**RESEARCH PAPER**

# Standardizing Arjuna Extracts: HPLC, Phytochemical, and Antioxidant Analysis for Consistent Quality

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**ABSTRACT**
Objectives: The study was undertaken to develop and validate a reliable HPLC method for the quantitative estimation of the flavonoid quercetin in different extracts of Terminalia arjuna, with an emphasis on supporting quality evaluation and standardization of herbal formulations. Methods: The raw drug was procured, processed, and subjected to extraction to obtain aqueous, ethanolic, and milk-based decoction (Arjuna Ksheer Paka) extracts. Preliminary phytochemical screening was performed to identify major classes of phytoconstituents. Total phenolic and flavonoid contents were estimated using standard colorimetric methods, while antioxidant activity was assessed by $IC_{50}$ determination (DPPH assay). HPLC method development was carried out using a C18 column with a mobile phase consisting of 0.1% formic acid and acetonitrile (60:40 v/v), followed by method validation. Findings: Phytochemical analysis confirmed the presence of flavonoids, polyphenols, cardiac glycosides, and related constituents. Total phenolic content was found to be 142.46 ± 0.50, 482.47 ± 1.52, and 58.20 ± 5.77 mg GAE/100 g for aqueous, ethanolic, and milk-based extracts respectively, while total flavonoid content was 179.21 ± 0.62, 89.81 ± 7.23, and 51.23 ± 0.77 mg QE/g. Antioxidant activity carried out by DPPH assay showed $IC_{50}$ values of 33.77 ± 0.43, 17.19 ± 1.50, and 31.40 ± 1.95 µg/ml for the respective extracts. The validated HPLC method enabled accurate quantification of quercetin, which was found to be 2.10 ± 1.17 mg per 400 mg of extract. Novelty: The novelty of this study lies in the integration of a validated HPLC method for quercetin estimation with comparative phytochemical and antioxidant profiling of aqueous, alcoholic, and milk-based Terminalia arjuna extracts. Inclusion of the traditional Arjuna Ksheer Paka formulation offers a unique insight into classical preparations**..**

**Keywords**: Quercetin, HPLC, T.Arjuna, standardisation, Arjuna Ksheer Paka..
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## INTRODUCTION

*Terminalia arjuna* (Roxb.) Wight & Arn., commonly known as Arjuna, is an important medicinal tree indigenous to the Indian subcontinent and widely distributed in the tropical regions of Central and Southern India. It predominantly grows along riverbanks and in dry deciduous forests. Botanically belonging to the family Combretaceae, the tree attains a height of 20–25 m and is characterized by a spreading crown, smooth grey bark, oblong–conical fruits and small yellowish flowers. The stem bark is the most therapeutically valuable part and has been extensively described in classical Ayurvedic texts such as Charaka Samhita and Ashtanga Hridaya, where it is indicated for the management of cardiac disorders. Vagbhata was among the earliest scholars to recommend Arjuna bark powder for cardiovascular ailments, and it remains a key ingredient in several cardiotonic formulations used in Ayurveda today. (1,2)

Phytochemical investigations reveal that the bark of *T. arjuna* contains a wide array of bioactive constituents including flavonoids (quercetin, kaempferol), phenolic acids, hydrolysable tannins, triterpenoid saponins (arjunic acid, arjunolic acid), glycosides and phytosterols. (3) These compounds are responsible for its potent antioxidant, anti-inflammatory, hypolipidemic and cardioprotective properties. Contemporary pharmacological studies have demonstrated its efficacy in ischemic heart disease, hypertension, hyperlipidaemia and atherosclerosis, along with hepatoprotective, antithrombotic, antibacterial, antifungal and antiviral activities. (4-7) The antioxidant capacity of *T. arjuna* has also been well documented, suggesting its role in mitigating oxidative stress–mediated cardiovascular damage. (8)

Despite the substantial therapeutic promise, quality assurance of *T. arjuna*-based herbal formulations remains inadequate. Most published studies employ aqueous or hydro-alcoholic extracts without establishing validated standardization parameters, leading to batch-to-batch variability and compromised reproducibility. Regulatory agencies increasingly emphasize the need for chromatographic fingerprinting and marker-based quantification to ensure safety, efficacy and global acceptability of herbal medicines. (9,10) However, limited efforts have been directed toward the HPLC-based standardization of *T. arjuna* using reliable phytochemical markers such as quercetin. Quercetin being a biologically

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active compound exerts significant anti-oxidant potential thereby warranting its use as not just an analytical marker but also a biomarker.

The present study therefore aims to develop and validate an HPLC method for the standardization of *Terminalia arjuna* bark using quercetin as a marker compound, along with comprehensive physicochemical and phytochemical evaluation, thereby contributing toward improved quality control and scientific validation of this important cardioprotective medicinal plant.

**Methodology: -**

**Collection and authentication:** Dried pieces of *T.arjuna* bark were procured from Dawa Bazar, Mumbai, India.. The sample was authenticated by Dr. Harshad Pandit., former Head of Department of Botany, Guru Nanak Khalsa college, Mumbai (sample specimen #: sdk p 019215853).

**Morphological and microscopical evaluation:** Whole pieces as well as a powdered sample of *T.arjuna* bark was examined to study morphological and microscopical characters.

**Extraction:** Twenty grams of the powdered drug was initially defatted with 200 mL of petroleum ether for 24 hours. The defatted residue was subsequently extracted with 200 mL of distilled water, stirred thoroughly, and ultrasonicated for 20 minutes at 25 °C. The mixture was then filtered, and the insoluble residue was discarded. The filtrate obtained was concentrated on a steam bath, and the dried extract was retained. The percentage yield of the extract was calculated as the mean of 3 independent measurements (triplicate).

The ethanolic extract was prepared following the same procedure, with 95% ethanol used as the extraction solvent in place of distilled water.

**Preparation of Arjuna Ksheer Paka:** 200g of previously defatted *T.arjuna* bark powder was added to a mixture containing 800ml milk and 3200 ml distilled water. The resulting mixture was boiled for 8 hours until a 1/4th reduction in volume was observed. It was filtered and the filtrate was evaporated to dryness in a steam bath.

**Phytochemical evaluation** (11,12)**:** The extracts obtained were subjected to the following evaluation tests as described in below table:

| Secondary metabolite | Test |
|---|---|
| Test for Volatile Oil | Sudan III solution was added to a small amount of extract. Development of a red colour is obtained by globules if volatile oil is present |
| Test for Acidic compounds | 10ml warm water was added to the extract and filtered. The filtrate obtained was further tested to determine its nature using a litmus paper |
| Tests for tannins | a) Ferric chloride test: A solution of 5% alcoholic ferric chloride was added to the extract.<br>A blue black precipitate indicates presence of hydrolysable tannins and condensed tannins show a brownish green colouration<br>b) Lead acetate test: Lead acetate solution (25%- basic) was added to the extract.<br>Precipitation with lead acetate indicates presence of tannins. |
| Tests for carbohydrates | a) Molisch's test: A few drops of alcoholic α-naphthol was added to the extract followed by a few drops of conc. sulphuric acid is added from the sides of the test tube.<br>Formation of a purple or violet coloured ring at the interface indicates the presence of carbohydrates.<br>b) Barfoed's test: To1 ml of extract, 1ml of Barfoed's reagent was added and heated gently in a water bath.<br>Formation of a red precipitate of cupric oxide indicates presence of monosaccharides. Disaccharides on prolonged heating (about 10 min) may also show a similar reaction due to conversion into monosaccharides. |
| Tests for alkaloids: | a) Dragendorff's reagent– Red to brown precipitate with potassium bismuth iodide.<br>b) Mayer's reagent- Cream coloured precipitate with potassium mercuric iodide solution.<br>c) Wagner's reagent - Reddish- brown precipitate with iodine potassium iodide solution<br>d) Hager's reagent -Yellow precipitate with saturated picric acid solution |
| Test for Proteins: | a) Heat test: The aqueous extract was boiled on a water bath.<br>If coagulation occurs, it indicates that proteins are present.<br>b) Biuret test: To 2ml of the aqueous extract, 2ml Biuret reagent was added<br>Development of a violet colour indicates proteins are present. |
| Test for Glycosides: | a. Cardiac glycosides:<br>Baljet's test: The extract was treated with picric acid solution.<br>Orange colouration indicates presence of cardiac glycosides. |

| | |
|---|---|
| | Legal's test: The extract was treated with pyridine. To this, an alkaline solution of sodium nitroprusside was added.<br>A red colouration indicates presence of cardiac glycosides.<br>b. Saponin glycosides: The froth-formation test is commonly performed to detect presence of saponin glycosides. In this test, 2ml of extract was taken in a test tube, distilled water was added and the test tube was shaken. |
| Test for Flavonoids: | a. Alkaline reagent test: The extract was treated with NaOH solution.<br>Appearance of an intense colour which turns colourless on addition of dilute HCl, indicates presence of flavonoids.<br>b. Zinc hydrochloride test: The methanolic extract was treated with conc HCl.<br>A pinch of zinc dust was added.<br>A red colouration indicates presence of flavonoids. |

**Thin Layer Chromatography:** Thin layer chromatography was carried out for treated extracts on pre-coated silica gel 60 F254 plates using Quercetin as standard. The mobile phase utilised was Toluene, ethyl acetate, acetic acid (5:5:1). The plates were dried and examined under white and ultraviolet light.

**Total Phenol Content:** The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent. (13,14)
1 ml aliquots of gallic acid having concentrations 20,40,60,80 and 100 ppm were mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml of saturated sodium carbonate solution. The resulting solutions were kept in the dark for 1 hour and the absorbance was measured spectrophotometrically to obtain the calibration curve of the standard compound. The aqueous and ethanolic extracts of *T.arjuna* were taken at concentrations of 50 ppm each and treated similarly. Total phenolic content was then calculated using the following formula:

**Total Flavonoid Content** (13,14)**:** The Total flavonoid content of the extracts was determined using aluminium chloride assay using quercetin as standard. 0.5 ml aliquots of quercetin having concentrations 20,40,60,80 and 100 ppm were mixed with 1.5ml distilled water, 0.1 ml potassium acetate, 0.1 ml 10% AlCl3. The volume was made up to 10 ml using distilled water. The resulting solutions were allowed to rest at RT for 30 mins and the absorbance was measured spectrophotometrically to obtain the calibration curve of the standard compound. The aqueous and ethanolic extracts of *T.arjuna* were taken at concentrations of 50 ppm each and treated similarly. Total flavonoid content was then calculated using the following formula: C x V/ W x 1000 where C: concentration of quercetin obtained, V: volume of solution, W: mass in grams. The final result was expressed in quercetin equivalent mg/g.

***In vitro* antioxidant potential** (13,14)**:** Determination of antioxidant activity was determined by DPPH assay using ascorbic acid as standard. 1ml each of methanolic solutions of ascorbic acid having concentrations 10,20,40,80 and 100 ppm were mixed with 2ml standard DPPH reagent and kept in the dark for 20 minutes. 1ml each of all extracts was treated similarly. Absorbance of all solutions as well as DPPH reagent were measured at 517 nm and percent scavenging(inhibition) was calculated using the formula: $(A_0- A_1/A_0)$ x 100 where $A_0$ represents absorbance of control and $A_1$ represents absorbance of sample. This was further plotted against concentration of sample and $IC_{50}$ values were calculated using the formula : $IC_{50} = 50( b/a)$ where b: intercept and a:slope

**HPLC method Development** (15-17)
The method was carried out on Shimadzu LCMS-8060 NX spectrophotometer, using ACE Generix C18 (5) 250 x 4.6 mm column. The mobile phase used was Phase A: Water with 0.1% formic acid and Phase B: Acetonitrile (60:40 v/v) with a gradient mode at the flow rate of 1 mL/min.Column temperature was maintained at 25º C and detection was carried out at 254 nm.

**HPLC method validation**

**Linearity**
Using the optimised HPLC method the linearity study was carried out in the range of 5 to100ppm. Standard solution of Quercetin was prepared and diluted to the linear concentration range for the construction of a calibration curve. The linear concentration range was between 5 to 100 ppm, and the calibration curve was plotted between peak area versus linear concentration of the standard quercetin.
solution was made and refrigerated for the purpose of developing and validating the analytical procedure.

**Precision**
Inter-day and intraday precision of the HPLC method was determined at mid-point concentrations i.e., 40 ppm in replicates of 6. And the % RSD was calculated.

**Accuracy**
Mid-point concentration of 40 ppm was spiked with quercetin standard in the percentages 75%, 100% and 125% respectively. And the percent recovery was calculated to determine the Accuracy

**Specificity**
Specificity parameter was evaluated by overlaying the solvent chromatogram with Quercetin's chromatogram to identify any other interfering peak.

**System suitability**
Triplicate of 100 ppm standard quercetin solution was performed to obtain system suitability results in the terms of area under curve, theoretical plates, and tailing factor.

**Robustness**

The robustness parameter was assessed by slight modification in the chromatographic conditions viz mobile phase composition, column temperature and flow rate. The change in the retention time was monitored.
The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the signal-to-noise (S/N) ratio, using criteria of 3:1 for LOD and 10:1 for LOQ, respectively.

**Applications of Developed HPLC method**

**12.1 Preparation of *T.arjuna* aqueous extract for HPLC analysis**

A 400mg sample of T arjuna aqueous extract was measured and then dissolved in 1000 µL of MS grade methanol. The sample underwent vortexing for a duration of 2 minutes, after which it was subsequently filtered using a 0.2 µm Whatman syringe filter. Quantitative analysis was conducted using a solution of the extract that was clear and filtered.

**Results and Discussion: -**

**Morphological and microscopical evaluation:** Pieces of Arjuna bark obtained were approximately 9 x8 x1 cm in dimensions. Outer and inner surfaces are greyish brown. Transverse section of arjuna bark shows the presence of cork cells followed by cortical parenchyma containing calcium oxalate crystals. Phloem fibres and medullary rays were also observed. Microscopical evaluation of the powder revealed presence of rosette shaped calcium oxalate crystals, starch grains and lignified fibres.

**Preparation and evaluation of aqueous and ethanolic bark extract**

The extracts were prepared as described and the total yield obtained was calculated and compared as in Table 1:

Table 1: Comparative yield of extracts of *T.Arjuna*

| **Extract** | **Percent Yield ( %w/w)** |
|---|---|
| Aqueous extract of T.arjuna | 43.3 ± 0.25 |
| Hydroalcoholic extract of T.arjuna | 32.54 ± 0.38 |
| Arjuna Ksheer Pakam | 36.44 ± 0.65 |
| Alcoholic extract of T.arjuna | 20.24 ± 0.17 |

| Test | Aqueous enriched extract of *T.Arjuna* | Ethanolic extract of *T.Arjuna* | Arjuna Ksheer Paka |
|---|---|---|---|
| Cardiac glycosides | Present | Present | Present |
| Saponins | Present | Present | Present |
| Flavonoids | Present | Present | Present |
| Tannins and polyphenols | Present | Present | Present |
| Volatile oils | Absent | Absent | Absent |
| Carbohydrates | Present | Present | Present |
| Alkaloids | Absent | Present | Absent |
| Proteins | Absent | Absent | Present |

**Phytochemical Evaluation of prepared extracts**

The extracts revealed the presence of phenolic compounds, saponins, flavonoids, cardiac glycosides and carbohydrates as mentioned in Table 2

Table 2: Phytochemical screening of the extracts of *T.Arjuna*

**Thin Layer Chromatography**: Examination of the plates was carried out. Spots bearing Rf 0.73 were obtained for the reference and the extract tracks. TLC plate was visualized under short UV at 254 nm.


**Figure 1: TLC of *T.Arjuna* extract with Quercetin as standard**

**Total Phenolic content :** Total phenolic contents for aqueous enriched extract, ethanolic extract and Arjuna Ksheer Paka were found to be 142.46± 0.50 mg GAE/100 g ,482.47±1.52, 58.20± 5.77 mg GAE/100 g

**Total Flavonoid content :** Total flavonoid contents for aqueous enriched extract, ethanolic extract and Arjuna Ksheer Paka were found to be 179.21± 0.62 QE mg/g, 89.818±7.23 QE mg/g, 51.23± 0.77 QE mg/g

***In vitro* antioxidant potential:** $IC_{50}$ values for aqueous enriched extract, ethanolic extract and Arjuna Ksheer Paka were found to be 33.77± 0.43, 17.190±1.5, 31.4± 1.95 (µg antioxidant/ml DPPH)

**HPLC method development**

**Table 3. Optimized HPLC conditions.**

| | |
|---|---|
| Stationary Phase | ACE Generix C18 (5) 250 x 4.6 mm |
| Mobile Phase | A: Water with 0.1% formic acid (60:40 v/v)<br>B: Acetonitrile |
| Mode | Gradient |
| Flow rate | 1 mL/min. |
| Detection | 254 nm using UV detector |
| Injection volume | 10 µL. |
| Column Oven Temperature | 25º C |

**HPLC method validation**

**Linearity and Range**

HPLC method linearity was determined in the range of 5 to 100 ppm as shown in the Fig 2, Table 4. The coefficient of correlation (R2) value of 0.9993 was observed for the graph between concentration and peak areas which indicates the good linear relationship.

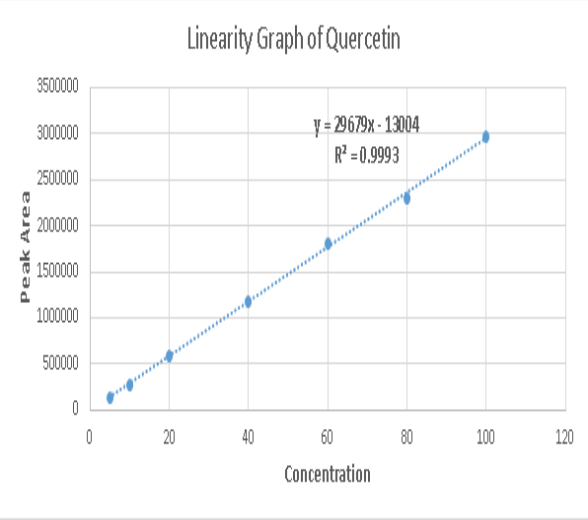


**Fig 2. Linearity Graph of Quercetin**

**Table 4. Summary of linear regression data of Quercetin.**

| | Parameters | Results |
|---|---|---|
| Linearity (n=7) | Regression equation | y = 29679x - 13004 |
| | Regression coefficient R2 | 0.9993 |
| | Correlation coefficient | 0.9996 |
| | Slope | 29679 |
| | Intercept | -13004 |

Limit of detection and Limit of quantitation using standard quercetin was found to be 0.748 ppm and 5 ppm respectively.

**Method Precision**

The precision was calculated by evaluating Quercetin standard solutions in 6 replicates of mid-point concentration i.e., 40 ppm. As per the results obtained in Table 5 indicates that the %RSD and average SD for Inter-day and Intra-day precision was less than 2 which means the method is precise and within the acceptable limits.

**Table 5. Inter-day, Intra-day precision of Quercetin.**

| | Parameters | Results |
|---|---|---|
| 1.Inter-day precision (n=6) | Retention time (mins)<br>Average ± SD<br>% RSD | 5.06 ± 0.01<br>0.11 |
| | Area<br>Average ± SD<br>% RSD | 1179098 ± 8964<br>0.76 |
| 2.Intra-day (1st batch) precision (n=6) | Retention time (mins)<br>Average ± SD<br>% RSD | 5.063 ± 0.01<br>0.01 |
| | Area<br>Average ± SD<br>% RSD | 1064282 ± 8353 0.78 |
| 3.Intra-day (2nd batch) precision (n=6) | Retention time (mins)<br>Average ± SD<br>% RSD | 5.06 ± 0.01<br>0.11 |
| | Area<br>Average ± SD<br>% RSD | 1135049 ± 5749<br>1.0 |

**Accuracy**

Accuracy was determined by the standard addition technique, in which the Quercetin sample was spiked with the Quercetin standard in the varying percentage viz, 75, 100, and 125% respectively (n=3). The percentage recovery was calculated and was found to be 81.27 ± 0.27, 78.35 ± 0.18, and 78.77 ± 0.35% respectively. The results showed that the suggested method is reliable and reproducible.

**Specificity**

Specificity was determined to evaluate how accurately and specifically the analyte of interest can be separated and identified from the extraneous matter. The chromatogram presented in Fig 3,4,5 indicates that the method is considered to be specific for the estimation of Quercetin.

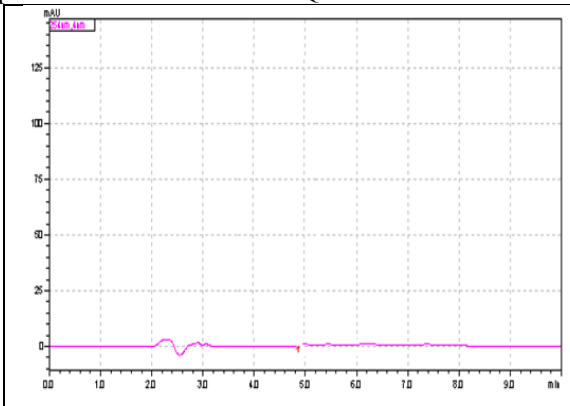

Fig 3. HPLC chromatogram of blank solution (Solvent).

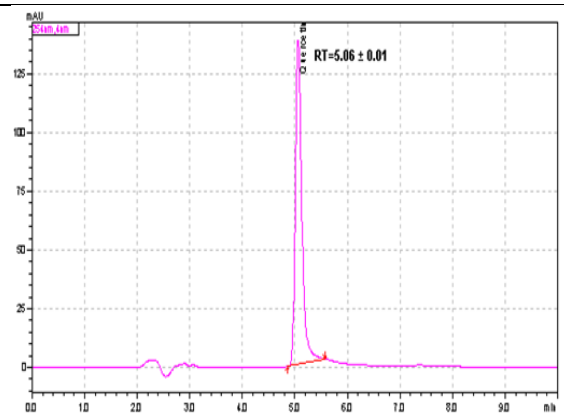


Fig 4. HPLC chromatogram of standard Quercetin.

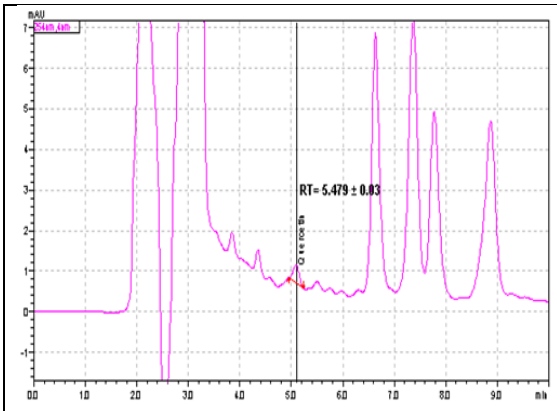


Fig 5. HPLC chromatogram of *T arjuna* extract.

**System suitability**

System suitability testing was done to check whether the overall operating system method was performed properly. Parameters including retention time, tailing factor and number of theoretical plates were calculated. And the result indicated were in the limits as presented in Table 6.

Table 6. System suitability for Quercetin

| | Concentration of standard Quercetin | Retention time | Area Under Curve | Theoretical plate | Tailing Factor |
|---|---|---|---|---|---|
| Average (n=3) | 100ppm | 5.052 | 32,40,897 | 47,762 | 1.471 |
| SD | 0 | 0.006158 | 92,995.85 | 691.05836 | 0.073074 |
| %RSD | 0 | 0.12189 | 2.869448 | 1.446872 | 4.969282 |

**Robustness**

Robustness of an analytical method is its capacity to remain deliberate even in the small variations in chromatographic conditions such as flow rate, mobile phase ratio and column temperature. Robustness data as shown in Table 7 indicates that the method is robust since %RSD values were less than 2 which is in the acceptable limits of the validation.

**Table 7. Robustness study**

| Parameters | Changes | Retention Time | | Theoretical plates | | Tailing factor | |
|---|---|---|---|---|---|---|---|
| | | Average ±SD | %RSD | Average ±SD | % RSD | Average ±SD | %RSD |
| Flow rate | (0.9 ml/min) | 5.554 ± 0.03 | 0.54 | 55943 ± 311.36 | 0.55 | 1.491 ± 0.04 | 2.68 |
| | (1.1 ml/min) | 4.629 ± 0 | 0 | 51367 ± 145.32 | 0.28 | 1.362 ± 0.03 | 2.02 |
| Mobile phase ratio | (58:42 v/v) | 4.629 ± 0 | 0 | 52122 ± 129.63 | 0.248 | 1.367 ± 0 | 0 |
| | (62:38 v/v) | 5.611 ± 0 | 0 | 56295 ± 62.12 | 0.11 | 1.364 ± 0.0 | 0 |
| Column oven temperature | (23°C) | 5.188 ± 0.01 | 0.19 | 55775 ± 258.78 | 0.46 | 1.308 ± 0.01 | 0.76 |
| | (27°C) | 4.956 ± 0.01 | 0.20 | 53663 ± 261.51 | 0.48 | 1.314 ± 0.03 | 2.28 |

**10. Applications of validated HPLC method.**

The quantification of Quercetin in the aqueous root extract of *T arjuna* was conducted using an approved technique. The findings were documented in Table 8.

Table 8: Analytical parameters of *T.Arjuna* extract

| Sr No. | Sample | Concentration (mg) ± SD | %RSD | Standard deviation | Standard error | Retention time (Mins) | Peak Area |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | *T arjuna* Extract (400 mg) | 2.10 ± 1.17 | 5.55 | 1.17 | 6.74 | 5.479 ± 0.03 | 49415 ± 42463 |

**DISCUSSION**

The therapeutic importance of *Terminalia arjuna* has long been recognized in traditional medicine, yet its integration into modern pharmaceutical systems continues to be hindered by the absence of standardized analytical protocols. In this investigation, a reproducible quality control framework was developed by combining phytochemical evaluation with RP-HPLC quantification of quercetin, selected as a chemical marker. Comparative extraction analysis revealed that ethanol provided a markedly higher yield than water, reflecting the stronger affinity of polyphenolic compounds for moderately polar solvents. This outcome is consistent with previous reports that alcoholic solvents effectively disrupt plant cell matrices, thereby facilitating the release of flavonoids, phenolic acids, and triterpenoids from *T. arjuna* bark. Sharma et al. similarly demonstrated enhanced polyphenol recovery with ethanolic extraction, underscoring ethanol's suitability for preparing standardized botanical extracts. (18)

Phytochemical screening confirmed the presence of flavonoids, tannins, phenolic compounds, and triterpenoids in both aqueous and ethanolic extracts, though these constituents were more abundant in the ethanolic fraction. The elevated levels of total phenolics and flavonoids observed in this study are particularly significant, as these metabolites are directly linked to antioxidant and cardioprotective activities. Singh et al. reported a strong correlation between phenolic concentration and free radical scavenging potential, thereby validating the functional relevance of these compounds. (19)

The RP-HPLC method established here achieved satisfactory resolution and specificity for quercetin, with acceptable linearity and repeatability, making it suitable for routine application. The recovery values obtained for the herbal drug samples ranged between 78% and 81%. Although these values are lower than those typically reported for synthetic pharmaceutical formulations, they are considered acceptable for complex herbal matrices. The reduced recovery can be attributed to matrix-associated effects, including strong interactions of the analyte with tannins, polysaccharides, and other co-extractive phytoconstituents, which may limit complete extraction. Importantly, the recoveries were consistent across concentration levels with low variability, indicating good method reproducibility. Therefore, the proposed method is deemed suitable for routine quantitative estimation and quality control of the herbal drug. Sanjay et al. highlighted that validated HPLC techniques provide greater reliability for herbal drug standardization compared to spectrophotometric assays alone. (20) The adoption of quercetin as a marker compound further strengthens the regulatory framework for quality assurance of *T. arjuna*.

Overall, the integration of extraction efficiency, phytochemical profiling, and chromatographic fingerprinting presented in this study offers a scientifically validated basis for the quality evaluation of *Terminalia arjuna* bark. The proposed analytical framework is expected to contribute meaningfully toward harmonized quality control practices and facilitate the incorporation of *T. arjuna*-based formulations into evidence-based phytopharmaceutical systems.

**CONCLUSION:**

A simple yet efficient reverse-phase high-performance liquid chromatography (HPLC) technique was developed to detect and quantify the presence of Quercetin from the Extracts of *T arjuna.* The HPLC technique that has been developed is straightforward, accurate, efficient, cost-effective, and precise. Additionally, it was verified in accordance with ICH requirements. The HPLC technique that has been developed exhibits promptness, making it well-suited for both quantitative analysis and quality control of extracts and herbal formulations derived from *T arjuna* species.

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