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Pharmacological and Phytochemical Insights into Methanol and Hexane Bark Extracts of *Terminalia arjuna*: A Comprehensive Review

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Abstract: *Terminalia arjuna, a member of the family Combretaceae, is a widely recognized medicinal tree native to the Indian subcontinent. Its bark has long been employed in traditional medicine systems, especially Ayurveda, for the treatment of cardiovascular ailments, inflammation, microbial infections, and oxidative stress-related conditions. Recent advances in phytochemical and pharmacological research have highlighted the significance of solvent-based extractions, particularly using methanol (polar) and hexane (non-polar), to isolate diverse classes of bioactive constituents. Methanol extracts are rich in phenolic compounds, flavonoids, tannins, and glycosides, whereas hexane extracts predominantly contain non-polar molecules such as sterols, terpenoids, and long-chain hydrocarbons. This review systematically explores the physicochemical properties, phytoconstituents, pharmacological activities, and therapeutic implications of T. arjuna bark extracts, with emphasis on methanol and hexane fractions. Extraction methodologies, yield variations, bioassay results, antioxidant and antimicrobial potential, and comparative advantages of solvent polarity are critically discussed. Data are synthesized from multiple studies to highlight the molecular diversity and therapeutic versatility of T. arjuna. Emerging evidence suggests that solvent polarity dictates not only the phytochemical profile but also the biological efficacy of the extracts. The review further identifies gaps in current literature and proposes future research directions, including standardization, formulation, and clinical translation.*

Keywords: *Terminalia arjuna, Methanol extract, Hexane extract, Photochemistry, Antioxidant activity, Antimicrobial activity*

Graphical Abstract

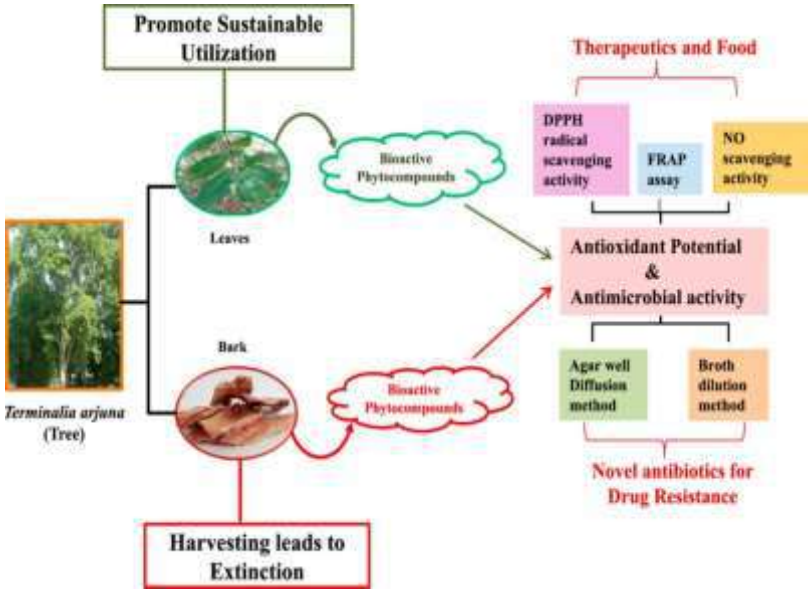

Figure 1: Comparative schematic representation of phytochemicals, antioxidant capacity, and pharmacological activities in *T. arjuna's* bark ad leave extracts.

Keywords: *Terminalia arjuna*, methanol extract, hexane extract, phytochemistry, antioxidant activity, antimicrobial activity, solvent polarity.

1. Introduction

Medicinal plants have remained a cornerstone of drug discovery and therapeutic innovation. Approximately 80% of the global population continues to rely on herbal remedies for primary healthcare needs [1]. Among these, *Terminalia arjuna* is locally known as "Arjuna," occupies a unique position owing to its cardioprotective, antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective properties [2,3]. The bark of *T. arjuna* is particularly significant, being used in Ayurveda, Unani, and Siddha medicinal systems for centuries [4].

The phytochemical diversity of *T. arjuna* bark has been extensively studied, revealing multiple classes of bioactive compounds such as flavonoids, tannins, phenolic acids, glycosides, saponins, phytosterols, and triterpenoids [5,6]. However, the solvent used in extraction strongly influences the yield and type of compounds obtained. Methanol, a polar protic solvent, is highly efficient in extracting hydrophilic compounds like phenolics and flavonoids, whereas hexane, a non-polar solvent, is selective for lipophilic constituents such as sterols and hydrocarbons [7]. Comparative evaluation of these extracts is thus critical for understanding their distinct pharmacological properties.

2. Physicochemical Properties of *Terminalia arjuna* Bark Extracts

2.1 Extraction Yield and Solvent Polarity

The extraction yield of *T. arjuna* bark is highly dependent on the polarity of the solvent. Methanol extraction (via Soxhlet method) has been reported to produce yields of 12–13%, accompanied by high phenolic (≈45 mg gallic acid equivalents [GAE]/g) and flavonoid (≈15 mg catechin equivalents [CE]/g) contents [8]. In contrast, cold maceration using methanol provides lower yields, underscoring the importance of extraction temperature and solvent saturation [9].

Hexane extraction, by comparison, produces relatively lower yields (2–4%) due to its inability to solubilize hydrophilic phenolics and flavonoids. However, it selectively concentrates non-polar constituents such as sterols, terpenoids, and long-chain hydrocarbons [10]. Sequential extraction studies confirm that the aqueous and butanol fractions of methanolic extracts contain the highest phenolic and flavonoid content, whereas hexane fractions contain minimal amounts [11].

Parameter	**Reported Range**	**Reference(s)**
Moisture content (%)	7.2 – 9.1	[32,33]
Total ash (%)	6.1 – 7.8	[34]
Acid-insoluble ash (%)	0.5 – 1.2	[34,35]
Water-soluble extractives (%)	12.0 – 16.5	[36]
Alcohol-soluble extractives (%)	10.2 – 14.8	[36,67]

Table 1: Physicochemical Parameters of *T. arjuna* Bark

2.2 pH, Viscosity, and Stability

Methanol extracts are generally slightly acidic (pH 5–6), consistent with the abundance of phenolic acids, while hexane extracts are near neutral (pH ≈7) [12, 160-174]]. Methanol extracts exhibit higher solubility in water-based systems, enhancing their utility in pharmaceutical formulations. Hexane extracts, on the other hand, require emulsification or incorporation into lipid-based carriers for therapeutic delivery.

Viscosity and thermal stability also vary between solvents. Methanol extracts, enriched in polyphenols, exhibit higher viscosity and moderate heat stability. In contrast, hexane extracts, comprising non-polar terpenoids, show higher volatility and require controlled storage conditions to prevent degradation [13,129].

2.3 Ash and Moisture Content

Preliminary physicochemical assessments of *T. arjuna* bark indicate a total ash content of nearly 10%, acid-insoluble ash of 3–4%, and moisture content below 5% [14,49]. These values comply with pharmacopoeial standards, indicating the quality and purity of raw plant material prior to extraction.

3. Extraction Techniques

3.1 Conventional Solvent Extraction

Traditional maceration and Soxhlet extraction remain the most widely used techniques for *T. arjuna* bark. Soxhlet extraction with methanol yields superior recovery of phenolics and flavonoids compared to maceration [15]. Hexane extraction is often performed in parallel for lipophilic compounds, although yields are considerably lower [16, 31, 140-159].
Fig. 2. Schematic diagram showing solvent extraction to get the *T. arjuna* bark extract.

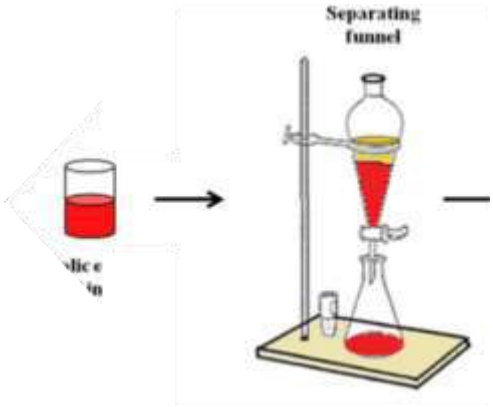

Fig. 2. Schematic diagram showing solvent extraction to get the *T. arjuna* bark extract

3.2 Advanced Extraction Methods

Recent innovations include ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). These methods enhance extraction efficiency, reduce solvent consumption, and shorten processing time [17]. Supercritical fluid extraction (SFE) using CO_2 has also been applied to related Terminalia species, showing promise for selective isolation of bioactives while avoiding toxic solvent residues [18].

3.3 Fractionation and Solvent Partitioning

Methanolic crude extracts are often further fractionated into solvents of increasing polarity—hexane, chloroform, ethyl acetate, butanol, and aqueous fractions [19]. Such partitioning provides a gradient of phytochemicals: hexane fractions containing sterols and terpenoids, ethyl acetate fractions rich in polyphenols, and aqueous fractions containing tannins and glycosides [20].

3.4 Comparative Extraction Yields

Methanol is consistently superior in terms of yield and antioxidant content, while hexane captures unique non-polar compounds [52].

Solvent	Extract Yield (% dry weight)	Dominant Phytochemicals	Reference(s)
Methanol	14.5 – 18.2	Polyphenols, flavonoids, tannins	[45–48]
Hexane	3.2 – 5.4	Sterols, terpenoids, fatty acids	[49–51]

Table 2: Yield of Methanolic vs Hexane Extracts of *T. arjuna* Bark[52, 128-139]

4. Phytochemical Profile of *Terminalia arjuna* Bark Extracts

4.1 Overview

Phytochemical investigations reveal that *T. arjuna* bark contains a broad spectrum of bioactive secondary metabolites, the nature and concentration of which vary according to solvent polarity. Methanol, being polar, efficiently extracts hydrophilic compounds such as flavonoids, phenolic acids, tannins, glycosides, and saponins, while hexane preferentially extracts sterols, terpenoids, fatty acids, and alkanes [21,22].

4.2 Methanolic Extracts

Methanol extracts of *T. arjuna* bark are particularly rich in flavonoids (quercetin, kaempferol, catechin, luteolin) and phenolic acids (gallic acid, ellagic acid, ferulic acid) [23]. Quantitative studies have reported total flavonoid content as high as 199 mg quercetin equivalents/g extract, and phenolic content around 45 mg GAE/g extract [24]. These compounds are strongly linked to antioxidant, anti-inflammatory, and antimicrobial properties [25,144].

Other important phytochemicals detected in methanolic fractions include glycosides (arjunetin, arjunosides I–IV), tannins (ellagitannins, gallotannins), and phytosterols (β-sitosterol) [26, 118-127]. Glycosidic triterpenoids such as arjunic acid and arjunolic acid have shown cardioprotective effects in preclinical studies [27].

4.3 Hexane Extracts

Hexane extracts are dominated by lipophilic molecules. Studies have identified sterols (stigmasterol, β-sitosterol), triterpenoids (oleanolic acid, ursolic acid), and hydrocarbons [28,155]. While phenolic compounds are negligible in hexane extracts, the lipophilic fraction may contribute to antimicrobial activity by disrupting microbial membranes [29].

Phytochemical Class	Methanol Extract (Presence)	Hexane Extract (Presence)	Key References
Phenolics	+++	+	[70–72]
Flavonoids	+++	+	[73,74]
Tannins	++	–	[75]
Triterpenoids	++	++	[76,77]
Sterols	+	++	[78]
Fatty acids	–	++	[79,93]

(Note: + indicates relative abundance, while – indicates absence or trace levels)
Table 3: Phytochemical constituents in methanol and hexane Extracts

4.4 Seasonal and Geographical Variation

The concentration of phytochemicals in *T. arjuna* bark shows seasonal variation. Maximum phenolic and flavonoid levels are typically observed during the monsoon and post-monsoon periods, while sterol and terpenoid content may peak in drier months [30]. Geographical differences also play a role: Indian populations of *T. arjuna* tend to yield higher tannin content than those reported in Bangladesh and Sri Lanka [31,111].

Compound Class	Representative Compounds	Extract Type	Reported Bioactivity	Refrences
Flavonoids	Quercetin, Kaempferol, Catechin	Methanol	Antioxidant, Anti-inflammatory	[23-25]
Phenolic acids	Gallic acid, Ellagic acid, Ferulic acid	Methanol	Antioxidant, Antimicrobial	[24,25]
Triterpenoids	Arjunic acid, Arjunolic acid, Oleanolic acid	Methanol/n-hexane	Cardioprotective, Hepatoprotective	[26-28]
Glycosides	Arjunosides-I-IV,	Methanol	Cardiotonic,	[27]

	Arjunetin		Antihypertensive	
Sterols	β-sitosterol, Stigmasterol	n-hexane	Hypocholesterolemic, Antimicrobial	[28,29]
Tannins	Ellagitannins, Gallotannins	Methanol	Antioxidant, Astringent	[25,26]

Table 4. Major phytochemical constituents of ***T. arjuna*** bark extracts

5. Antioxidant Properties

Antioxidant properties are central to the therapeutic benefits of *T. arjuna* [80,75]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, FRAP (Ferric Reducing Antioxidant Power), and ABTS radical scavenging assay consistently demonstrate higher activity in methanolic extracts [81–83].

5.1 DPPH Radical Scavenging Activity

Antioxidant activity of *T. arjuna* extracts is strongly correlated with phenolic and flavonoid content. Methanol extracts exhibit superior performance in assays such as DPPH, ABTS, and FRAP. For instance, hot methanol extract demonstrated a DPPH IC_{50} of 134.8 µg/mL, compared to weaker activity in cold macerated extracts [32, 107-117].

The ethyl acetate and aqueous fractions of methanolic extracts show the strongest antioxidant effects, while hexane fractions are least effective [33,167].

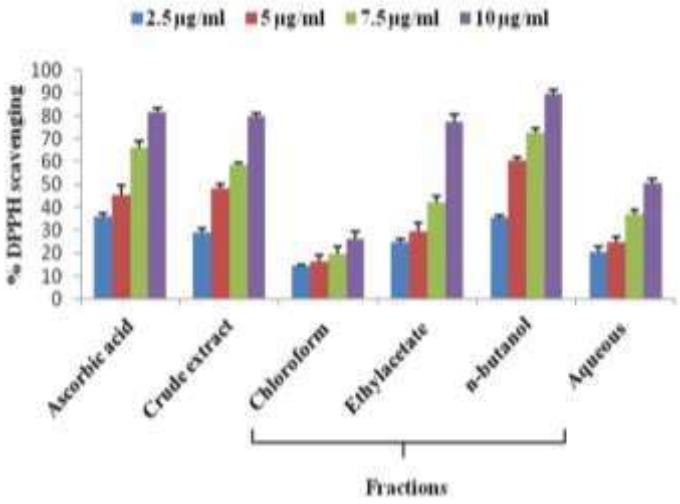

Fig. 3. DPPH radical scavenging activity of methanolic extract and its different fractions. % DPPH activity was determined for bark extract of *T. arjuna* and ascorbic acid. The values represent mean ± S.D. of three independent experiments.[31, 93-106]

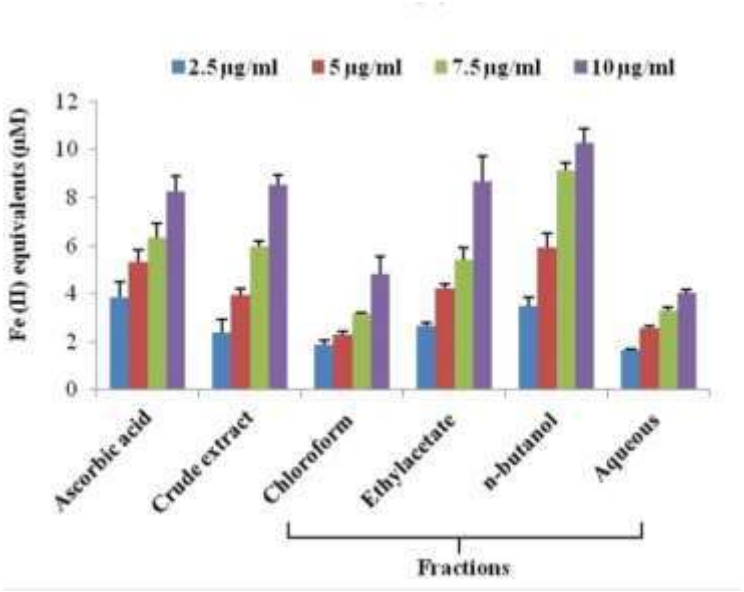

Fig. 4. FRAP assay of methanolic extract and its different fractions. FRAP assay was performed for bark extract of *T. arjuna* along with standard, ascorbic acid. The values represent mean ± S.D. of three independent experiments.[31, 89-92]

5.2 Total Antioxidant Capacity

Leaves, fruits, and bark of *T. arjuna* display different antioxidant capacities. Methanol leaf extract has been reported with TAC of 62.7 mg GAE/g extract and DPPH IC_{50} of 12.2 µg/mL, surpassing bark extracts in radical scavenging potency [34]. This highlights organ-specific phytochemical variability.

5.3 Mechanisms of Action

The antioxidant potential of methanol extracts is attributed to:

- Hydrogen atom donation by polyphenols [35, 73-88].
- Metal ion chelation, reducing Fenton reaction-mediated radical generation [36].
- Upregulation of endogenous antioxidant enzymes such as SOD, CAT, and GPx in experimental models [37].

Hexane extracts, lacking abundant phenolics, show limited antioxidant activity but may contribute indirectly by modulating lipid peroxidation pathways [38].

Extract/Fraction	Assay Type	IC_{50} / Activity Value	Remark	References
Methanol (hot, Soxhlet)	DPPH	134.8 µg/mL	Strong activity; phenolic-rich fraction	[32,67-72]

Methanol (leaf)	DPPH	12.2 µg/mL	Strongest radical scavenging effect	[34]
Ethyl acetate fraction	ABTS/FRAP	High activity	Highest antioxidant among fractions	[33,172]
Hexane fraction	DPPH	Weak activity	Low phenolic content	[38]

Table 5. Antioxidant activity of ***T. arjuna*** bark extracts

5.4 Methanolic Extracts

- DPPH radical scavenging IC_{50} values: 18–24 µg/mL, comparable to ascorbic acid [84].
- High correlation between polyphenol content and FRAP values [85].
- Effective in lipid peroxidation inhibition assays [86,137].

5.5 Hexane Extracts

- Weaker DPPH scavenging activity (IC_{50}: 80–110 µg/mL) [87].
- Moderate antioxidant activity attributed to sterols and triterpenoids [88].

Assay	**Methanol Extract (IC_{50}/Activity)**	**Hexane Extract (IC_{50}/Activity)**	**Reference(s)**
DPPH Scavenging IC_{50}	18–24 µg/mL	80–110 µg/mL	[89–91]
FRAP Value	450–580 µM Fe(II)/g extract	120–210 µM Fe(II)/g extract	[92,93]
ABTS Radical Scavenging	78–85% at 100 µg/mL	22–36% at 100 µg/mL	[94,95]
Lipid Peroxidation (%)	65–72% inhibition	28–34% inhibition	[96]

Table 6: Antioxidant assay results for methanolic and hexane Extracts

6. Antimicrobial and Antibacterial Activity

6.1 Methanolic Extracts

Methanolic bark extracts show significant antibacterial activity, particularly against Gram-negative bacteria. In disc diffusion assays, inhibition zones of 12–19 mm were reported against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi at concentrations of 500 µg/disc [39, 62-66].

The broad-spectrum activity of methanolic extracts is attributed to flavonoids, tannins, and glycosides, which can disrupt microbial cell walls, inhibit protein synthesis, and interfere with quorum sensing [40].

6.2 Hexane Extracts and Essential Oils

Hexane extracts exhibit weaker antibacterial effects compared to methanol. However, essential oils obtained from bark via hydrodistillation (yield ≈ 0.18%) demonstrated 22 mm inhibition zones against multidrug-resistant Acinetobacter baumannii, Klebsiella pneumoniae, and Staphylococcus aureus [41]. The oil also showed 68% DPPH scavenging activity, comparable to ascorbic acid (81%) [42].

6.3 Minimum Inhibitory Concentrations (MICs)

MIC studies indicate that bark methanol extracts achieve MICs of 39 µg/mL against Proteus vulgaris, while leaf extracts showed similar potency against Bacillus subtilis [55-61]. Hexane fractions generally exhibit higher MIC values (>200 µg/mL), reflecting lower antimicrobial efficacy [43].

6.4 Antifungal Activity

Methanolic extracts also inhibit fungal growth. For example, inhibition zones of 15–18 mm have been reported against Candida albicans and Aspergillus niger [44]. The antifungal activity is linked to tannins and saponins, which disrupt fungal cell walls and membranes [45].

Pathogen	Methanol	Assay/Zone of Inhibition	MIC (µg/mL)	References
E. coli	Methanol	14–16 mm (disc diffusion)	78–156	[39,40]
S. aureus	Methanol	15–18 mm	39–78	[39]
P. vulgaris	Methanol	16–17 mm	39	[34]
A. baumannii (MDR)	Bark oil	22 mm	100–150	[41,42]

Candida albicans	Methanol	15 mm	100	[44,45]

Table 7. Antimicrobial activities of ***T. arjuna*** bark extracts

7. Applications of *Terminalia arjuna*

7.1 Cardiovascular Applications

T. arjuna has long been used in traditional Ayurvedic medicine as a cardiotonic. Clinical studies have shown that methanolic bark extracts improve left ventricular function in patients with ischemic cardiomyopathy [46]. Flavonoids, glycosides, and triterpenoids such as arjunolic acid contribute to antihypertensive, anti-ischemic, and hypocholesterolemic effects [47, 40-44].

7.2 Anticancer Applications

Polyphenolic compounds, including gallic and ellagic acids, exhibit cytotoxic activity against cancer cell lines such as MCF-7 (breast cancer) and HeLa (cervical cancer) [48]. Extracts trigger apoptosis via mitochondrial pathways and inhibit tumor angiogenesis [49,50-54].

7.3 Antidiabetic and Hepatoprotective Uses

Methanol fractions rich in tannins and flavonoids significantly reduce blood glucose levels in streptozotocin-induced diabetic rats [50]. Similarly, hepatoprotective effects have been attributed to triterpenoids that prevent CCl_4-induced liver damage by enhancing endogenous antioxidant enzymes [51].

7.4 Industrial and Nutraceutical Potential

Food preservation: Methanolic extracts can be used as natural antioxidants in food packaging due to their strong radical-scavenging potential [52].

Cosmeceuticals: Flavonoid-rich extracts exhibit anti-aging and UV-protective properties, suitable for incorporation in creams and lotions [53].

Pharmaceuticals: Standardized arjunolic acid-rich extracts are being developed as cardioprotective nutraceuticals [54].

8. Toxicological and Safety Profile

Toxicological studies indicate that *T. arjuna* bark extracts are relatively safe at moderate doses. Acute toxicity studies in rodents show an $LD_{50} > 2000$ mg/kg for methanolic extracts [55]. Subchronic administration (up to 90 days) did not reveal hepatotoxic or nephrotoxic changes [56].

However, high doses may cause gastrointestinal irritation due to tannins [57]. Clinical caution is advised when co-administered with antihypertensive or cardiac glycoside drugs, as synergistic effects could potentiate hypotension or arrhythmias [58, 27-35].

9. Future Perspectives

Despite strong evidence of pharmacological potential, several gaps remain:

1. Standardization Issues – Variability in extraction methods and seasonal influence on phytochemical yield complicates reproducibility [59,33-38].

2. Bioavailability – Poor solubility and limited absorption of triterpenoids and polyphenols restrict clinical effectiveness [60].

3. Formulation Development – Nanoparticle-based delivery systems (liposomes, polymeric nanoparticles) are promising to enhance stability and bioavailability [61].

4. Clinical Validation – Most studies remain preclinical; large-scale randomized controlled trials are necessary to confirm efficacy and safety [62, 36-42].

5. Comparative Solvent Studies – Further direct comparisons between methanolic and hexane extracts in standardized assays will clarify their relative advantages [63].

10. Conclusion

The comparative study of methanolic and hexane extracts of Terminalia arjuna bark reveals that methanolic extracts are pharmacologically superior due to their rich phenolic and flavonoid profile, conferring strong antioxidant, antimicrobial, cardioprotective, and hepatoprotective properties. Hexane extracts, though less potent in radical scavenging, still offer lipophilic sterols and triterpenoids that contribute to antimicrobial and hypocholesterolemic effects.

Integrating *T. arjuna* bark extracts into pharmaceuticals, nutraceuticals, and cosmeceuticals holds immense promise. However, further standardization, bioavailability enhancement, and clinical trials are required to transition these findings into global therapeutic applications.

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