides	Present
4	Flavonoids	Present
5	Phenols	Present
6	Phytosterols	Present
7	Proteins	Absent
8	Saponins	Present
9	Tannins	Present
10	Terpenoids	Present

Table 2. Components detected in GC-MS analysis of *T. arjuna* bark ethanoilc extract

S. No.	RT Value	Name of the Compound	Chemical Formula	Molecular Weight	Area %
1	5.987	Catechol	C6H6O2	110	8.43
2	6.930	5-Hydroxymethylfurfural	C8H10O4	170	6.85
3	7.155	Cyclohexane, (1-methylethyl)-	C9H18	126	2.38
4	10.280	1-Tridecene	C13H26	182	7.71
5	10.482	Hexadecane, 1-chloro-	C16H33Cl	260	5.56
6	11.590	Cyclohexane, octyl-	C14H28	196	4.58
7	12.826	2,4-Di-tert-butylphenol	C14H22O	206	3.36
8	14.801	9-Eicosene, (E)-	C20H40	280	10.51
9	17.172	Dodecane, 2,6,11-trimethyl-	C15H32	212	2.48
10	19.764	Acenaphthene, 5-acetyl-	C14H12O	196	22.70
11	21.786	Cyclopentaneundecanoic acid, methyl ester	C17H32O2	268	4.38
12	22.441	8-Methylnonanoic acid	C10H20O2	172	3.38
13	23.114	Eicosanoic acid, ethyl ester	C22H44O2	340	9.50
14	25.077	2(1H)-Naphthalenone, octahydro-8a-methyl-, trans-	C11H18O	166	3.81
15	32.276	Diisooctyl phthalate	C24H38O4	390	4.37

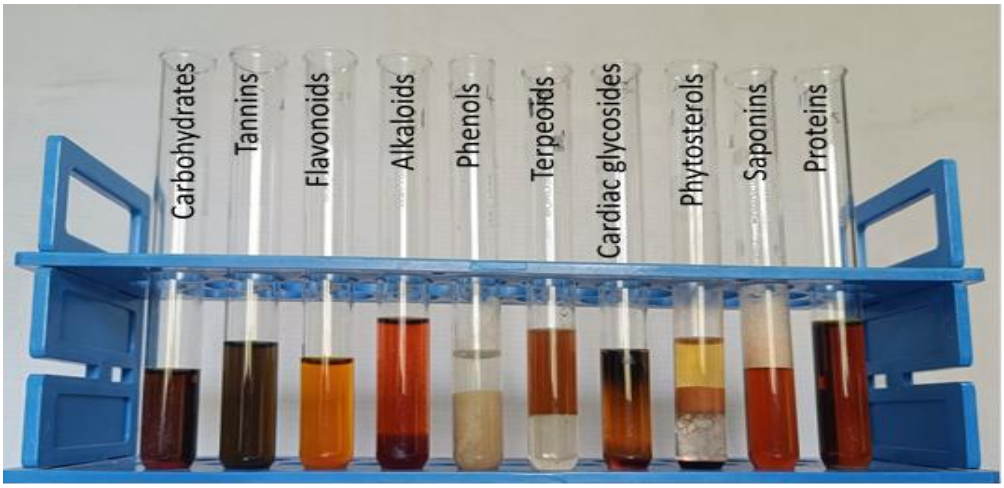
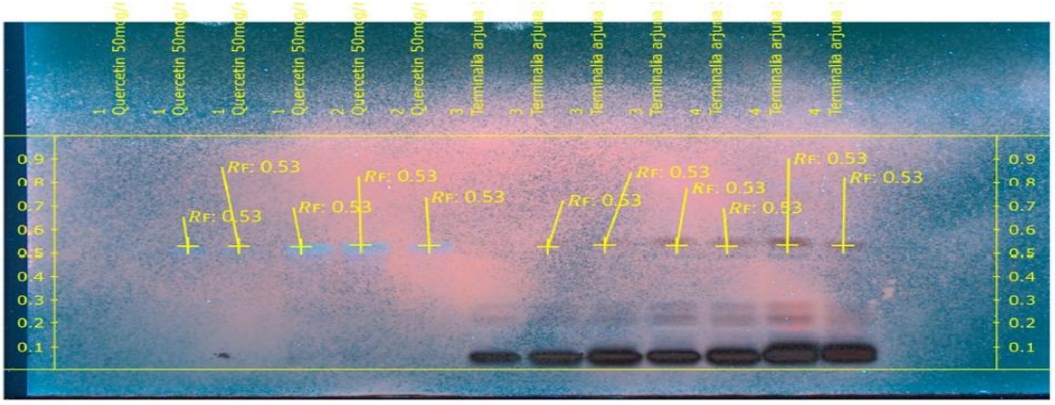
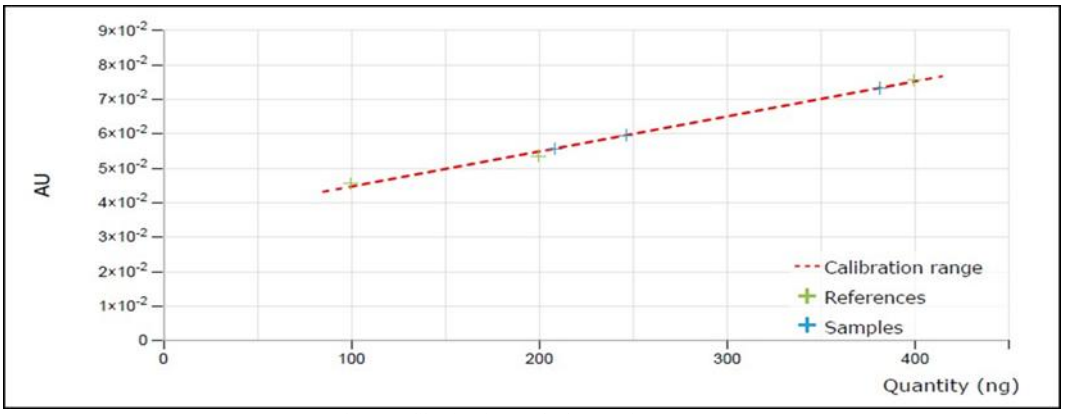


Figure 1: Phytochemical analysis of *T. arjuna* bark ethanolic extract



a) HPTLC fingerprint of *T. arjuna* bark extract and quercetin standard at 366 nm (Derivatized)



b) Calibration for standard quercetin at 366 nm

Figure 2: HPTLC quantification of quercetin in *T. arjuna* bark extract [a) HPTLC fingerprint and b) Calibration curve]

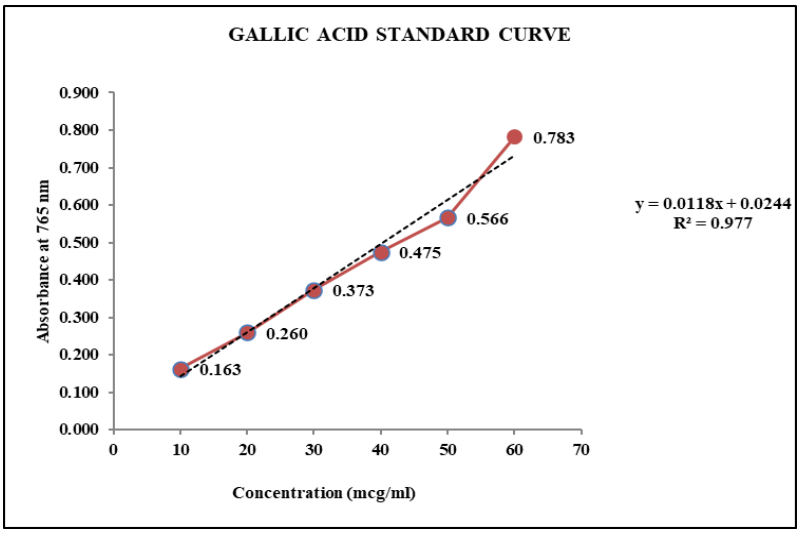


Figure 3: Standard curve for estimation of total phenol content in *T. arjuna* bark extract using gallic acid

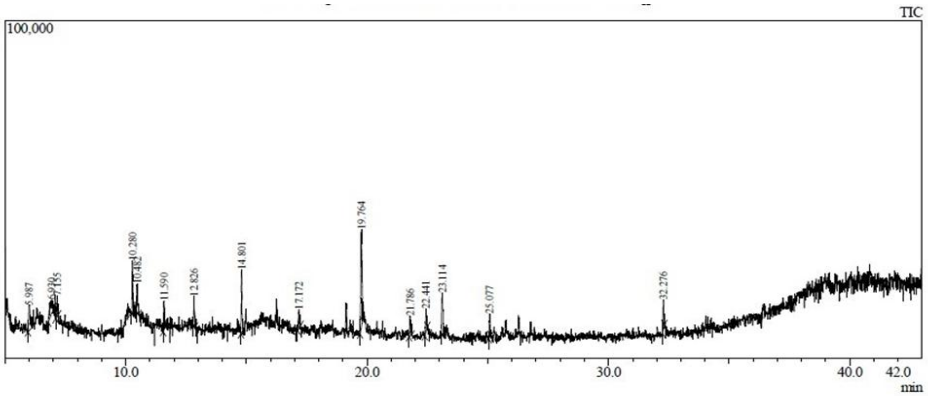


Figure 4: GC-MS Chromatogram of *Terminalia arjuna* bark extract

Gas chromatography-mass spectroscopic (GC-MS) analysis

The GC-MS analysis of ethanolic extract of *T. arjuna* bark ethanolic extract revealed the presence of 15 major compounds as depicted in Figure 4. The retention time, name, molecular formula, molecular weight, percentage area is presented in Table 2. Among them, catechol and 2,4-di-ter-butyl phenol exhibit noticeable pharmacological significance.

DISCUSSION

T. arjuna, commonly known as arjun, is a traditional medicinal plant widely distributed throughout the South Asian region. It exhibits a broad spectrum of biological activities, including antioxidant, antimutagenic, antidiabetic, antimicrobial effects and possesses significant cardioprotective potential [11]. The percentage yield of *T. arjuna* bark ethanolic extract (17.15%) obtained in our study is consistent with the findings of Shukla *et al*., who reported the percentage yield of hydroethanolic extract of *T. arjuna* was 18.5% of the dried powder [12]. In contrast, Akhter *et al.* [5] reported a relatively higher yield of 35.70%, which might be attributed to different extraction conditions [4]. Preliminary phytochemical analysis revealed presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phytosterols, saponins, tannins and terpenoids present in the ethanolic extract of *T. arjuna* bark. The secondary metabolites present in the plant materials are primarily responsible for their therapeutic properties. The findings are in agreement with those of Anamika *et al.* except for the presence of lactones [13]. Such variation in the phytochemical composition might correspond to differences in the geographical origin, soil type and climatic conditions. Quercetin, a natural flavonoid which is present in adequate amounts in *T. arjuna* bark extract as determined by HPTLC, is primarily responsible for its antioxidant, anti-inflammatory, cardioprotective, antihypertensive and anticancer properties. It scavenges reactive oxygen species, stabilizes cellular membranes and inhibits lipid peroxidation, thereby conferring protection against oxidative damage [14]. These mechanisms are relevant in conditions where oxidative stress and inflammatory cascade play a central role. The presence of phenolic compounds also contributes significantly to its therapeutic potential owing to its antioxidant, antidislipidaemic, hypocholesterolemic activity. By preventing oxidative modification of low-density lipoproteins and improving lipid profiles, phenolics further enhance the cardioprotective property of *T. arjuna* [15]. The alkaloid content obtained in the present study was considerably higher than that reported by Kamalla *et al*. [16]. The enhanced alkaloid yield underscores the therapeutic richness and pharmacological significance of *T. arjuna* bark. Further chemical characterization using GC-MS analysis demonstrated that the *T. arjuna* bark extract was rich in polyphenolic compounds such as catechol and 2,4-di-tert-butylphenol. These phenolic compounds are well recognized for their strong antioxidant activity, along with anti-inflammatory, anti-diabetic, anticancer and antimicrobial property [17, 18]. The presence of such bioactive molecules further substantiates the therapeutic relevance of *T. arjuna* and supports its use in managing oxidative stress mediated pathological conditions. Overall, the findings of the present study reinforce the pharmacological significance of *T. arjuna* bark as a rich source of bioactive phytochemicals. The combined presence of flavonoids, phenols, alkaloids and other secondary metabolites suggest a synergistic mechanism of action.

CONCLUSION

The present investigation substantiates the phytochemical richness of *T. arjuna* bark, confirming its potential source of therapeutically active compounds. The identification and quantification of bioactive components including phenols, flavonoids and alkaloids reaffirm the plant's multifaceted pharmacological relevance. This study bridges the empirical knowledge with scientific evidence, transforming age-old ethnopharmacological claims into quantifiable scientific data.

Acknowledgements

The authors are grateful to Tamil Nadu Veterinary and Animal Sciences University and Veterinary College and Research Institute, Namakkal for providing facilities to carry out the research.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

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REFERENCES

1. Anita B, Renganayaki V. Analysis of phytoconstituents present in *Terminalia arjuna* bark extract using spectroscopic techniques. Int J ChemTech Res. 2019;12(2):189-96.
2. Amalraj A, Gopi S. Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: A review. J Tradit Complement Med. 2016;7(1):65-78.
3. Dwivedi S, Chopra D. Revisiting *Terminalia arjuna* – an ancient cardiovascular drug. J Tradit Complement Med. 2014;4(4):224.
4. Dube N, Nimgulkar C, Bharatraj DK. Validation of therapeutic anti-inflammatory potential of Arjuna Ksheera Paka– a traditional Ayurvedic formulation of *Terminalia arjuna*. J Tradit Complement Med. 2016;7(4):414-20.
5. Akhter S, Hossain MD, Aminul H, Mohammad S, Mohiuddin B. Phytochemical screening, antibacterial, antioxidant and cytotoxic activity of the bark extract of *Terminalia arjuna*. Eur J Sci Res. 2012;86(4):543-52.
6. Biradar B, Sonawane B, Barge S, Kharade S. Antimicrobial activity and phytochemical analysis of orange (*Citrus aurantium* L.) and pineapple (*Ananas comosus* (L.) Merr.) peel extract. Ann Phytomed. 2016;5(2):156-60.
7. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th ed. London: Tindall and Co.; 1983. p.343-83.
8. Patel DK, Patel K, Dhanabal SP. Development of bioanalytical parameters for standardization of *Terminalia arjuna*. J Acute Dis. 2013;2(4):287-91.
9. Makkar HPS, Blummel M, Borowy NK, Becker K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agric. 1993;61:161-65.
10. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall Ltd.; 1973. p.49-188.
11. Zafar F, Jahan N, Asi MR, Zafar WU. Nanosuspension enhances dissolution rate and oral bioavailability of Terminalia arjunabark extract *in vivo* and *in vitro*. Asian Pac J Trop Biomed. 2020;10(4):164-71.
12. Shukla SK, Sharma SB, Singh UR. Pre-treatment with α-tocopherol and *Terminalia arjuna* ameliorates pro-inflammatory cytokines, cardiac and apoptotic markers in myocardial infarcted rats. Redox Rep. 2015;20(2):49-59.
13. Anamika S, Singh S, Banweer J, Samundre P. Phytochemical screening and antibacterial activity of *Terminalia arjuna*. JETIR. 2024;11(1):169-82.
14. Chen WJ, Cheng Y, Li W, Dong XK, Wei JL, Yang CH, *et al*. Quercetin attenuates cardiac hypertrophy by inhibiting

mitochondrial dysfunction through SIRT3/PARP-1 pathway. Front Pharmacol. 2021;12:739615.
15. Saha A, Pawar VM, Jayaraman S. Characterisation of polyphenols in *Terminalia arjuna* bark extract. Indian J Pharm Sci. 2012;74(4):339-347.
16. Kamalla R, Remya M, Ensha Lomiya MA, Raguvaran R, Jambagi KM, Dosar S. Qualitative and quantitative phytochemical analysis of *Terminalia arjuna*. Int J Adv Biochem Res. 2021;8:936-939.
17. Surana K, Ahire A, Bhawar S, Jeughale P, Aher K, Ahire C, *et al*. Catechol: important scaffold in medicinal chemistry. Medicopharmaceutica. 2023;1(1):47-57.
18. Zhao F, Wang P, Lucardi R, Su Z, Li S. Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. Toxins (Basel). 2020;12(1):35.

HOW TO CITE THIS ARTICLE
Keerthana S, Sankar P, Arivuchelvan A, Gopal K. Analytical elucidation of phytoconstituents in *Terminalia arjuna* bark through HPTLC and GC-MS approaches. J Phytopharmacol 2026; 15(1):20-25. doi: 10.31254/phyto.2026.15103