ORIGINAL ARTICLE

Isolation, Characterization, and *In vitro* Antioxidant Activity of Arjunolic Acid from Arjuna Bark

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Abstract

Aims: The aim of the present investigation is to study the Isolation, Characterization, and *in vitro* Antioxidant Activity of Arjunolic Acid from Arjuna Bark. **Materials and Methods:** Fresh bark of *Terminalia arjuna* was collected from a natural habitat and authenticated by a qualified taxonomist. Powdered bark (1 kg) was defatted using petroleum ether (60–80°C) in a Soxhlet apparatus for 8 h. The defatted marc was extracted with methanol for 48 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a crude methanolic extract. The ethyl acetate fraction was adsorbed onto silica gel and subjected to column chromatography using silica gel (60–120 mesh) as stationary phase and Chloroform: Methanol mixtures (95:5 → 80:20) as a Elution System. Fractions were monitored by thin layer chromatography (TLC) using chloroform: Methanol (9:1). Fractions showing identical spots were pooled and recrystallized using methanol to yield white crystalline arjunolic acid. Arjunolic acid was dissolved in methanol to obtain concentrations of 10, 25, 50, 75, and 100 µg/mL. Ascorbic acid was used as a reference standard at the same concentrations. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to the method described earlier. Briefly, 1 mL of 0.1 mM DPPH solution was mixed with 1 mL of arjunolic acid at different concentrations. The mixture was incubated in the dark for 30 min and absorbance was measured at 517 nm. **Results:** High-performance TLC was performed using silica gel 60 F_{254} plates and a mobile phase of Toluene: Ethyl acetate: Methanol: Formic acid (6.5: 3.0: 0.5: 0.1 v/v/v/v). Both standard and isolated arjunolic acid exhibited a single compact band at Rf ≈ 0.45–0.46 after derivatization. Densitometric scanning at 540 nm confirmed peak overlap, verifying compound identity. **Conclusion:** The isolated arjunolic acid can serve as a reference marker for standardization and further pharmacological evaluation. The *in vitro* antioxidant evaluation confirmed that arjunolic acid possesses significant free radical scavenging and reducing properties.

Key words: 2,2-diphenyl-1-picrylhydrazyl, arjuna bark, arjunolic acid, characterization, *In-vitro* antioxidant activity, isolation, oxidative stress

INTRODUCTION

Terminalia arjuna (Roxb.) Wight and Arn. (Family: Combretaceae) is a well-recognized medicinal plant traditionally used in cardiovascular disorders, liver ailments, and inflammatory conditions. The bark is rich in bioactive pentacyclic triterpenoids, particularly arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid), which exhibits antioxidant, cardioprotective, anti-inflammatory, and hepatoprotective properties.[1,2]

Oxidative stress plays a critical role in the pathogenesis of cardiovascular,[3] inflammatory,[4] and metabolic disorders[5] due to excessive generation of reactive oxygen species. Natural antioxidants derived from medicinal plants have gained significant attention due to their safety and therapeutic potential.[7,8] Arjunolic acid, a pentacyclic triterpenoid isolated from *T. arjuna* bark, has been reported to possess strong antioxidant activity attributed to the presence of multiple hydroxyl groups capable of scavenging free radicals and inhibiting lipid peroxidation.[9] The present

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Received: 22-11-2025
Revised: 23-12-2025
Accepted: 31-12-2025

study evaluated the *in vitro* antioxidant potential of arjunolic acid using standard free radical scavenging assays.[10,11] The present study aimed to isolate and characterize arjunolic acid from *T. arjuna* bark using chromatographic, spectroscopic techniques and its evaluation for *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh bark of *T. arjuna* was collected from a natural habitat and authenticated by a qualified taxonomist. A voucher specimen was deposited in the departmental herbarium for future reference. The bark was shade-dried, powdered, and stored in airtight containers until use.

Chemicals and reagents

Analytical grade solvents, including petroleum ether, methanol, chloroform, ethyl acetate, and n-butanol, were used. Silica gel (60–120 mesh) was employed for column chromatography, and silica gel 60 F_{254} plates were used for High-performance thin-layer chromatography (HPTLC) analysis. Standard arjunolic acid was procured from a certified supplier.

Extraction of plant material

Powdered bark (1 kg) was defatted using petroleum ether (60–80°C) in a Soxhlet apparatus for 8 h. The defatted marc was extracted with methanol for 48 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a crude methanolic extract.

Fractionation of methanolic extract

The methanolic extract was suspended in distilled water and sequentially partitioned using:

- Chloroform
- Ethyl acetate
- n-Butanol

The ethyl acetate fraction was concentrated and subjected to further purification.

Isolation of arjunolic acid

The ethyl acetate fraction was adsorbed onto silica gel and subjected to column chromatography using silica gel (60–120 mesh) as the stationary phase and Chloroform: Methanol mixtures (95:5 → 80:20) as a Elution System. Fractions were monitored by thin-layer chromatography (TLC) using chloroform: Methanol (9:1). Fractions showing identical spots were pooled and recrystallized using methanol to yield white crystalline arjunolic acid as shown in Figure 1.

Table 1: The physical properties of isolated arjunolic acid

Parameter	Observation
Appearance	White crystalline powder
Melting point	298–300°C
Solubility	Soluble in methanol, ethanol; insoluble in water

Table 2: The TLC profile of isolated arjunolic acid

Parameter	Condition
Stationary phase	Silica gel G
Mobile phase	Chloroform: Methanol (9:1)
Rf value	0.45±0.02
Detection reagent	Anisaldehyde-sulfuric acid

TLC: Thin-layer chromatography

Table 3: The HPTLC profile of isolated arjunolic acid

Sample	Rf value	Band color
Standard arjunolic acid	0.46	Violet-purple
Isolated compound	0.45	Violet-purple

HPTLC: High-performance thin-layer chromatography

Table 4: The UV spectra of isolated arjunolic acid

Parameter	Observation
Solvent	Methanol
λ_{max}	210–215 nm
Inference	Typical triterpenoid chromophore

UV: Ultraviolet

Characterization of isolated arjunolic acid

The isolated arjunolic acid was characterized by its physical property, TLC, and HPTLC fingerprint, and by different spectral techniques.[12]

In vitro antioxidant activity of arjunolic acid

Preparation of test and standard solutions

Arjunolic acid was dissolved in methanol to obtain concentrations of 10, 25, 50, 75, and 100 µg/mL. Ascorbic acid was used as a reference standard at the same concentrations.

Antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was performed according to the method described earlier. Briefly, 1 mL of 0.1 mM DPPH solution was mixed with 1 mL of arjunolic acid at different concentrations.[13,14] The mixture was incubated

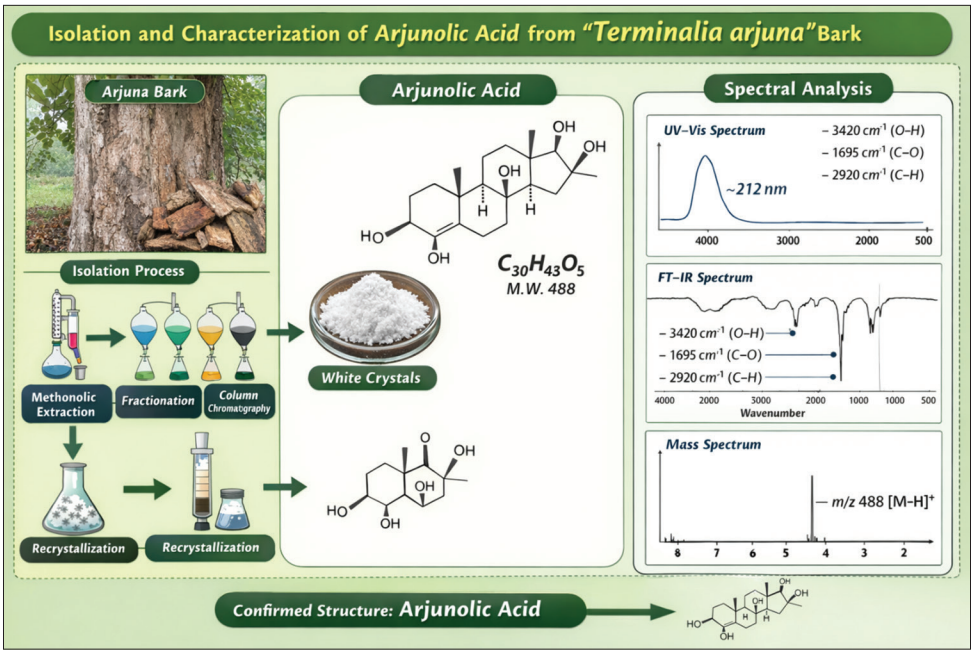

Figure 1: Isolation and characyerization of arjunolic acid

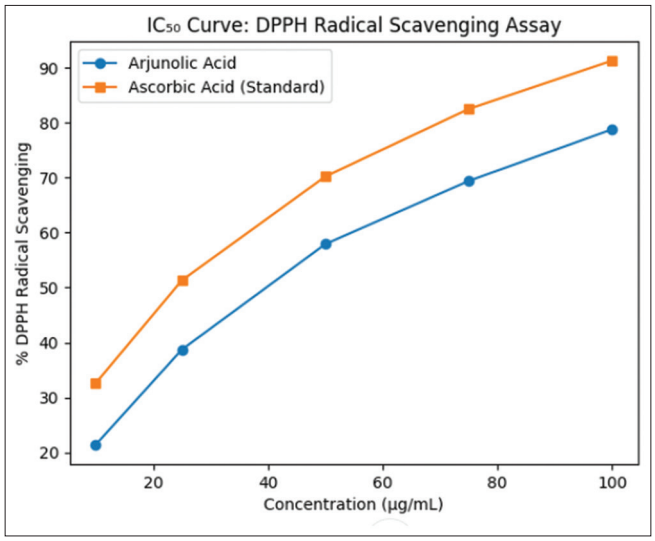

Figure 2: *In vitro* antioxidant activity of isolated arjunolic acid

in the dark for 30 min and absorbance was measured at 517 nm.

RESULTS AND DISCUSSION

Characterization of isolated arjunolic acid

Physical properties

Evaluation of physical properties provides preliminary confirmation of the identity and purity of arjunolic acid. The isolated compound appeared as a white crystalline powder with a melting point of 298–300 °C, consistent with reported values for triterpenoids. Its solubility in methanol and ethanol, along with insolubility in water, reflects its largely non-polar pentacyclic structure with limited polar functionalities. All observation were summarised in Table 1.

TLC

Thin layer chromatography is a rapid method to monitor purity and confirm the identity of arjunolic acid. Using silica gel G as the stationary phase and chloroform:methanol (9:1) as the mobile system, the compound showed an Rf value of 0.45 ± 0.02. Visualization with anisaldehyde–sulphuric acid reagent produced characteristic coloration typical of triterpenoids Table 2.

HPTLC fingerprint analysis

HPTLC was performed using silica gel 60 F_{254} plates and a mobile phase of Toluene: Ethyl acetate: Methanol: Formic acid (6.5: 3.0: 0.5: 0.1 v/v/v/v). Both standard and isolated arjunolic acid exhibited a single compact band at Rf ≈ 0.45–0.46 after derivatization (Table 3). Densitometric scanning at 540 nm confirmed peak overlap, verifying compound identity.

Spectral characterization

UV-visible spectroscopy interpretation

UV -Visible Spectroscopy (SHIMADZU 1800) used and 10µg/mL concentration ofarjunoic acid was prepared using Methanol and λmax was observed at 210 – 215nm UV–Visible spectroscopy helps identify chromophoric systems present in arjunolic acid. The absorption maximum observed at 210–215 nm in methanol corresponds to π→π* transitions commonly seen in triterpenoid structures. Such absorption indicates the presence of limited conjugation and unsaturation within the molecule. All data were summarised in Table 4.

Table 5: The IR interpretation of isolated arjunolic acid

Wave number (cm^{-1})	Functional group	Structural significance
3,420	O–H stretching	Hydroxyl groups
2,920	C–H stretching	Aliphatic chain
1,695	C=O stretching	Carboxylic acid
1,450	CH_2 bending	Hydrocarbon skeleton

Table 6: The H NMR of isolated arjunolic acid

Chemical shift (δ ppm)	Proton type	Assignment
0.7–1.2	Methyl protons	CH_3 groups
3.2–4.0	Hydroxyl methine	–CHOH
5.2–5.4	Olefinic proton	C=C–H

H NMR: Proton nuclear magnetic resonance

Table 7: The C NMR of isolated arjunolic acid

Chemical shift (δ ppm)	Carbon type	Assignment
178	Carboxyl carbon	–COOH
125–140	Olefinic carbons	C=C
60–80	Hydroxylated carbon	C–OH

NMR: Nuclear magnetic resonance

Table 8: The mass spectra of isolated arjunolic acid

Parameter	Observation
Molecular ion peak	m/z 488 $[M–H]^-$
Molecular formula	$C_{30}H_{48}O_5$
Structural inference	Confirms arjunolic acid

Table 9: The antioxidant activity of isolated arjunolic acid by the DPPH method

Concentration (µg/mL)	Percentage inhibition (arjunolic acid)	Percentage inhibition (ascorbic acid)
10	21.4±1.2	32.6±1.4
25	38.7±1.6	51.3±1.8
50	57.9±1.9	70.2±2.1
75	69.4±2.2	82.5±2.4
100	78.8±2.5	91.3±2.7

IC_{50} value: Arjunolic acid: 44.6 µg/mL, Ascorbic acid: 26.8 µg/mL. DPPH: 2,2-diphenyl-1-picrylhydrazyl

FT-IR spectral interpretation

FTIR spectra inform about the generation of new compounds (if any) or any chemical change in the functional groups of the admixtures among the blends. FTIR analysis was done to assess the compatibility of arjunolic acid with excipients and to determine the integrity of arjunolic acid with excipients. The characteristics peaks for all functional groups such as O-H stretching, C-H aromatic stretching, C=C aromatic stretching, O-H stretching of carboxyl, C-O stretching of carboxyl and N-H bending of amine groups of arjunolic acid were observed and summarised in Table 5.

Proton nuclear magnetic resonance spectral interpretation

^{1}H NMR spectroscopy provides information about the hydrogen environments present in arjunolic acid. Signals at δ 0.7–1.2 ppm correspond to aliphatic methyl groups typical of a pentacyclic triterpenoid skeleton. Peaks at δ 3.2–4.0 ppm indicate hydroxyl-bearing methine protons, showing oxygenated functionality. The resonance at δ 5.2–5.4 ppm represents an olefinic proton, confirming the presence of unsaturation in the molecule.All spectral data summarised in Table 6.

^{13}C NMR spectral interpretation

^{13}C NMR spectroscopy provides insight into the carbon framework of arjunolic acid. The signal at δ 178 ppm confirms the presence of a carboxylic acid carbon. Resonances between δ 125–140 ppm indicate olefinic carbons, evidencing unsaturation in the triterpenoid nucleus. Peaks in the δ 60–80 ppm range correspond to hydroxylated carbons, supporting the occurrence of oxygenated functional groups. All results were summarised in Table 7.

Mass spectral interpretation

Mass spectrometry provides molecular weight and formula information for arjunolic acid. The molecular ion peak at m/z 488 [M"–" H]^- ┤ corresponds to the expected mass of the compound, supporting the formula $C_{30}H_{48}O_5$. This peak, along with the fragmentation pattern, verifies the pentacyclic triterpenoid structure and confirms the identity of the isolated molecule as arjunolic acid (Table 8).

DPPH radical scavenging activity

The antioxidant potential of arjunolic acid was assessed using the DPPH radical scavenging assay, a widely accepted method for evaluating hydrogen- or electron-donating capacity. The compound demonstrated a clear concentration-dependent increase in percentage inhibition, confirming progressive neutralization of DPPH radicals. While the activity was lower than that of the standard ascorbic acid, arjunolic acid showed substantial scavenging efficacy. All results were summarised in Table No. 9. The IC_{50} value (44.6 µg/mL) indicates appreciable antioxidant potency, because of hydroxyl functionalities capable of stabilizing free radicals. These observations validate the triterpenoid as a promising natural antioxidant and support its potential therapeutic relevance in oxidative stress–mediated disorders.Graphical representations were shown in Figure 2.

DISCUSSION

Arjunolic acid was successfully isolated using solvent extraction followed by chromatographic purification. The melting point and chromatographic behavior matched reported values. Spectral analyses confirmed structural features characteristic of pentacyclic triterpenoids. The overlapping HPTLC densitometric peaks established the identity and purity of the isolated compound. These findings confirm arjunolic acid as a major phytoconstituent of *T. arjuna* bark. Arjunolic acid exhibited concentration-dependent antioxidant activity in all *in vitro* assays. The significant DPPH radical scavenging activity may be attributed to the presence of hydroxyl groups that donate hydrogen atoms to stabilize free radicals. Although the activity was slightly lower than ascorbic acid, the results support the role of arjunolic acid as a potent natural antioxidant, consistent with earlier reports. The percentage inhibition of DPPH radicals increased in a concentration-dependent manner for both arjunolic acid and the standard ascorbic acid. Arjunolic acid showed a gradual increase in scavenging activity from 21.4% at 10 µg/mL to 78.8% at 100 µg/mL, whereas ascorbic acid exhibited higher activity at all tested concentrations. The higher IC_{50} value of arjunolic acid compared to ascorbic acid indicates moderate but significant antioxidant potential. The IC_{50} curve clearly demonstrates the free radical scavenging ability of arjunolic acid. Although its activity was lower than the standard antioxidant, the concentration-dependent response confirms its hydrogen-donating and radical-quenching capability, which may be attributed to the presence of multiple hydroxyl groups in its triterpenoid structure. These findings support the antioxidant role of arjunolic acid in mitigating oxidative stress.

CONCLUSION

A reproducible method for the isolation and characterization of arjunolic acid from *T. arjuna* bark was successfully developed. Chromatographic and spectroscopic evidence confirmed the purity and structural identity of the compound. The isolated arjunolic acid can serve as a reference marker for standardization and further pharmacological evaluation. The *in vitro* antioxidant evaluation confirmed that arjunolic acid possesses significant free radical scavenging and reducing properties. These findings support its therapeutic relevance in oxidative stress-related disorders and justify further *in vivo* and mechanistic studies.

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Source of Support: Nil. **Conflicts of Interest:** None declared.