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PRELIMINARY PHYTOCHEMICAL STUDIES OF TERMINALIA ARJUNA BARK EXTRACT VIA SOXHLET AND COLD MACERATION

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ABSTRACT

The study investigates the phytochemical profile of *Terminalia arjuna* bark (Commercially available powder) extracts prepared using two extraction methodssuch as Soxhlet extraction and cold macerationwith solvents of varying polarity (ethanol, methanol, ethyl acetate, hexane, and aqueous). Preliminary phytochemical screening was conducted to detect bioactive compounds. The results demonstrated that Soxhlet extraction enabled a wider recovery of phyto-constituents compared to cold maceration. Ethanol and methanol extracts were especially rich in phenols, tannins, steroids, alkaloids, while aqueous extracts consistently showed phenols, tannins, steroids, alkaloids, and saponins. Hexane extracts predominantly alkaloids. Cold maceration, although effective in extracting some alkaloid, tannin, saponin and phenol compounds, was less efficient overall, yielding a narrower phytochemical spectrum. These results confirm that *Terminalia Arjuna* bark is a potent source of diverse phytoconstituents with potential pharmacological importance, and that Soxhlet extraction may be regarded as a superior technique for the effective recovery of bioactive compounds.

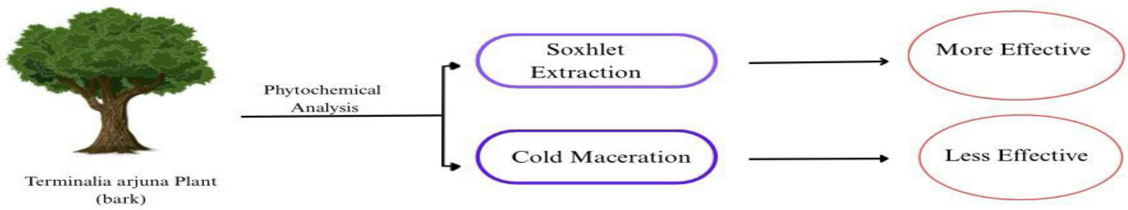

Fig : Graphical Abstract

INTRODUCTION

Medicinalplants have long been recognized as one of the most significant sources of therapeutic agents for humankind.[1] Since ancient times, herbal preparations and plant-derived remedies have played a crucial role in the prevention and treatment of various diseases. According to the World Health Organization (WHO)[2], nearly 80% of the world's population still relies on plant-based medicines for primary healthcare needs, highlighting the continuing importance of medicinal plants as reservoirs of bioactive compounds. These phytoconstituents, often secondary metabolites[3] are not directly involved in plant growth and development but contribute to their defence mechanisms against pathogens, pests, and environmental stress. Importantly, they are also responsible for the pharmacological properties that make plants valuable in traditional and modern medicine.

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In this context, *Terminalia Arjuna*[4], a well-known medicinal plant belonging to the family Combretaceae, has been extensively used in Ayurveda, Siddha, and Unani systems of medicine. Its bark, in particular, is valued for its cardioprotective, antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Previous studies have attributed these activities to a rich composition of bioactive compounds, including triterpenoids (arjunolic acid, arjunic acid), flavonoids, tannins, and glycosides.Despite its traditional use and pharmacological importance, comparative studies on the influence of different extraction methods and solvents on the phytochemical profile of *T. arjuna* bark remain limited.

Therefore, the present study was designed to carry out a preliminary phytochemical investigation[5]of *Terminalia arjuna* bark extracts prepared using Soxhlet extraction and cold maceration with solvents of varying polarity (ethanol, methanol, ethyl acetate, hexane, and aqueous). By comparing the two extraction techniques, the study aims to determine their relative efficiency in recovering diverse classes of phytoconstituents. This comparative analysis not only provides insights into the optimal extraction strategy for *T. arjuna* but also contributes to a deeper understanding of how extraction conditions influence phytochemical yield and diversity. Ultimately, such information is essential for standardizing herbal preparations, ensuring reproducibility, and facilitating the development of plant-based therapeutic agents.

MATERIALS AND METHODS

Collection of Sample

Commercially available *Terminalia arjuna* bark powder was procured from a certified herbal supplier. The material was authenticated by a Botanist, Dr. Rahul B. Kamble, Department of Botany, Dr. Ambedkar College, Deekshabhoomi, Nagpur.

Preparation of Extract

Soxhlet Extraction: The extraction yield of the extracts from plants species is highly depends on the solvent polarity, which determines both qualitatively and quantitatively the extracted compounds. 50 g of bark powder was subjected to Soxhlet extraction sequentially with (non polar to polar solvents) such as hexane, ethyl acetate, ethanol, methanol, and distilled water in a Soxhlet apparatus for 72 h followed by concentrated in a rotator evaporator under reduced pressure at temperature 40-50°C. Extracts were filtered and concentrated under reduced pressure using a rotary evaporator, then stored at 4 °C.

Cold Maceration: A 50 gmof bark powder was macerated in each solvent (non-polar to polar solvents) hexane,ethyl acetate, ethanol, methanol, distilled water for 72 hours at room temperature with intermittent shaking. Extracts were filtered, concentrated, and preserved at 4 °C.

Phytochemical Screening

Preliminary phytochemical tests were conducted according to standard protocols[6-7]for detecting:

- **Phenols and Tannins** – Ferric chloride test
- **Flavonoids** – Shinoda and Alkaline reagent tests
- **Steroids and Terpenoids**- Salkowski and Liebermann–Burchard tests
- **Alkaloids** – Mayer's and Wagner's test
- **Saponins** – Froth test
- **Coumarins** – UV fluorescence test
- **Gums and Mucilage** – Ruthenium red test
- **Volatile oils** – Spot test
- **Fixed oils** – Stain test

RESULTS AND DISCUSSION

Phytochemical Screening Results

Phytochemical Screening Results (+/-) Soxhlet Extractionin different solvents

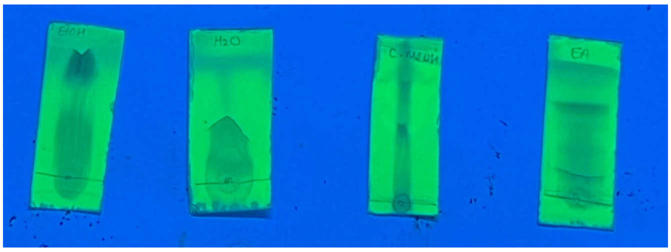
Sr. No.	Phytoconstituents	Ethanol	Methanol	Ethyl Acetate	Hexane	Aqueous
1	Phenol	+	+	-	-	+
2	Tannin	+	+	+	-	+
3	Flavonoid	-	-	-	-	-
4	Steroids	+	-	+	-	+
5	Alkaloid	+	+	+	+	+
6	Terpenoid	-	-	-	-	-
7	Saponin	+	-	-	+	+
8	Coumarin	-	-	-	-	-
9	Gum and Muci-lage	-	-	-	-	-
10	Volatile Oil	-	-	-	-	-
11	Fixed Oil	-	-	-	-	-

Phytochemical Screening Results (+/-) Cold Maceration in different solvents

Sr. No.	Phytoconstituents	Ethanol	Methanol	Ethyl Acetate	Hexane	Aqueous
1	Phenol	-	+	-	-	+
2	Tannin	+	+	-	-	+
3	Flavonoid	-	-	-	-	-
4	Steroids	+	-	-	-	+
5	Alkaloid	+	+	+	+	+
6	Terpenoid	-	-	-	-	-
7	Saponin	-	-	-	-	+
8	Coumarin	-	-	-	-	-
9	Gum and Mucilage	-	-	-	-	-
10	Volatile Oil	-	-	-	-	-
11	Fixed Oil	-	-	-	-	-

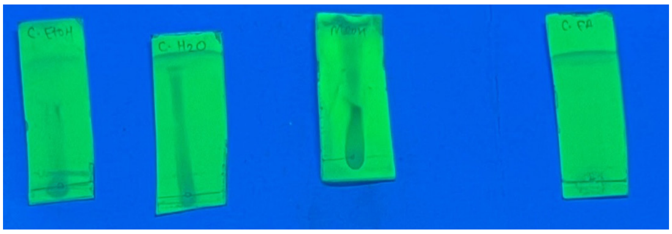
TLC Observation[8]

TLC profiling of the Soxhlet and cold maceration extracts of *Terminalia arjuna* bark was carried out on TLC plates (Figure 1 and 2). The plates were developed using a solvent system of Methanol:Ethylacetate (1:4, v/v).



Soxhlet Extraction

Figure 2.TLC profile of Terminalia arjuna extracts (a) ethanol (b) aqueous (c) methanol (d) ethyl acetate visualized solvent system of Methanol:Ethyl acetate (1:4, v/v).



Cold Maceration

Figure 3.TLC profile of Terminalia arjuna extracts (a) ethanol (b) aqueous (c) methanol (d) ethyl acetatein visualized solvent system of Methanol:Ethyl acetate (1:4, v/v).

Post-derivatization TLC visualization

To further confirm the nature of phytoconstituents, developed TLC plates were sprayed with specific derivatizing reagents. Anisaldehyde reagent produced brown to violet coloured bands in ethanol and methanol Soxhlet extracts, confirming the presence of triterpenoids, steroids, and saponins. Ninhydrin reagent revealed characteristic brown spots, indicating the presence of amino groups and alkaloid-like compounds. These results provide additional evidence supporting the preliminary phytochemical screening data.

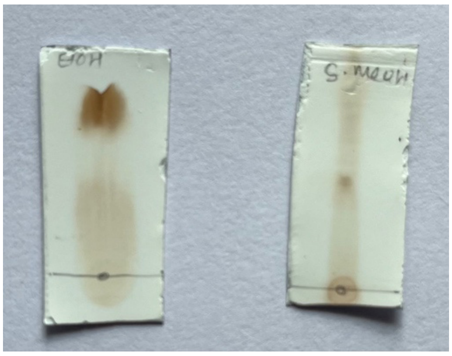


Figure 3(a). TLC profile of Terminalia arjuna ethanol and methanol extractsrespectively visualized with ninhydrin reagent (Soxhlet) Soxhlet)

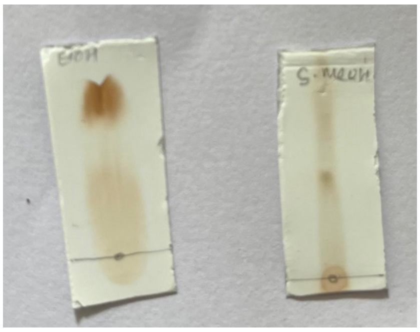
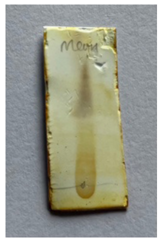


Figure 3 (b).TLC profile of Terminalia arjuna ethanol and methanol extractsrespectively visualized with anisaldehyde reagent (Soxhlet)

Table 1. Phytochemical Detection by TLC Using Different Reagents (Methanolic Cold Maceration Extract)

Reagent	Observation	Possible Compounds	TLC Analysis
Ninhydrin	Brownish spot	Amino group containing compounds (amino acids, peptides, alkaloids)	
Iodine vapour (I_2)	Light brown/ yellow spot visible	Terpenoids, steroids, fatty acids (nonpolar to mid-polar compounds)	
Anisaldehyde	Brownish/ greyish spot	Flavonoids, phenolics, terpenoids, glycosides	

DISCUSSION

The results demonstrate that Soxhlet extraction yielded a broader spectrum of phytochemicals across solvents compared to cold maceration. Ethanol and methanol extracts were particularly rich in phenols, tannins, alkaloids, and steroids, compounds known for antioxidant and cardioprotective effects. Aqueous extracts consistently contained phenols, tannins, alkaloids, and saponins, aligning with the traditional Ayurvedic practice of using aqueous decoctions.Hexane extracts showed predominant alkaloid recovery in Soxhlet, while cold maceration yielded fewer constituents overall. The poor performance of hexane in cold maceration can be attributed to its non-polar nature and lack of exhaustive solvent penetration.The superiority of Soxhlet extraction is attributed to continuous hot solvent recycling, which enhances solubility and extraction of bioactive compounds. However, cold maceration may still be useful for preserving thermolabile compounds, though in smaller quantities.These findings reinforce the pharmacological potential of *T. arjuna*, particularly ethanol and methanol extracts, which may serve as rich sources of therapeutic compounds.

The TLC profiling of *Terminalia arjuna* bark extracts obtained through Soxhlet and cold maceration methods revealed the presence of diverse classes of phytoconstituents. Soxhlet extracts developed in the solvent system of Methanol:Ethylacetate (1:4, v/v). showed clear separation of bands under UV light (Fig. 2). Ethanol and methanol extracts displayed prominent bands, while the aqueous extract exhibited fewer and faint spots, indicating a lower concentration of extractable secondary metabolites. The ethyl acetate extract also showed well-defined bands, reflecting the presence of semi-polar compounds.

Post-derivatization analysis further supported the preliminary phytochemical screening. Anisaldehyde spray reagent produced characteristic brown to violet bands in Soxhlet ethanol and methanol extracts, suggesting the presence of triterpenoids, steroids, and saponins. Ninhydrin treatment resulted in brownish spots, confirming amino group–containing compounds such as amino acids or alkaloids. These findings are consistent with earlier reports on *T. arjuna* bark phytochemistry, which highlight the abundance of triterpenoids and phenolic compounds.

Cold maceration methanolic extract also exhibited distinct TLC spots after derivatization (Fig. 3 and Table 1). Ninhydrin revealed the presence of nitrogenous compounds, iodine vapour confirmed terpenoids and steroids, and anisaldehyde indicated flavonoids, glycosides, and phenolics. The results demonstrate that even under cold extraction, methanol efficiently extracts a wide range of phytoconstituents. However, the intensity and sharpness of bands were comparatively less prominent than Soxhlet extracts, which may be attributed to the higher extraction efficiency of continuous hot percolation.

Overall, the TLC results validate the phytochemical diversity of *T. arjuna* bark and confirm that both Soxhlet and cold maceration extracts contain bioactive secondary metabolites such as flavonoids, phenolics, terpenoids, steroids, glycosides, and alkaloids. These findings provide strong experimental support to the preliminary phytochemical screening and highlight methanol as a suitable solvent for the extraction of multiple bioactive constituents.

CONCLUSION

The present study confirmed that *Terminalia arjuna* bark contains a wide array of phytoconstituents, including phenols, tannins, steroids, alkaloids and saponins. Soxhlet extraction consistently demonstrated superior recovery of these compounds across solvent systems, particularly with ethanol and methanol, compared to cold maceration.The results validate the medicinal potential of *T. arjuna* and highlight the importance of selecting suitable extraction methods and solvents for maximizing phytochemical yield. Further quantitative and chromatographic analyses (HPTLC, GC-MS, LC-MS) are recommended to precisely characterize and isolate the active compounds responsible for pharmacological activities.

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