Natural Resources for Human Health

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Original Article

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Received 20 November 2025
Revised 19 January 2026
Accepted 29 January 2026
Available Online 30 June 2026

Edited by Kannan RR Rengasamy

KEYWORDS:
Liver disease
Terminalia arjuna
Anti-oxidant activity
Hepatoprotection
Network pharmacology

https://doi.org/10.53365/nrfhh/217525
eISSN: 2583-1194

Exploration of HPTLC and LC-MS metabolomic profiling and network pharmacology–based biomolecular targets of Terminalia arjuna (Roxb. ex DC.) Wight & Arn. in the treatment of hepatic complications

Arti sinoria[1], Garima Garg[1], Nitin Kumar[2]

[1]Department of Pharmacognosy, IIMT College of Medical Sciences, IIMT University, Ganga Nagar, Meerut, India
[2]Department of Pharmacognosy, IIMT College of Pharmacy, Greater Noida, UP, India

ABSTRACT: *Background*: Treatment for hepatic complications still remains a challenging issue for healthcare professionals due to lack of multitargeted and biomolecular-based therapeutic approaches. Moreover, contemporary therapeutic approaches often fail to achieve widespread and sustained utilization due to limitations related to affordability, accessibility and availability. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., a well-known Indian medicinal plant, is recognized for its antioxidant, anti-inflammatory, and cardioprotective properties. *Objectives*: This study aims to explore the metabolomic profiling (HPTLC and LC-MS) and network pharmacology–based biomolecular targets of *T. arjuna* in the treatment of hepatic disease. *Methodology*: Pharmacopoeial assessment was conducted to establish quality standards, and DPPH antioxidant activity was analyzed to evaluate free radical scavenging. Phytochemical insights and biomolecular approaches were revealed through HPTLC and LC-MS technique and network pharmacology analysis. Network pharmacology and gene ontology analyses further examined the plant's multitargeted effects on hepatic disorders. *Results*: The results confirmed that *T. arjuna* meets pharmacopoeial quality standards, with notable DPPH radical scavenging activity (IC_{50} = 182.2 ± 1.846 µg/mL). Metabolomic analysis revealed significant marker compounds, while network pharmacology identified TP53, IL6, and HFE as primary targets interacting with gallic acid, ellagic acid, arjunolic acid, procyanidin, and catechin. These interactions regulate liver carcinoma, hepatocellular carcinoma risk, hypogammaglobulinemia, and liver cirrhosis. *Conclusion*: The study concludes that *T. arjuna* effectively modulates TP53, IL6, and HFE expression, providing a promising natural approach for liver disorders. However, further preclinical and clinical studies are needed to bridge existing scientific gaps and validate its therapeutic efficacy.

1. INTRODUCTION

Liver disease, or hepatotoxicity, involves progressive hepatocyte damage, leading to fibrosis or hepatitis. The liver's detoxification process is crucial, as it metabolizes drugs, toxins, and xenobiotics. Severe liver diseases affect bile secretion, which is essential for digestion. Liver failure and hepatitis can be caused by infections, autoimmune disorders, excessive

* Corresponding author.
E-mail address: sinoriya.arti2@gmail.com (Arti Sinoria)

alcohol use, and toxic substances, including antibiotics, chemotherapeutics, and chlorinated hydrocarbons. Drug abuse is a leading cause of liver diseases, contributing to morbidity and mortality by harmful substances such as thioacetamide, carbon tetrachloride, and certain medications like paracetamol can damage liver cells, affecting lipid membranes, proteins, and nucleic acids (Ali et al., 2023; Salar et al., 2023).

Liver disease remains a major contributor to global mortality, with a significant rise in fatalities over the past decades. According to the Global Burden of Disease 2019 study, cirrhosis and other chronic liver diseases claimed 1.26 million lives in 2019, reflecting a 13% increase since 1990. In addition, liver cancer resulted in approximately 830,000 deaths in 2020, accounting for 8.3% of all cancer-related fatalities worldwide. Viral hepatitis, particularly HBV and HCV, continues to be a critical concern, leading to an estimated 1.3 million deaths annually. Alcohol-associated liver disease (ALD) affects around 3.3 million individuals each year, contributing to 5.9% of global deaths. Mortality rates vary significantly by region, with Mongolia experiencing high mortality of patients with liver cancer at the rate of 71.0 per 100,000 people, compared to 6.6 in the United States ((Gan et al., 2025; Ghulam et al., 2022).).

Modern therapeutic strategies continue to be constrained by issues of affordability, availability and accessibility and are further limited by the occurrence of significant adverse drug reactions. Medicinal plants are widely regarded as effective, affordable, and accessible options for hepatoprotection. Traditional medicine systems such as Ayurveda, Siddha, and Unani rely on plant-based treatments. Herbal drugs have gained popularity due to their safety and efficacy. *Terminalia arjuna*, a medicinal tree belonging to the Combretaceae family, is widely recognized for its therapeutic properties, traditionally. It is native to the Indian subcontinent, thrives in tropical and subtropical regions, particularly along riverbanks and dry forests in India, Bangladesh, and Sri Lanka. Phytochemical studies reveal that *T. arjuna* (Roxb. ex DC.) Wight & Arn. is rich in bioactive compounds, including flavonoids, tannins, glycosides, saponins, and triterpenoids. It exhibits cardioprotective, antihypertensive, and antidiabetic properties (Bachheti et al., 2022; Dev et al., 2021; Saha et al., 2012; Uddin et al., 2021).

To comprehensively evaluate metabolomics-based quality assessment and hepatoprotective potential, the study is aimed to explore the quality aspects of *T. arjuna* using modern analytical techniques such as high-performance thin-layer chromatography (HPTLC) and liquid chromatography-mass spectrometry/gas chromatography-mass spectrometry (LC-MS/GC-MS) (Gautam et al., 2021, 2023). These two techniques are the most advanced, robust, and sensitive chromatographic techniques. HPTLC gives the visual chromatographic pattern of phytochemicals present in the plant matrix while LC-MS demonstrates the components present in the medicinal plants. LC-MS-based metabolomics and network pharmacology combinedly provide the molecular insights via exploring the molecular genes targets associated with the treatment of liver disease. Network pharmacology outcomes are hypothetically validating and evaluating the effect of phytochemicals in the treatment of hepatic disease or liver disease (Gaurav, 2022; Gaurav et al., 2022, 2023; Salar et al., 2023). However, this is the first study that is being reported which specially targets the multitargeted and therapeutic effect of *T. arjuna* in the treatment of liver disease. The findings could support the development of new, plant-based hepatoprotective therapies, leveraging the natural, readily available, and cost-effective properties of medicinal plants to address liver diseases with minimal side effects.

2. MATERIAL AND METHODS

2.1. Chemicals, reagents, and reagents

Chemicals and Reagents: Methanol, ethanol, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH), HPTLC, LC-MS, UV-Vis spectrophotometer, rotary evaporator, analytical balance, centrifuge, and micropipettes. These materials and tools are essential for phytochemical screening, antioxidant (DPPH) activity assay, total phenol and flavonoid content determination, and advanced chromatographic and spectrometric analyses of *T. arjuna*.

2.2. Collection and authentication of plant

The collection and authentication of *T. arjuna* (Report code: 368) plants from CCRAS, Jhansi, India, involve meticulous botanical identification and verification processes. Plant samples are collected from specific geographical locations, ensuring authenticity through morphological and anatomical characterization. Authentication at CCRAS adheres to rigorous botanical standards, confirming the species' identity based on established taxonomic criteria. This meticulous approach guarantees that the plant material used in research is scientifically validated, maintaining the integrity and reliability of studies investigating *T. arjuna's* pharmacological properties. The authentication no. of the plant was 368.

2.3. Macroscopy and microscopy studies

The methodology for powder microscopy studies on *T. arjuna* (bark) involved a detailed examination of the plant

materials using both visual and microscopic techniques. For macroscopic analysis, the specimens were collected and observed for distinct features such as size, shape, color, and texture of the bark of *T. arjuna*. The plants were identified based on their characteristic morphological traits. For powder microscopy, both plant materials were dried, finely powdered, and mounted on slides using appropriate solvents. The prepared slides were then examined under a light microscope to observe cellular structures, including epidermal cells, trichomes, and vascular bundles. The presence of specific anatomical features, such as starch grains and other inclusions, was noted. All observations were recorded with high-resolution photographs for record purpose (Prakash Chaudhary et al., 2018).

2.4. Pharmacopoeial standards examination

In this study, loss on drying (LOD) was determined using a hot air oven at 105°C until a constant weight was achieved. Approximately, 1 g of *T. arjuna* (bark) powder was weighed, dried, and cooled in a desiccator, and reweighed to calculate moisture content. For total ash determination, 1 g of the sample was incinerated in a muffle furnace at 550°C until complete oxidation. The remaining inorganic residue was weighed, and acid-insoluble ash was determined by boiling the ash with 2N HCl, filtering, washing, drying, and re-incinerating the residue at 550°C. For alcohol-soluble extractives, 5 g of bark powder was macerated in 100 mL alcohol for 24 hours, filtered, evaporated, and dried at 105°C. Similarly, water-soluble extractives were determined by macerating in 100 mL distilled water, filtering, and drying. The final residues represented the respective extractive contents (Prakash Chaudhary et al., 2018).

2.5. Preparation of extract

About 200 g of *T. arjuna* (Bark) powder was accurately weighed and soaked in 1 L of distilled water in a glass container for 72 hours with occasional stirring. The maceration process allowed the efficient extraction. After maceration, the mixture was filtered using filter paper to separate the liquid extract from the plant residue. The extract was then concentrated using a water bath at 80°C until a viscous residue remained (Gaurav et al., 2022).

2.6. Phytochemical evaluation

The phytochemical analysis of *T. arjuna* (bark) was performed using standard methods. Phytosterols were detected via the Salkowski test, where a reaction with H_2SO_4 produced a reddish-brown color in the chloroform layer. Triterpenoids were identified using Liebermann–Burchard's test, forming a brown ring and deep red coloration. Saponins were confirmed by a foam test, showing persistent foam for 10 minutes. Alkaloids reacted with Dragendorff's reagent, forming an orange-red precipitate. Carbohydrates were identified using Molisch's test, producing a reddish-violet ring. Flavonoids formed a yellow precipitate with lead acetate, while lactones produced a deep red color in Legal's test. These analyses followed established protocols to determine the phytochemical composition of the sample (Rusmana et al., 2017; Sandhya Kumari and Singara Charya, 2017).

2.7. DPPH free radical scavenging activity

The DPPH antioxidant activity of *T. arjuna* aqueous extract was assessed using ascorbic acid as a standard reference across concentrations (62.5–1000 µg/mL). Firstly, a 0.1 mM DPPH solution in methanol was prepared. Sample solutions of *T. arjuna* extract and standard solutions of ascorbic acid were then prepared in methanol at the specified concentrations. Each solution (1 mL) was mixed with 2 mL of the DPPH solution and incubated in the dark for 30 minutes. After incubation, the absorbance of each solution was measured at 517 nm using a spectrophotometer, with a water blank serving as reference. Percentage inhibition of DPPH radicals was calculated using absorbance values, and the results were used to plot inhibition (%) versus concentration (µg/mL) curves for both *T. arjuna* extract and ascorbic acid. This method enables comparison of antioxidant activity, typically expressed through IC_{50} values or inhibition percentages, between *T. arjuna* and ascorbic acid, as a standard antioxidant compound (Gaurav et al., 2022, 2023; Salar et al., 2023).

2.8. HPTLC analysis

A sample (50 mg) of *T. arjuna* extract was accurately weighed and dissolved in 2 mL of methanol via proper vortexing and vigorous shaking. Thereafter, the sample was filtered using PEP Syring filter of 0.22µ and applied on the TLC plate using Camag Linomat HPTLC applicator. The application volume of the sample for each track was maintained at 4 µL.

Following sample application, the TLC plates were air-dried and subsequently developed in a pre-saturated TLC chamber containing a solvent system of toluene: ethyl acetate: glacial acetic acid (4 :5: 1, v/v/v) for Terminalia arjuna.

The development chamber was allowed to saturate for 15 minutes prior to plate development.

The plates were developed to migration distances of 80 mm and 70 mm respectively. After development, the plate was taken off from the chamber and dried using an air drier and visualized under Camag UV visualizer at day light, short wavelength (254 nm), and log wavelength (366 nm), followed by scanning using TLC scanner 4 at 254 nm and 366 nm. The chromatogram of the developed plate was recorded.

Thereafter, the plate was derivatized with anisaldehyde sulfuric acid reagent and heated to visualize the bands on each track. The images of the derivatized TLC plate was captured at 366 nm and day light view followed by scanning of derivatized plate at 366 nm and 520 nm. Each imaged and developed chromatogram were recorded for data analysis (Gaurav et al., 2020).

2.9. LC-MS analysis

For LC-MS analysis of *T. arjuna extract,* a stock solution with a concentration of 1 mg/mL was prepared by dissolving 100 mg of the sample in 100 mL of methanol. The analysis was conducted using an ACQUITY HPLC system (Waters Corp., USA) equipped with an Eclipse Plus C18, 4.6 mm × 150 mm, (5 µm) column, a binary solvent delivery system, an auto-sampler, and a tunable mass spectrometer detector controlled by Empower software (Waters, Manchester, UK). The mobile phase comprised acetonitrile (A) and water (B) in a 70:30 (v/v) ratio, operated in isocratic mode for compound separation. The flow rate was maintained at 1 mL/min, and the sample injection volume was set at 10 µl per run. The nebulizer and cone gas flow rates were adjusted to 500 L/h and 50 L/h, respectively, while the source temperature was kept at 100°C. The capillary voltage was set at 3.0 kV, and the cone voltage at 40 kV. Data acquisition and spectral analysis were performed using Mass Lynx V4.1 software (Waters, USA). Metabolite identification was conducted by evaluating the m/z values, which were cross-referenced with existing literature, the MassBank database, and PubChem for tentative characterization (Ibrahim et al., 2021; Salar et al., 2023).

2.10. Network pharmacology

2.10.1. Selection of genes

Genes associated with liver disease were retrieved from the GeneCards database (Table 1, Supplementary file). Searches were conducted using the keywords "liver disease," and results were filtered based on relevance scores, expression levels, and literature citations. Genes with high confidence and strong associations with the respective diseases were prioritized. Functional annotations and involvement in related biological pathways were reviewed for accuracy. Duplicates and nonspecific genes were excluded. A total of 97 genes were selected for each condition, ensuring comprehensive coverage of disease-relevant genes for further analysis, such as pathway enrichment and network pharmacology studies (Gautam, 2022).

2.10.2. Compounds and protein interaction study

The network pharmacology study was examined to determine the polypharmacological role of chemical constituents of *T. arjuna* in liver disease using network pharmacological approaches. This study was performed using a bioinformatic computational tool, that is, Cytoscape version 3.9.1 using an internal supported app, namely, STRING and STICH. In brief, for this analysis, 97 numbers of the genes were selected from gene card data based on their function in liver disease. Each gene was evaluated for its ligand and protein interaction and integrated for its partial and significant interaction. During the analysis, the genes that showed no interaction were removed during the interpretation analysis of the network. The developed network was imported with each partial and significant interaction (Gaurav, 2022; Gaurav et al., 2022; Gautam, 2022; Salar et al., 2023).

2.11. Gene ontology analysis

To perform this study, the Metascape tool was used to determine the multitargeted and therapeutic effect of metabolites of *T. arjuna* in liver disease. In this analysis, the genes that showed prominent or even partial interaction with the constituents of *T. arjuna* were selected and analyzed for determining their pathophysiological role via exploring the different pathways involved in the treatment of hepatic disease. The gene expression analysis related to *H. sapience* was characterized and gene ontology results were assessed to determine

Table 1
Extractive yield of *Terminalia arjuna* through the maceration method.

Parameters	*Terminalia arjuna* (Bark)	
Extractive yield	**Trial 1**	**Trial 2**
Initial weight of the drug (g)	201.47	205.26
Extractive weight of the drug (g)	46.97	50.00
Percentage of extractives (w/w)	23.31	24.36
Average weight (w/w)	23.84 ± 0.737	

pathways involved in the pathophysiology while DisGeNet analysis was assessed to determine the role of metabolites related to genes in liver disease and associated disorders (Gautam, 2022).

3. RESULTS AND DISCUSSION

The extraction of *T. arjuna* was found to be 23.845 ± 0.737% and 17.195 ± 1.638, respectively, aligning with the standards set by the Ayurvedic Pharmacopoeia of India, Part-1, Vol. 2, pages 17–18. This match confirms the extract's quality and consistency with established Ayurvedic benchmarks, ensuring its suitability for therapeutic applications as specified in traditional Indian medicine guidelines (Table 2).

3.1. Macroscopy and microscopy studies

3.1.1. Macroscopy and microscopic characterization of T. arjuna

T. arjuna has distinct macroscopic characteristics that aid in its identification and use in pharmacognosy. The outer bark of *T. arjuna* is typically gray to pale brown, with a smooth texture in young trees, which becomes rough and fissured in older trees. The inner bark is reddish-brown to pinkish, providing a stark contrast to the outer layer.

The outer surface of the bark is rough and scaly, marked with numerous vertical and horizontal fissures. Upon peeling, the inner bark reveals a fibrous texture. The bark has a faint, characteristic odor and astringent taste, which is indicative of its high tannin content. The bark thickness varies depending on the age of the tree, ranging from a few millimeters to over a centimeter in mature specimens. The bark is typically available in flat or slightly curved pieces, with lengths varying from a few centimeters to several decimeters, depending on how it is harvested and processed. The outer surface may show patches of lichens and mosses, especially in trees growing in humid environments. The inner surface is smooth and fibrous, often showing longitudinal striations. When broken, the bark shows a short, granular fracture on the outer side and a fibrous fracture on the inner side. These macroscopic characteristics are crucial for the preliminary identification and authentication of *T. arjuna* bark in herbal medicine and pharmacognostic studies (Table 3 and Figure 1).

The microscopy of *T. arjuna* bark powder reveals several distinct features that are crucial for its identification and quality control in pharmacognostic studies. The images display various microscopic structures, including fibers, sclereids, and parenchyma cells. The fibers are elongated, thick-walled, and often show tapering ends, which are characteristic of the supportive tissues in the bark. The presence of sclereids, which are thick-walled, lignified cells, provides structural support and contributes to the toughness of the bark. These sclereids appear as irregularly shaped, densely packed structures, indicative of their role in providing mechanical strength.

In addition, the parenchyma cells are visible, showcasing thin-walled structures that are involved in storage and basic metabolic functions. These cells often contain starch grains

Table 2
Extractive yield of Terminalia arjuna through the maceration method.

Parameters	*Terminalia arjuna* (Bark)	
Extractive yield	**Trial 1**	**Trial 2**
Initial weight of the drug (g)	201.47	205.26
Extractive weight of the drug (g)	46.97	50.00
Percentage of extractives (w/w)	23.31	24.36
Average weight (w/w)	23.84 ± 0.737	

Table 3
Macroscopic characteristics of *Terminalia arjuna* bark.

Characteristic	Description
Color	Outer: Gray to pale brown; Inner: Reddish-brown to pinkish
Texture	Outer: Rough, scaly; Inner: Smooth, fibrous
Odor	Faint, characteristic
Taste	Astringent
Thickness	Varies from a few millimeters to over a centimeter
Shape and Size	Flat or slightly curved pieces, varying lengths
Surface Characteristics	Outer: Vertical and horizontal fissures, patches of lichens and mosses; Inner: Longitudinal striations
Fracture	Outer: Short, granular; Inner: Fibrous

Figure 1. Macroscopic view of arjuna bark.

or other storage compounds, which can be seen as small granules within the cells. The presence of these granules, especially under polarized light, can confirm the identity of the bark powder. In some of the images, vascular elements like tracheids and vessels can be observed, which are essential for the conduction of water and nutrients within the plant. Moreover, the powder shows fragments of the epidermis, which is the outer protective layer of the bark. The epidermal cells are polygonal and may contain cuticular layers that help in protecting the inner tissues. The microscopy also reveals the presence of calcium oxalate crystals, which are common in many plant tissues and appear as bright, fringent structures under polarized light (Figure 2).

3.2. Pharmacopoeial standards examination

The analysis of *T. arjuna* (Bark) was conducted to determine its physicochemical properties, including LOD, total ash, acid insoluble ash, and extractive values. The results indicate that the LOD was found to be 4.01±0.44%, which suggests a relatively low moisture content, essential for preventing microbial growth and ensuring the stability of the plant material during storage.

The total ash content was determined to be 10.57 ± 0.52%, reflecting the presence of inorganic residues after incineration. This parameter is crucial in assessing the purity and quality of herbal drugs, as excessive ash content may indicate contamination or adulteration. The acid insoluble ash, measured at 1.92 ± 0.17%, represents the portion of ash that remains undissolved in acid, indicating the presence of silica and other earthy materials, often linked to environmental contamination or improper handling during processing.

The alcohol-soluble extractive value was recorded at 13.63 ± 0.63%, signifying the presence of bioactive compounds that are soluble in organic solvents, such as flavonoids, alkaloids, and glycosides. This parameter plays a significant role in evaluating the therapeutic potential of the bark. The water-soluble extractive value was found to be 19.64 ± 0.32%, highlighting the solubility of various phytoconstituents in aqueous mediums, which may contribute valuable insight into the quality, purity, and potential therapeutic efficacy of *T. arjuna* bark, supporting its traditional use in herbal medicine while ensuring compliance with standard quality parameters (Table 4 and Figure 3).

3.3. Phytochemical evaluation

The phytochemical analysis of the *T. arjuna* (Bark) revealed a diverse array of constituents as indicated by various chemical tests. Alkaloids, detected using the Dragendroff's test, were confirmed present (+), suggesting potential pharmacological activity. Carbohydrates, identified through the Molisch's test,

Table 4.
LOD, total ash, and acid insoluble ash of *Terminalia arjuna* (Bark).

Parameters	Results (%, w/w)
LOD	4.01 ± 0.44
Total ash	10.57 ± 0.52
Acid insoluble ash	1.92 ± 0.17
Alcohol soluble extractive	13.63 ± 0.63
Water soluble extractive	19.64 ± 0.32

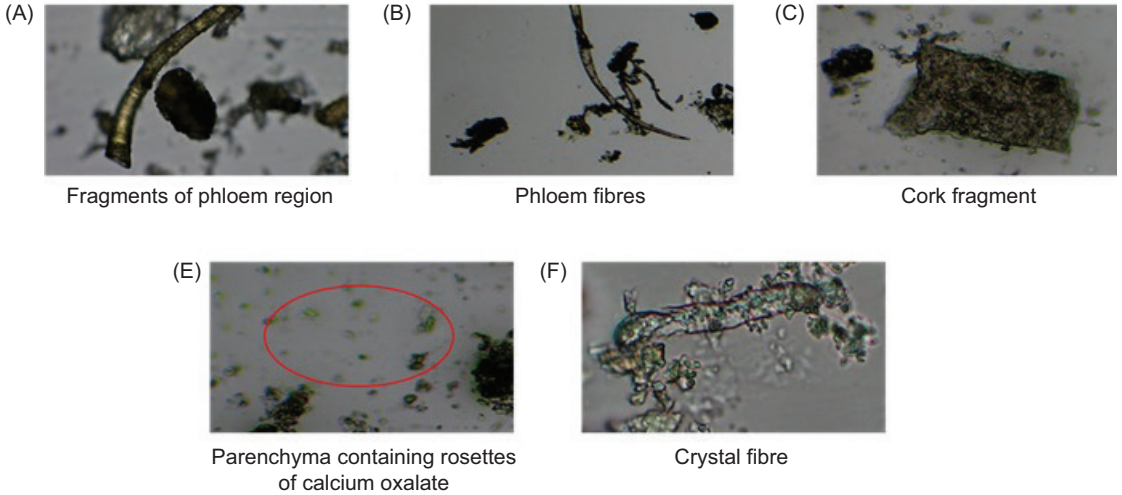


Figure 2. Powder microscopy of arjuna bark.

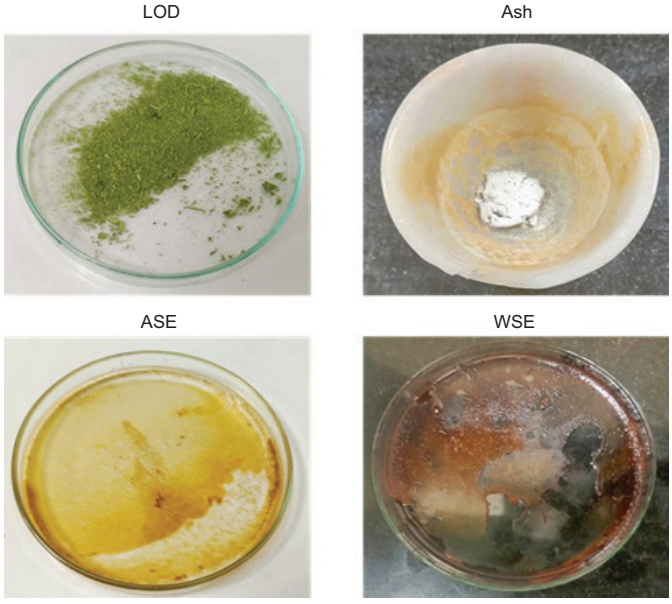


Figure 3. Experimental snaps for LOD, ash, ASE and WSE.

were also found (+), indicating the sample's nutritional value. Flavonoids, revealed by the lead acetate test (++), imply antioxidant properties beneficial for health. Glycosides, identified by the Keller–Killiani test (++), may contribute to therapeutic effects due to their bioactive nature. Lactones, detected using the Legal's test (+), suggest potential medicinal properties, while phenolic compounds and tannins, confirmed by the 5% FeCl3 test (++), indicate antioxidant and anti-inflammatory potentials. Phytosterols, identified by the Salkowski reaction (+), are known for their cholesterol-lowering effects. Proteins, detected by the Ninhydrin test (+), highlight the sample's nutritional content. Saponins, found through the Foam test (+), indicate potential detergent and medicinal properties. Triterpenoids, identified by Liebermann–Burchard's test (+), suggest anti-inflammatory and antimicrobial activities. Moreover, the presence of these phytoconstituents underscores the sample's rich chemical composition with various bioactive compounds beneficial for health and potential therapeutic applications. Further studies could explore specific biological activities and mechanisms of action associated with these phytochemicals to harness their full potential in medicine and nutrition (Table 5).

3.4. DPPH free radical scavenging activity

DPPH anti-oxidant activity of *T. arjuna* (Bark) extract was performed as per the referenced protocol. The study evaluated the antioxidant potential of *T. arjuna* by assessing its DPPH free radical scavenging activity at various concentrations,

Table 5
Preliminary phytochemical analysis of Terminalia arjuna (Bark) extract.

S. No	Phytoconstituents	Tests	*Terminalia arjuna (Bark)*
1.	Alkaloids	Dragendroff's test	–
2.	Carbohydrates	Molisch's test	+
3.	Flavonoids	Lead Acetate test	+
4.	Glycosides	Keller–Killiani test	+
5.	Phenolic compounds and tannins	5% $Fecl_3$ Test	+
6.	Phytosterols	Salkowski reaction	–
7.	Proteins	Ninhydrin test	+
8.	Saponins	Foam test	+
9.	Triterpenoids	Liebermann–Burchard's test	+

using ascorbic acid as a standard. The results showed a concentration-dependent increase in antioxidant activity for all tested samples. Ascorbic acid exhibited the highest scavenging activity, with values ranging from 58.39% at 62.5 µg/mL to 93.78% at 1000 µg/mL, and an IC_{50} value of 180.4 µg/mL.

Among the plant extracts, *T. arjuna* demonstrated stronger antioxidant activity. At 62.5 µg/mL, *T. arjuna* showed 31.39% scavenging activity, increasing to 74.76% at 1000 µg/mL, with an IC_{50} value of 182.2 ± 1.846 µg/mL. These findings indicate that *T. arjuna* extracts possess antioxidant properties, though *T. arjuna* is more effective. The observed free radical scavenging activity suggests that these plants could be valuable natural sources of antioxidants. The outcome of the study has been represented in Table 6 and Figure 4.

These findings suggest that while ascorbic acid is a more potent antioxidant at lower concentrations, the aqueous extract shows considerable radical scavenging activity, particularly at higher concentrations. This highlights the potential of the aqueous extract as a natural antioxidant source, beneficial for developing health supplements or therapeutic agents. The effectiveness of the aqueous extract in scavenging

Table 6
DPPH free radical anti-oxidant activity.

Concentration (µg/mL) of DPPH free radical scavenging activity (%)	Ascorbic acid	*Terminalia arjuna*
62.5	58.394 ± 0.739	31.3892 ± 0.485
125	69.347 ± 0.934	46.3272 ± 0.945
250	83.347 ± 1.734	59.2934 ± 0.782
500	89.346 ± 0.993	71.8923 ± 0.956
1000	93.784 ± 1.128	74.7621 ± 0.838
IC_{50} value	180.4 ± 1.399	182.2 ± 1.846

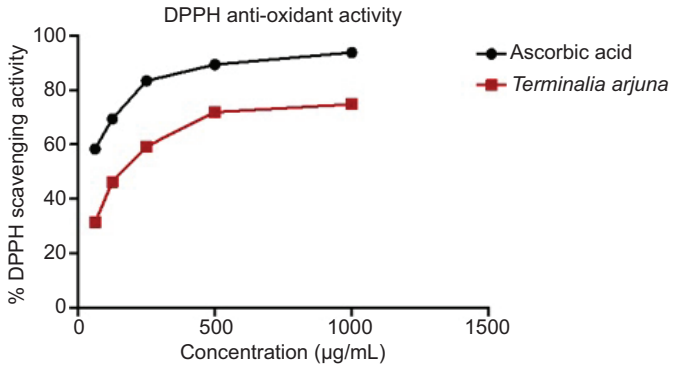


Figure 4. DPPH free radical anti-oxidant activity.

radicals indicates that it contains bioactive compounds capable of donating electrons to neutralize free radicals. The slight variations in standard deviations across concentrations demonstrate the consistent performance and reliability of the aqueous extract's antioxidant properties.

Moreover, both ascorbic acid and the aqueous extract exhibit significant radical scavenging activities in a concentration-dependent manner. Although ascorbic acid demonstrates higher efficacy across all concentrations, the aqueous extract shows promising antioxidant potential, especially at higher concentrations. These results support the use of the aqueous extract as an effective natural antioxidant, potentially contributing to the development of alternative therapeutic agents aimed at combating oxidative stress–related diseases.

3.5. HPTLC analysis

The HPTLC analysis of *T. arjuna* bark extract was performed to confirm the presence of ellagic acid and to quantify its relative concentration. The study was conducted using CAMAG instrumentation under controlled chromatographic conditions. Silica gel 60 F_{254} plates (E. Merck KGaA, Germany) were used as the stationary phase. The mobile phase system consisted of toluene: ethyl acetate: methanol: formic acid in the ratio of 10: 9: 6: 5 (v/v/v/v), which provided good separation and resolution of the phytoconstituents. The chromatogram was developed in a twin-trough glass chamber of 10 × 10 cm size, pre-saturated with the mobile phase vapors. The solvent front was allowed to migrate up to 80 mm. After development, the plate was air-dried in an oven at 60°C for 5 min before detection. Detection was carried out at 254 nm using a CAMAG TLC Scanner 3 equipped with a deuterium lamp (D_2). The scanning speed was maintained at 20 mm s^{-1} with a data resolution of 100 µm $step^{-1}$. The plates were visualized under UV light at 254 nm to detect fluorescent or quenching zones corresponding to ellagic acid and other phenolic compounds present in the extract. Digital documentation of the developed plate was performed using a CAMAG Reprostar 3 system.

The chromatogram of the **standard ellagic acid** exhibited a single, well-defined peak at **Rf = 0.54,** with a maximum peak height of **305 AU** and an area of **11779.5 AU**. This peak served as the reference for identifying the same compound in the bark extract. The *T. arjuna* sample displayed a similar single peak at **Rf = 0.52**, with a maximum height of **282.6 AU** and an area of **11642.5 AU**, closely matching the standard. The near-identical Rf values (0.54 for standard vs. 0.52 for sample) and comparable peak shapes confirm the presence of **ellagic acid** as a major phenolic component in the bark extract.

The results indicate that the solvent system employed achieved a high degree of specificity for ellagic acid, as no overlapping peaks or impurities were observed near the Rf region of interest. The absence of tailing and the sharpness of the peaks reflect good plate activity and optimal saturation of the mobile phase chamber. The data validate the suitability of the chromatographic system for ellagic acid quantification in *T. arjuna* formulations.

The developed HPTLC plate was visualized and recorded at **254 nm** (Figure 5). The documentation shows distinct bands corresponding to standard ellagic acid and the test sample. Both exhibit prominent quenching zones at similar migration distances, confirming identity. The photographic documentation was taken under automatic exposure mode (966.23 ms, gain = 1.0) with a white balance adjustment (R: 1.40; G: 1.00; B: 1.20). The plate image demonstrates clear separation and

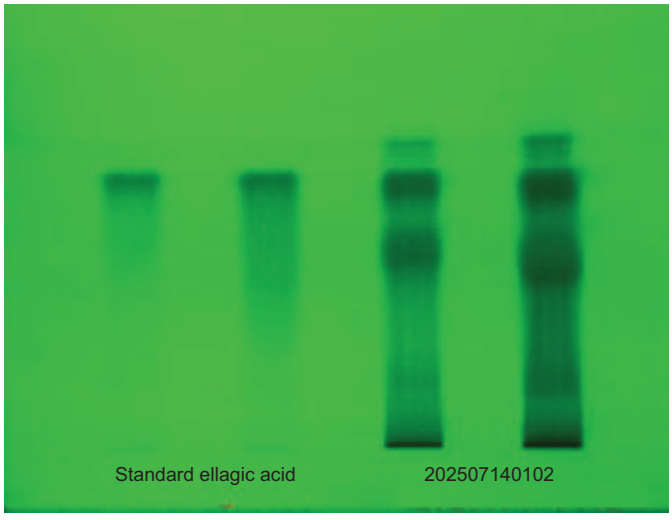


Figure 5. Developed TLC chromatograms of *Terminalia arjuna* standard ellagic acid visualized at 254 nm.

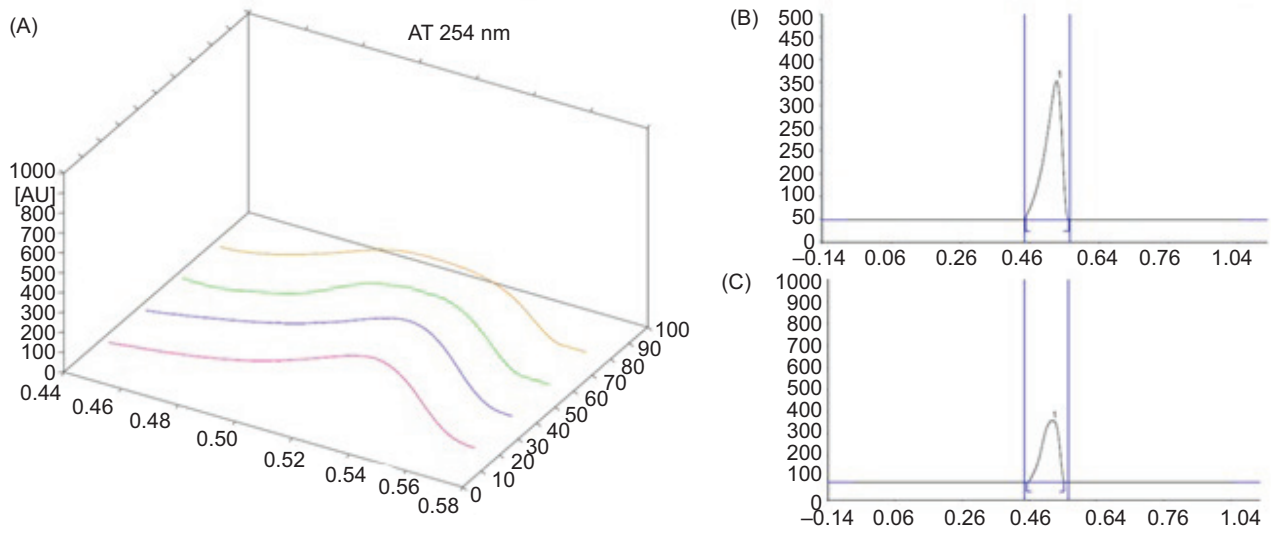


Figure 6. HPTLC chromatographic profiles of standard and sample tracks scanned at 254 nm. (A) Three-dimensional overlay chromatogram showing all peaks at various concentrations; (B) and (C) represent densitograms of track 1 (standard) and track 3 (sample), respectively, exhibiting a distinct peak at Rf value 0.46 corresponding to ellagic acid.

Table 7
Details of *Terminalia arjuna* standard ellagic acid at 254 nm R_f value and its peak area and standard assigned compound.

Sample	Rf value	Peak height (AU)	Area	Assigned compound
Ellagic acid	0.54	305	11779.5	Ellagic acid
T. arjuna bark extract	0.52	282.6	11642.5	Ellagic acid

intensity proportional to the applied concentration. The close correlation of Rf values between the standard and the extract demonstrates the authenticity of ellagic acid within the *T. arjuna* bark matrix. The high degree of similarity in both peak height and area signifies that ellagic acid is present in considerable quantity and remains stable under the experimental conditions. The chromatographic fingerprint also aligns with previously published research reporting ellagic acid as one of the major polyphenolic antioxidants responsible for the cardioprotective and hepatoprotective activity of *T. arjuna*.

This analysis further supports that HPTLC is an efficient, rapid, and cost-effective technique for the qualitative and quantitative estimation of ellagic acid in complex herbal matrices. The validated method ensures repeatability, specificity, and precision suitable for routine quality control and standardization of herbal formulations containing *T. arjuna* bark extract.

3.6. LC-MS analysis of *T. arjuna*

The LC-MS analysis of *T. arjuna* revealed the presence of ten bioactive compounds, each identified based on their mass-to-charge (m/z) values, ionization modes, and comparison with theoretical masses. The detected compounds included gallic acid, catechin, arjunolic acid, terminic acid, corilagin, ellagic acid, ethyl gallate, embelin, arjungenin, and procyanidin. Gallic acid (m/z 169.42) was detected in [M-H]- mode, consistent with the values reported by Manu et al. (2019). Ethyl gallate (m/z 175.11) was identified in [M-Na]+ mode, as previously described by Saha et al. (2012). Catechin (m/z 273.04) and procyanidin (m/z 593.51), detected in [M-NH4]- and [M-H]- modes respectively, are known for their cardioprotective effects.

Triterpenoids such as arjunolic acid (m/z 488.29) and arjungenin (m/z 502.30) were identified in [M-H]- and [M-2H]+ modes, respectively, corroborating the results of Carvalho et al. (2022). Terminic acid (m/z 473.45), detected in [M+H]+ mode, matches findings from Md. (2013). Corilagin (m/z 635.35) and ellagic acid (m/z 303.47) were observed in [M-H]+ and [M+H]+ modes. Embelin (m/z 255.15), identified in [M-K] mode, was consistent with data reported by Md. (2013) and Bachheti et al. (2022). The LC-MS profiling demonstrated a rich phytochemical composition comprising phenolic acids, flavonoids, triterpenoids, and ellagitannins. These compounds are pharmacologically significant, particularly for their antioxidant, cardioprotective, and anti-inflammatory properties. The presence of gallic acid, catechin, and procyanidin highlights the plant's potential for managing oxidative stress and cardiovascular diseases (Amalraj and Gopi, 2017; Manu et al., 2019; Saha et al., 2012).

Triterpenoids like arjunolic acid and arjungenin validate *T. arjuna's* traditional use in treating heart ailments

(Carvalho et al., 2022). Ellagic acid and corilagin underscore its anti-cancer potential, while embelin points to its role in combating microbial infections (Bachheti et al., 2022; Md., 2013). The detected masses aligned closely with theoretical values, confirming the accuracy of the identification. This analysis affirms the therapeutic relevance of *T. arjuna*, supporting its extensive use in traditional and modern medicine.

3.7. Network pharmacology study of *T. arjuna*

The network pharmacology study of *T. arjuna* focuses on the interaction of its bioactive compounds with target proteins, the association of identified genes with diseases, and the results of DisGeNET analysis as compound–protein interaction, gene-disease association network, and a bar chart of DisGeNET analysis. The network reveals the interactions between bioactive compounds in *T. arjuna* such as gallic acid, ellagic acid, arjunolic acid, procyanidin, and catechin—and their target proteins, including TP53, HFE, and IL6. These interactions highlight the therapeutic potential of the compounds in modulating key biological pathways. Gallic acid and ellagic acid appear to play a central role in this network, as evidenced by their multiple interactions with TP53 and IL6, which are crucial proteins involved in inflammation, cellular repair, and immune responses. The inclusion of HFE, a gene associated with iron homeostasis, suggests the potential of *T. arjuna* in managing conditions related to iron overload, such as hemochromatosis. In addition, the network provides insights into the complementary interactions between these compounds, suggesting a synergistic effect in targeting these proteins.

In the association between genes targeted by *T. arjuna* compounds and diseases, genes such as TP53, IL6, and HFE are linked to a wide range of liver-related disorders and systemic conditions. IL6, a pro-inflammatory cytokine, is prominently associated with liver cirrhosis, alcoholic liver disease, and chemical-induced liver injury, emphasizing its pivotal role in inflammatory pathways. TP53, a tumor suppressor gene, is linked to liver carcinoma and increased risk of hepatocellular carcinoma, indicating the potential anticancer properties of *T. arjuna*. HFE is associated with conditions such as hemochromatosis and abnormal glucose tolerance, reflecting its role in metabolic and iron-related diseases. The network also shows connections to diseases beyond the liver, such as inflammation and hyperpigmentation, suggesting broader therapeutic implications of the bioactive compounds.

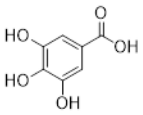
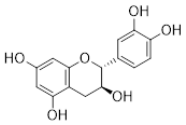
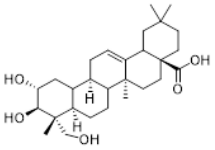
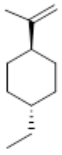
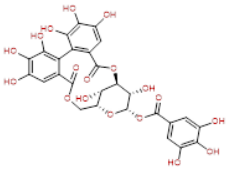
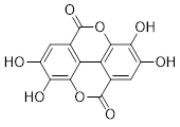
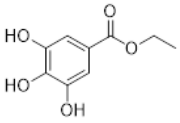
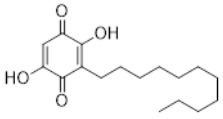
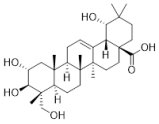
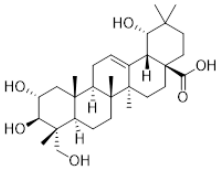
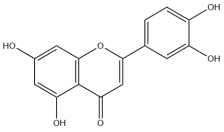
The DisGeNET analysis results are presented through a bar chart, showcasing diseases associated with the target genes. Chronic viral hepatitis, hemochromatosis type 1, acquired hypogammaglobulinemia, and liver cirrhosis (alcoholic and nonalcoholic) are among the most significant conditions identified. The high association with chronic viral hepatitis underscores the potential of *T. arjuna* in addressing liver-related viral infections. Similarly, its relevance to hemochromatosis highlights its therapeutic application in managing iron-related disorders. Other diseases, such as brain infarction, amyotrophic lateral sclerosis, and osteoarthritis, suggest a broader systemic impact of the bioactive compounds. This highlights the versatility of *T. arjuna* in targeting multiple diseases, potentially through the modulation of inflammation, oxidative stress, and metabolic pathways. The analysis collectively underscores the therapeutic potential of *T. arjuna* and its bioactive compounds in treating liver diseases and systemic disorders. The compound–protein interaction network (Figure 6A) highlights key molecular targets, while the gene–disease association (Figure 6B) connects these targets to specific conditions, and the DisGeNET analysis (Figure 6C) further validates these associations. This integrative approach demonstrates the importance of network pharmacology in understanding the multifaceted effects of traditional medicinal plants like *T. arjuna*. Further experimental and clinical studies are warranted to validate these findings and explore the precise mechanisms underlying its therapeutic potential (Figures 7 and 8).

The network pharmacology analysis of *T. arjuna* provides valuable insights into its therapeutic potential, particularly in liver diseases and systemic disorders. The results indicate that bioactive compounds such as gallic acid, ellagic acid, arjunolic acid, procyanidin, and catechin interact with key proteins, including TP53, IL6, and HFE, which play pivotal roles in various biological pathways. This highlights the multitargeted pharmacological effects of *T. arjuna* and its potential for managing complex diseases.

In the compound–protein interaction network, gallic acid and ellagic acid emerge as central compounds due to their multiple interactions with target proteins. TP53, a tumor suppressor gene, is crucial for cellular repair and apoptosis regulation, suggesting that these compounds may possess anticancer properties, particularly in liver carcinoma. IL6, a pro-inflammatory cytokine, is linked to inflammation and immune regulation, indicating that *T. arjuna* may alleviate inflammatory liver conditions such as cirrhosis and alcohol-induced liver injury. The interaction with HFE, associated with iron homeostasis, further supports its application in managing metabolic disorders like hemochromatosis. The complementary interactions of these compounds suggest potential synergistic effects, amplifying their therapeutic efficacy (Cota et al., 2019; Jawal et al., 2024; Verma and Jogdand, 2021).

Brighenti et al. highlight the critical role of IL-6 in linking chronic inflammation to tumorigenesis through p53 downregulation. IL-6 stimulates c-MYC translation, enhancing

Table 8
LC-MS metabolites profiling of *Terminalia arjuna*.

Sr. No.	Compound name	Chemical structure	Identified mass	Theoretical mass	Ionization mode	References
1.	Gallic acid		187.1	170.12	$[M+H_2O]^-$	(Manu et al., 2019)
2.	Catechin		251.2	290.26	$[M-K]^-$	(Amalraj and Gopi, 2017)
3.	Arjunolic acid		469.3	488.70	$[M- H_2O]-$	(Carvalho et al., 2022)
4.	Terminic acid		473.2	472.7	$[M+H]^+$	(Md., 2013)
5.	Corilagin		634.5	636.46	$[M-+2H]^+$	(Md., 2013)
6.	Ellagic acid		301.1	302.19	$[M-H]^+$	(Saha et al., 2012)
7.	Ethyl gallate		199.1	198.17	$[M+H]^+$	(Saha et al., 2012)
8.	Embelin		293.2	294.18	$[M-H]^-$	(Md., 2013), (Bachheti et al., 2022)
9.	Arjungenin		505.2	504.70	$[M+H]^+$	(Carvalho et al., 2022)
10.	Procyanidin		593.5	594.5	$[M-H]^-$	(Saha et al., 2012)
11.	Luteolin		284.2	286.24	$[M-2H]^-$	(Tahir et al., 2025)

ribosome biogenesis and promoting MDM2-mediated p53 degradation. This mechanism fosters epithelial–mesenchymal transition, increasing invasiveness and reducing apoptosis. Anti-inflammatory treatment reverses these changes, emphasizing its therapeutic potential (Brighenti et al., 2014).

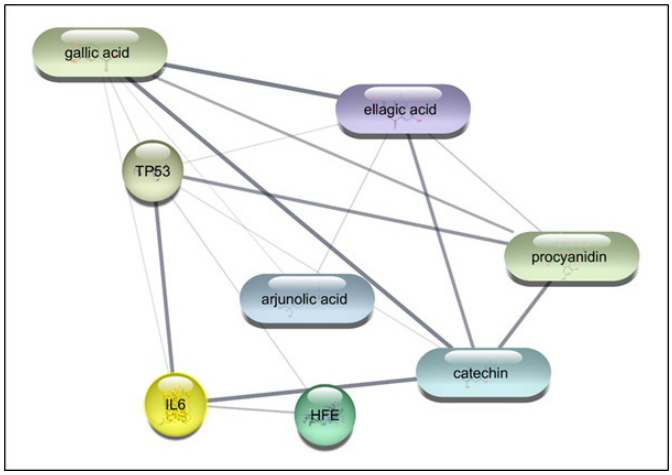


Figure 7. The role of metabolites from *Terminalia arjuna* in hepatotoxicity or liver disease. The network reveals the interactions between bioactive compounds in *T. arjuna* such as gallic acid, ellagic acid, arjunolic acid, procyanidin, and catechin—and their target proteins, including TP53, HFE, and IL6.

The gene–disease association network reinforces these findings by linking the target proteins to specific diseases. IL6 is prominently associated with liver cirrhosis, alcoholic liver disease, and chemical-induced liver injury, underscoring the role of inflammation in these conditions. Similarly, TP53's link to liver carcinoma highlights the relevance of *T. arjuna* in cancer prevention or treatment. HFE's association with hemochromatosis and abnormal glucose tolerance expands the therapeutic scope to metabolic and iron-related disorders. In addition, the network includes conditions such as inflammation and hyperpigmentation, suggesting systemic benefits beyond liver health (Mahendra, 2023).

The DisGeNET analysis further validates these associations by identifying chronic viral hepatitis, alcoholic liver cirrhosis, and hemochromatosis as highly significant conditions. The high relevance of *T. arjuna* in chronic viral hepatitis suggests its potential in managing viral liver infections. Furthermore, its association with systemic conditions such as amyotrophic lateral sclerosis and brain infarction underscores its broader therapeutic impact, likely mediated by its anti-inflammatory and antioxidant properties. *T. arjuna* demonstrates significant therapeutic potential through its bioactive compounds targeting multiple proteins and pathways. The integrative findings suggest its efficacy in treating liver diseases, metabolic disorders, and systemic conditions. Future research should focus on experimental validation and clinical trials to explore these promising therapeutic applications.

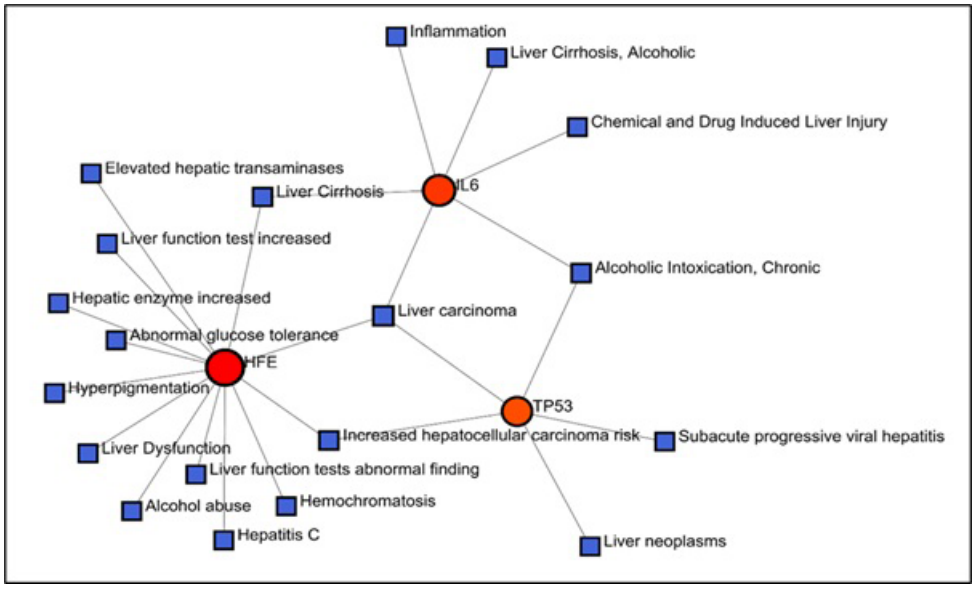


Figure 8. It represents the association between genes targeted by *Terminalia arjuna* compounds and diseases. Genes such as TP53, IL6, and HFE are linked to a wide range of liver-related disorders and systemic conditions. IL6, a pro-inflammatory cytokine, is prominently associated with liver cirrhosis, alcoholic liver disease, and chemical-induced liver injury, emphasizing its pivotal role in inflammatory pathways.

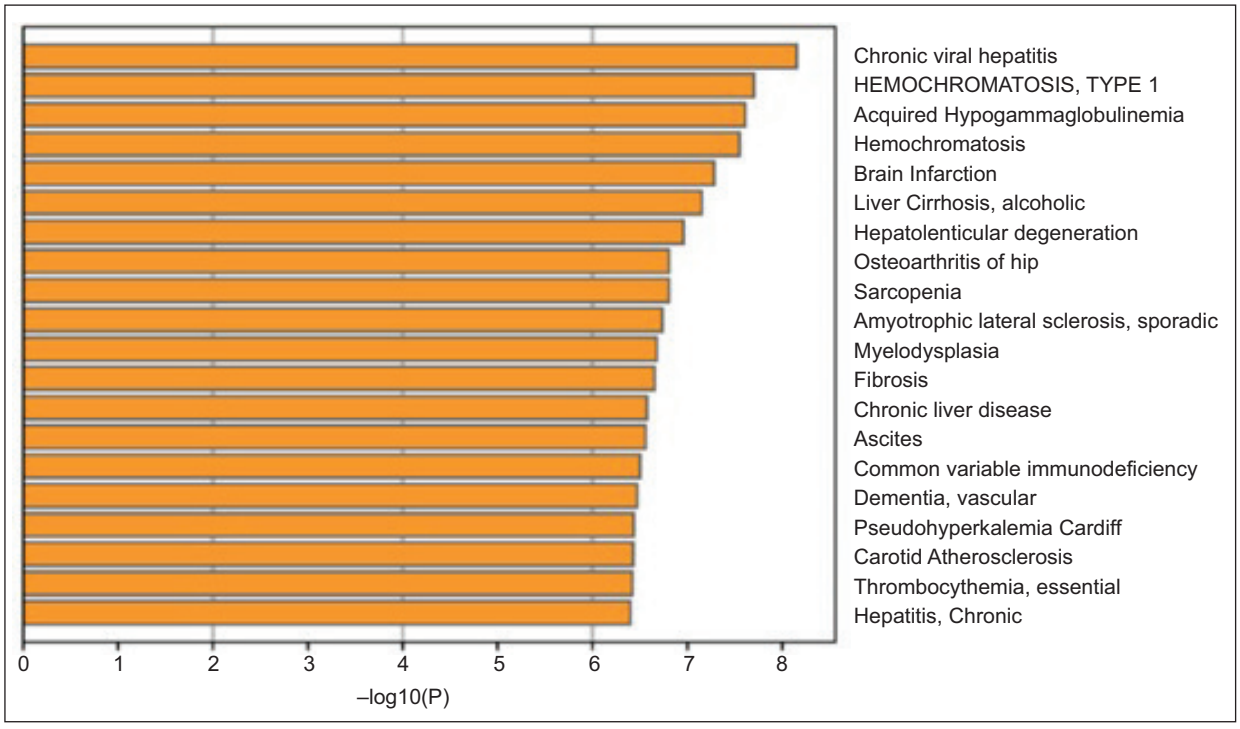


Figure 9. It represents DisGeNET analysis results through a bar chart, showcasing diseases associated with the target genes. Chronic viral hepatitis, hemochromatosis type 1, acquired hypogammaglobulinemia, and liver cirrhosis (Alcoholic and non-alcoholic liver cirrhosis).

Patanè et al. (2023) report the significant potential of catechins and their polymeric structures in addressing metabolic syndrome by mitigating its key risk factors: obesity, hypertension, and hyperglycemia. Flavanols effectively reduce oxidative stress and chronic inflammation, major contributors to metabolic dysfunction, through mechanisms tied to their flavonoid skeleton. In vitro and in vivo studies confirm their efficacy at specific doses, emphasizing their therapeutic viability. Moreover, albumin's role as a flavanol delivery system enhances its bioavailability and targeted action. These results suggest that dietary supplementation with flavanols could serve as a promising strategy to counteract multiple metabolic targets associated with metabolic syndrome (Patanè et al., 2023).

The study highlights gallic acid's protective effects against paraquat-induced liver toxicity. Paraquat significantly impaired hepatic antioxidant defenses, decreasing vitamin C, SOD, and CAT levels, while increasing oxidative stress markers (PC, MDA) and inflammatory cytokines like IL-1β. It also disrupted lipid profiles and liver function. However, gallic acid effectively reversed these toxic effects by enhancing antioxidant activity and reducing oxidative stress, inflammation, and lipid abnormalities. In addition, it improved hepatocyte morphology damaged by paraquat. These findings suggest that gallic acid strengthens the antioxidant defense system and may serve as a therapeutic agent to mitigate paraquat-induced liver damage, demonstrating its clinical potential (Nouri et al., 2021).

The findings demonstrate a strong correlation between gallic acid's antioxidant properties and its hepatoprotective effects. Paraquat-induced liver damage is characterized by oxidative stress, inflammation, and impaired liver function. Gallic acid counteracted these effects by enhancing antioxidant defenses, reducing oxidative stress markers, and mitigating inflammation. Its ability to improve hepatocyte morphology further supports its hepatoprotective role. By restoring antioxidant activity and reducing toxic impacts on the liver, gallic acid emerges as a potential therapeutic agent for liver protection, particularly in conditions of oxidative damage. This correlation underscores its utility in combating liver toxicity and preserving hepatic health (Kondeva-Burdina et al., 2023).

4. CONCLUSION

This study highlights the therapeutic potential of *T. arjuna* in managing hepatic complications through multitargeted biomolecular interactions. The pharmacopoeial assessment

confirmed its compliance with quality standards, while the DPPH assay demonstrated strong antioxidant activity, indicating its efficacy in combating oxidative stress. Metabolomic profiling using HPTLC and LC-MS identified key bioactive compounds, including gallic acid, catechin, and arjunolic acid. Network pharmacology analysis revealed that *T. arjuna* effectively interacts with critical targets such as TP53, IL6, and HFE, which play pivotal roles in liver pathogenesis, including liver carcinoma, cirrhosis, and hypogammaglobulinemia. These findings suggest that *T. arjuna* offers a promising natural alternative for hepatic disease treatment by regulating key molecular pathways. However, while the study provides strong preliminary evidence, further preclinical and clinical trials are essential to establish its efficacy and safety. Expanding research in this area could lead to affordable and accessible liver disease treatments based on natural compounds.

AUTHOR CONTRIBUTIONS

Arti Sinoria was responsible for data collection as well as the analysis and interpretation of the results. Dr. Garima Garg and Dr. Nitin Kumar contributed to drafting the manuscript and critically revising it for important intellectual content and approving the final version for publication.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, Ganga Nagar-250001, Meerut, India for providing the facilities and support to complete the study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

Nil.

ORCID

Arti sinoria 0009-0003-4295-1613
Garima Garg 0000-0001-9460-1840
Nitin Kumar 0000-0003-4473-6418

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